



A multi-compartment pulsatile drug delivery system for sequential release: in vitro and in vivo evaluation

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Abstract

This study presents an innovative, structurally engineered, pulsatile oral drug delivery platform that enables programmable, sequential drug release by integrating a structurally engineered three-dimensional (3D)-printed capsular device with conventional immediate-release (IR) tablets. Unlike coating-based or multilayer systems, which suffer from complex manufacturing and inconsistent release profiles, the multi-compartment capsule provides precise and reproducible control over the release timing through simple structural modifications. Each compartment accommodated intact IR tablets, allowing immediate release from the open-window sections and delayed release from the barrier-enclosed compartments. The lag time (T_{lag}) before drug release can be predictably tuned by adjusting the barrier wall thickness. The system was optimized for esomeprazole and naproxen, for which gastroprotection requires temporally separated exposure. The in vitro dissolution displayed immediate release of esomeprazole and delayed release of naproxen with T_{lag} of 11.33 ± 1.37 h. Both immediate absorption of esomeprazole and delayed absorption of naproxen were observed following oral administration in beagle dogs. The time to maximum plasma concentration (T_{max}) of esomeprazole was 0.67 ± 0.67 h, whereas the T_{max} of naproxen was 9.83 ± 2.40 h with a T_{lag} of 4.75 ± 1.17 h. These findings demonstrate that this modular 3D-printed platform, combined with conventional tablets, provides controlled and sequential drug delivery, thereby supporting patient-tailored therapeutic regimens.

Keywords Pulsatile release · Delayed release · Drug delivery system · 3D printing · Pharmacokinetics

Introduction

Oral drug delivery remains the predominant route of administration owing to its convenience, non-invasiveness, and cost-effectiveness. Over the decades, oral drug delivery systems have evolved significantly from conventional immediate-release formulations to several sophisticated modified-release systems, such as sustained-release, extended-release, and delayed-release formulations [1]. These advancements have substantially improved the therapeutic outcomes

by reducing fluctuations in plasma drug concentrations, minimizing adverse effects, decreasing dosing frequency, and enhancing patient adherence. However, despite these improvements, significant challenges remain in addressing the more complex drug delivery requirements, particularly those involving precise temporal control of drug release [2, 3].

Pulsatile or repeat-action drug delivery systems have emerged as a specialized approach designed to release drugs at distinct time points, creating pulses of drug release at predetermined time intervals [2, 4]. Unlike conventional sustained-release formulations, which aim to maintain constant plasma drug concentrations, these systems deliver the drug sequentially at well-defined drug-free intervals. Temporal control of drug release is particularly valuable for therapeutic applications that target conditions with circadian rhythms, therapies for developing biological tolerance, and delivery of multiple active pharmaceutical ingredients in a precisely timed manner [4, 5].

For example, the clinical need for pulsatile drug delivery systems is evident under conditions with prevailing night

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or early morning manifestations [6]. The pulsatile delivery of antihypertensive medications in cardiovascular disease treatment can efficiently manage the early morning surge in blood pressure when patients are at a higher risk for adverse cardiovascular events. Similarly, for asthma, which often worsens during the night and early in the morning, pulsatile delivery systems can release higher doses of bronchodilators during vulnerable periods. Moreover, repeated pulse delivery of antibiotics can potentially prevent the development of resistant bacterial strains [7].

Another important application of pulsatile delivery systems is in combination therapies, in which the sequential administration of drugs can prevent unwanted drug–drug interactions or optimize therapeutic outcomes. Pulsatile drug delivery systems can circumvent detrimental drug–drug interactions by sequentially releasing medications at distinct times after a single administration, which would otherwise require separate administration at specific time intervals [8]. Therefore, a pulsatile drug delivery system can improve patient compliance. An example of the improvement of therapeutic outcomes by a pulsatile drug delivery system is the combination of non-steroidal anti-inflammatory drugs (NSAIDs) and proton pump inhibitors (PPIs). Although NSAIDs such as naproxen effectively treat different inflammatory conditions, these are associated with serious gastrointestinal adverse effects [9, 10]. These side effects can be mitigated by co-administration of PPIs such as esomeprazole [9, 11]. However, the protective effect is optimized when PPI achieves sufficient acid suppression before NSAID exposure [12], which can be achieved using pulsatile drug delivery systems.

Several approaches have been employed to develop pulsatile drug delivery systems, including multi-layered tablets, coating technologies, and matrix systems with erosion or swelling mechanisms [4, 6]. However, these conventional methods often involve complex manufacturing processes requiring multiple steps, specialized equipment, and extensive optimization. Several of these systems suffer from premature drug release, inconsistent release profiles, high performance variability, and limited flexibility in adapting to different active pharmaceutical ingredients. Furthermore, scale-up and reproducibility issues frequently arise during industrial production, presenting significant barriers to commercialization.

A commercial example illustrating both the potential and limitations of current approaches is VIMOVO[®], a fixed-dose combination product containing immediate-release esomeprazole and enteric-coated naproxen [13]. Although VIMOVO[®] successfully reduces NSAID-associated gastric damage compared to naproxen alone, its formulation depends on conventional enteric coating technology, which provides limited control over the precise timing of naproxen release. Moreover, the manufacturing process for

such multi-component dosage forms is complex and offers minimal flexibility for customization based on individual patient needs.

Recent advances in three-dimensional (3D) printing technology have opened up new possibilities for pharmaceutical applications, offering unprecedented precision and flexibility in dosage form design. We demonstrated the potential of 3D printing technology to develop new controlled-release formulations by combining 3D-printed capsular devices with controlled-release functionality with conventional tablets [14, 15]. For example, a gastroretentive delivery system for acyclovir was developed using fused deposition modeling (FDM) 3D printing technology, which demonstrated enhanced gastric retention and improved bioavailability in beagle dogs [14]. In another study, the application of 3D printing for the development of customized oral drug delivery systems with controlled-release profiles was explored [15]. These investigations indicate the feasibility of using 3D printing to fabricate complex pharmaceutical formulations with tailored functionalities, suggesting its potential application in the creation of sophisticated pulsatile drug delivery systems.

Therefore, we designed and developed a modular 3D-printed pulsatile drug delivery system by combining a 3D-printed functional capsular device with conventional tablets. We hypothesized that a multi-compartment 3D-printed capsular device would enable the immediate release of one drug, followed by the sequential release of other drugs after predetermined lag times. The system was optimized using esomeprazole and naproxen as model drugs, which were designed with gastroretentive features to ensure that drug release occurred in the stomach, the primary site of both acid suppression and NSAID-induced damage. Both in vitro dissolution studies and in vivo pharmacokinetic evaluations were conducted in beagle dogs to characterize the performance of the developed system. The present approach may be promising for controlled-release technology, potentially overcoming the limitations of conventional manufacturing methods while offering new possibilities for optimized combination drug therapy.

Materials and methods

Materials

Esomeprazole magnesium (98%), naproxen sodium (98%), and lansoprazole (98%), an internal standard (IS), were acquired from Sigma-Aldrich Co. (St. Louis, MO, USA). Formic acid and ammonium formate were obtained from Sigma-Aldrich. High-performance liquid chromatography-grade (HPLC)-grade reagents, including triethylene amine, phosphoric acid, methanol, acetonitrile, and distilled water,

were purchased from J.T. Baker, Inc. (Phillipsburg, NJ, USA). Microcrystalline cellulose (MCC) and magnesium stearate were obtained from Whawon Pharmaceuticals. Co. (Seoul, Korea), and Samchun Chemical Co., Ltd. (Seoul, Korea). Polylactic acid (PLA) and polyvinyl alcohol (PVA) filaments were purchased from Raise3D Inc. (Irvine, CA, USA).

Preparation of pulsatile drug delivery systems

Inner tablets

Ketoprofen was used as a model drug during the development of the pulsatile drug delivery system, which was later optimized for esomeprazole and naproxen. For the ketoprofen IR tablet, dexketoprofen trometamol (30.0%) was used as the active pharmaceutical ingredient and was formulated using wet granulation with lactose (49.0%), microcrystalline cellulose (5.0%), low-substituted hydroxypropyl cellulose (10.0%), Kollicoat (5.0%), and magnesium stearate (1.0%).

Esomeprazole magnesium trihydrate (salt factor=0.90) and naproxen sodium (salt factor=0.91) were used as the active pharmaceutical ingredients for esomeprazole and naproxen IR tablets, respectively (Table 1). The esomeprazole IR tablet was formulated using lactose (81.39%) as the binder, crospovidone (4.88%) as the disintegrant, and sodium stearyl fumarate (0.27%) and magnesium stearate (1.36%) as lubricants. For naproxen IR tablets, microcrystalline cellulose (14.67%) was used as the diluent, polyvinylpyrrolidone K30 (1.18%) as the binder, sodium starch glycolate (5.79%) as the disintegrant, and magnesium stearate (4.81%) as the lubricant. The esomeprazole IR tablets were manufactured using direct compression. The ingredients were mixed in a polybag for 10 min before the lubricants were added in an additional mixing step. Naproxen IR

tablets were prepared by wet granulation, in which the active pharmaceutical ingredient was mixed with the diluent and disintegrant before adding a sufficient volume of ethanol containing polyvinylpyrrolidone K30. The kneaded mixture was passed through a 20-mesh sieve, dried at 60 °C for 60 min, and sieved again. After adding magnesium stearate, the granules were directly compressed using a hydraulic tablet press (Carver, Inc., Wabash, IN) with round-shaped punches (esomeprazole: 9.0 mm diameter, 3.0 mm height; naproxen: 5.0 mm diameter, 6.0 mm height) at a pressure of 1.0 ton.

Pulsatile drug release capsular device

The pulsatile drug-release capsular device was designed using computer-aided design (CAD) software (Rhino 6; Robert McNeel & Associates, Seattle, WA, USA) and fabricated using a FDM 3D printer (Raise3D Pro2; Irvine, CA, USA). The device structure featured an air pocket for gastro-floating, and two or three compartments printed with PLA and PVA filaments. The multi-compartment capsular device comprised an open window-type compartment for the immediate release of its inner tablet and other compartments enclosed by a water-soluble PVA filament for the second and third-timed releases of their respective inner tablets (Fig. 1A). Both PLA and PVA have established safety profiles for use in biomedical applications. PLA is Food and Drug Administration (FDA)-approved and Generally Recognized As Safe (GRAS) for medical use [16, 17]. PVA is poorly absorbed, does not bioaccumulate [18], and dissolves into nanosized particles that are readily eliminated from the gastrointestinal tract [19].

The printing parameters for the FDM 3D printer were optimized as follows: 0.15 mm for the first layer height, 215 °C nozzle temperature, 85 °C heat bed temperature, 0.25 mm extrusion width, 40 mm/s retraction speed, 80% infill density, 15 mm/s printing speed, and 6 mm/s first layer speed. The dual-extrusion system utilized a PLA filament for the main body and a PVA filament for the delayed-release compartments. During printing, the inner tablets were inserted into designated compartments to achieve sequential drug release. The final assembly constituted a complete pulsatile drug delivery system comprising a top air pocket and multiple compartments containing inner tablets (Fig. 2).

In vitro evaluation

In vitro dissolution tests

In vitro dissolution testing of the pulsatile drug delivery system was conducted according to the USP Apparatus 2 guidelines (paddle method) using a Distek Dissolution System 2500 coupled with an Evolution Dissolution Sampler 4300 (North Brunswick, NJ, USA). The dissolution medium

Table 1 Composition (w/w %) of esomeprazole and naproxen immediate-release (IR) tablets

	Esomeprazole	Naproxen
Active pharmaceutical ingredient*	12.10 (20 mg as esomeprazole)	73.55 (100 mg as naproxen)
Lactose	81.39	—
Crospovidone	4.88	—
Microcrystalline cellulose	—	14.67
Polyvinylpyrrolidone K30	—	1.18
Sodium starch glycolate	—	5.79
Sodium stearyl fumarate	0.27	—
Magnesium stearate	1.36	4.81
Total	100.0	100.0

*The salt factor of esomeprazole magnesium trihydrate and naproxen sodium is 0.90 and 0.91, respectively

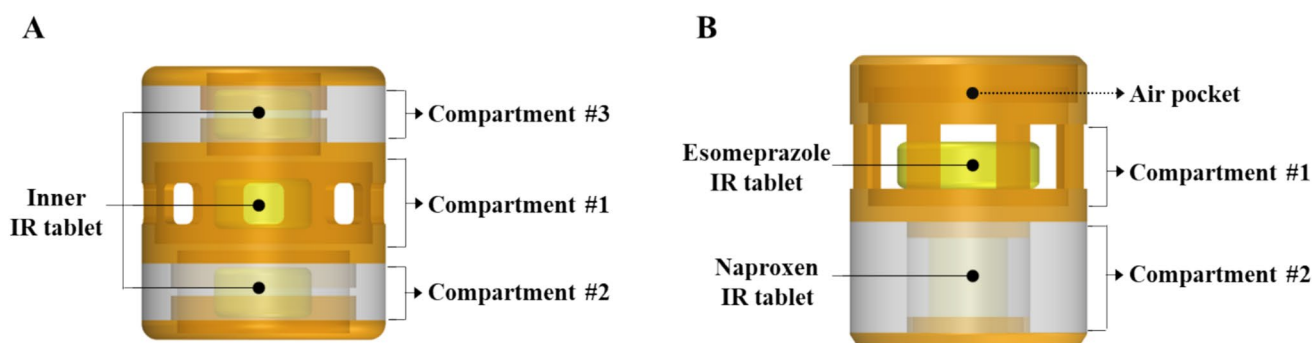


Fig. 1 Structure of the pulsatile drug delivery system consisting of a capsular device with three compartments (A) and two compartments containing inner immediate-release (IR) tablets (B)

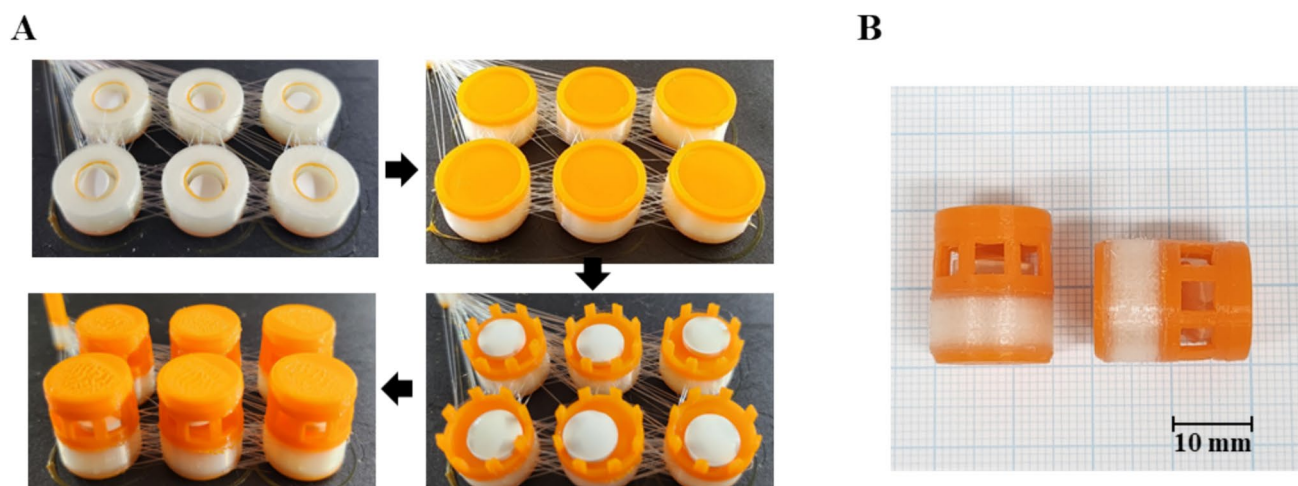


Fig. 2 Printing process of the pulsatile drug delivery systems using an FDM 3D printer with polylactic acid (PLA) and polyvinyl alcohol (PVA) filaments (A), and the completed system for esomeprazole and naproxen (B)

consisted of 900 mL of a phosphate buffer solution (pH 6.8) maintained at 37 ± 0.5 °C, with a paddle stirring speed of 50 rpm. Samples (2 mL) were collected at the predetermined time points. The medium was replaced with fresh medium after each sampling. The collected samples were filtered through a 45 μ m polyethylene syringe filter (Distek) before analysis. The lag time (T_{lag}) was determined as the time immediately preceding the first measurable increase in drug concentration in the dissolution medium. T_{80} was defined as the time at which 80% of the loaded drug was released into the dissolution medium.

HPLC analysis

The stock solution was prepared by dissolving dexketoprofen trometamol in methanol. The stock solution was serially diluted with methanol to prepare the working standard solutions. Calibration curves were constructed for ketoprofen (1.0–40 μ g/mL) to determine the drug

concentrations in the samples. The ketoprofen concentrations were quantified using a ultraviolet/visible Spectrophotometer (Ultrospec 2100 *pro*, Amersham Biosciences, Buckinghamshire, UK).

Standard esomeprazole magnesium trihydrate and naproxen sodium were dissolved in methanol to obtain stock solutions. These stock solutions were serially diluted with 50% acetonitrile to prepare working standard solutions. Calibration curves were constructed for esomeprazole (0.5–50 μ g/mL) and naproxen (5–500 μ g/mL). The esomeprazole and naproxen concentrations in the dissolution medium were quantified using a Waters Alliance 2695 HPLC system coupled with a Waters 2996 Photodiode Array Detector (Waters; Milford, MA, USA). Chromatographic separation was performed on a Luna C18 column (250 $\times$ 4.6 mm, 5 μ m, Phenomenex, Torrance, CA). The mobile phase comprised 0.1% triethylamine in water (adjusted to pH 3 using phosphoric acid) and acetonitrile (40:60, v/v). The flow rate was 1 mL/min, with a total run time of 7 min, and the column

temperature was maintained at 40 °C. A sample volume of 20 µL was injected, and detection was performed at 273.6 nm.

In vivo evaluation

Animal studies

The animal studies were approved by the Institutional Animal Care and Use Committee of HLB Biostep (KNOTUS IACUC 22-KE-0043, KNOTUS IACUC 23-KE-0207). Six male beagle dogs (16–18 months) from Orient Bio (Seongnam, Korea) were housed individually in stainless steel cages (W 895 × L 795 × H 765 mm) under controlled conditions (temperature: 23 ± 3 °C, relative humidity: $55 \pm 10\%$, 12-h light/dark cycle with 150–300 Lux illumination). The animals were fed a standard dog diet (300 g; Cargill Agri Purina, Seongnam, Korea) once daily, with water available ad libitum.

The in vivo performance of the pulsatile drug delivery system was evaluated in beagle dogs following oral administration of a barium sulfate-labeled system. Serial abdominal radiographs were obtained to track the sequential separation of each compartment, from which the release of the inner tablet was inferred in the gastrointestinal tract.

For the pharmacokinetic evaluation, an optimized pulsatile drug delivery system containing 20 mg esomeprazole and 100 mg naproxen was orally administered to beagle dogs under non-fasting conditions. The mouth of the animal was opened, and the drug delivery system was placed at the base of the tongue to allow natural swallowing. After confirming complete ingestion, 25 mL of water was administered using a syringe. All six animals successfully swallowed the devices without difficulty, and no adverse events (vomiting, abnormal excretion, or changes in mobility) were observed throughout the study. Blood samples (3 mL) were collected from the cephalic vein into heparinized tubes (5 IU/mL) at 0, 0.17, 0.33, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 24, 36, 48, 72, 96, 120, and 144 h after administration. The blood samples were centrifuged at $4,000 \times g$ at 4 °C for 10 min to obtain plasma, which was stored at -70 °C until analysis.

LC–MS/MS analysis

The plasma concentrations of esomeprazole and naproxen were simultaneously determined using a liquid chromatography–tandem mass spectrometry (LC–MS/MS) system consisting of an Agilent 1200 HPLC coupled with an Agilent 6430 triple-quadrupole mass spectrometer (Agilent Technologies; Santa Clara, CA, USA). Chromatographic separation was performed on a Luna CN column (150 × 2.0 mm, 3 µm, Phenomenex, Torrance, CA) with a Security Guard

Cartridge Kit (Phenomenex). The mobile phase consisted of 0.2% formic acid and 10 mM ammonium formate in water/acetonitrile (65:35, v/v). The flow rate was 0.3 mL/min, with a total run time of 6 min, and the column temperature was maintained at 40 °C. A sample volume of 2 µL was injected.

The mass spectrometer was operated in positive electrospray ionization mode with multiple reaction monitoring (MRM). The selected *m/z* transitions were 346.1 → 198.1 for esomeprazole, 231.1 → 185.0 for naproxen, and 370.0 → 252.0 for lansoprazole (IS). The MS parameters were as follows: gas flow, 10 L/min; gas temperature, 350 °C; nebulizer, 35 psi; fragmenter, 135 V; collision energy, 6 V for esomeprazole, 10 V for naproxen, and 5 V for IS. The dwell time was 200 ms for all transitions. Data acquisition and processing were performed using MassHunter Quantitative Analysis software (Agilent Technologies).

Stock solutions were prepared by dissolving the standard compounds, esomeprazole and naproxen, in methanol. Working standard solutions were prepared by serially diluting the stock solutions with methanol. The calibration ranges were 10–5000 ng/mL for esomeprazole and 100–50000 ng/mL for naproxen. Quality control (QC) samples were prepared at the lower limit of quantification (LLOQ) and at low, medium, and high concentrations of both analytes. The sample preparation involved protein precipitation. Briefly, 50 µL of IS working solution (1000 ng/mL), 50 µL of the appropriate working solution, and 50 µL of blank dog plasma were mixed with 350 µL of methanol. After vortexing for 10 min, the mixture was centrifuged at $15,000 \times g$ for 10 min. A 100 µL aliquot of the supernatant was mixed with 100 µL of distilled water and transferred to an autosampler vial for injection.

The assay was validated for specificity, linearity, sensitivity, accuracy, precision, process efficiency, and stability in accordance with the FDA guidelines [20]. Intra- and inter-day accuracy and precision were assessed using five replicates of quality control (QC) samples. Process efficiency was evaluated by assessing the extraction recovery and matrix effects using QC samples. Stability was assessed under several conditions, including short-term (4 h at room temperature), long-term (3 weeks at -70 °C), autosampler (24 h at 4 °C), and freeze–thaw (three cycles) conditions.

Noncompartmental analysis

Pharmacokinetic parameters were determined using the non-compartmental analysis. The parameters included lag time (T_{lag}), maximum plasma concentration (C_{max}), time to reach maximum concentration (T_{max}), terminal half-life ($t_{1/2}$), and the area under the plasma concentration–time curve from time zero to the last observation time point (AUC_{last}) and to infinity (AUC_{inf}). C_{max} and T_{max} were obtained directly from the plasma concentration–time profiles.

Statistical analysis

All data are presented as mean $\pm$ standard deviation unless otherwise specified. Linear regression analysis was performed to evaluate the correlations between the variables. The regression equation and coefficient of determination (R^2) were obtained for all data points using IBM SPSS Statistics version 28 (IBM Corp., Armonk, NY, USA).

Results

Development of the pulsatile drug delivery system

System design

A pulsatile drug delivery system was initially developed to achieve multiple timed releases of a drug using ketoprofen as the model drug. Figure 1 illustrates the structure of the pulsatile drug delivery system, which consisted of a capsular device with three or two compartments and inner IR tablets. Compartment #1 was designed with open windows for immediate release, whereas compartments #2 and #3 were enclosed in water-soluble PVA walls of varying thicknesses to enable the sequential delayed release of the inner tablets. The release times of the second and third tablets could be controlled independently and precisely by modifying the thickness of the PVA walls. Thicker PVA walls require more time to dissolve, thereby prolonging the lag time before drug release.

The system was fabricated using a dual-extrusion FDM 3D printer equipped with PLA and PVA filaments. PLA was used to construct the structural framework, and PVA was used as a dissolution-controlled barrier. Figure 2A illustrates the printing process. During printing, the IR tablets were manually inserted at predetermined stages. The IR tablet for

immediate release was placed during printing of the windowed structure, whereas the tablets for delayed release were inserted during PVA barrier extrusion.

Effect of PVA wall thickness

A series of in vitro dissolution studies was performed to demonstrate that the timing of drug release could be controlled by controlling the PVA wall thickness of the capsular device. Figure 3A shows a representative in vitro dissolution profile of ketoprofen from the pulsatile drug delivery system with PVA wall thicknesses of 1.5 mm in the second compartment and 3.0 mm in the third compartment ($n=4$). The system exhibited an initial immediate-release phase, followed by a clear stepwise release pattern. The second release occurred at 3.50 ± 0.63 h and the third release at 8.00 ± 0.93 h, corresponding to the lag times (T_{lag}) before the second and third releases, respectively.

Figure 3B shows the relationship between PVA wall thickness ranging from 1.2 mm to 3.6 mm and T_{lag} for drug release ($n=4-6$). As the PVA wall thickness increased, the lag time was progressively prolonged from 2.51 ± 0.59 h at 1.2 mm to 10.35 ± 0.58 h at 3.6 mm. Linear regression analysis of all the individual data points ($n=34$) confirmed a strong linear correlation ($R^2=0.943$; $p<0.001$). These data demonstrated a direct correlation between the thickness of the PVA barrier and the onset of drug release, supporting the feasibility of timing control through structural design.

In vivo X-ray imaging

Serial ventrodorsal (VD) and lateral (LAT) abdominal X-ray images were acquired following oral administration of the pulsatile drug delivery system in beagle dogs to visualize the release behavior in vivo. The capsular device in this study included PVA barriers of 1.5 mm and 3.0 mm in

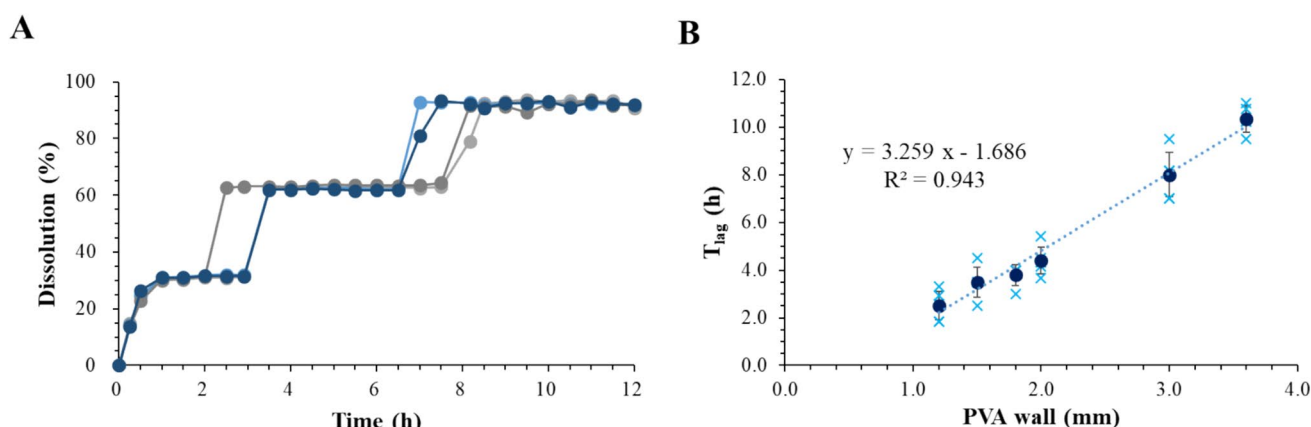


Fig. 3 Representative in vitro dissolution profiles of ketoprofen from the pulsatile drug delivery system with PVA wall thickness of 1.5 mm and 3.0 mm (A, $n=4$), and the lag time (T_{lag}) of dissolution as a function of PVA wall thickness, including additional data points (B, $n=4-6$)

compartments #2 and #3, respectively. At 0 h, the stomachs remained intact. At 4 h, the second compartment was separated from the main body, indicating that the PVA layer of compartment #2 had dissolved. After 8 h, the detachment of compartment #3 was visible in the VD view, and by 12 h, the third compartment was fully separated in both the VD and LAT views (Fig. 4). These *in vivo* observations are in good agreement with the *in vitro* dissolution results, where the second and third drug releases occurred at 3.50 ± 0.63 h and 8.00 ± 0.93 h, respectively.

Optimization of the pulsatile drug delivery system

Based on the results of the three-compartment pulsatile release system, the system was optimized for the sequential delivery of esomeprazole and naproxen. As shown in Fig. 1B, the modified system included an air pocket at the

top for gastroflotation and two compartments: compartment #1 for the immediate release of esomeprazole and compartment #2 for the delayed release of naproxen. The PVA wall thickness for the naproxen compartment was set to 3.6 mm to provide sufficient lag time. Figure 2B shows a fully assembled system containing esomeprazole and naproxen, with dimensions of 15.0 mm (diameter) $\times$ 18.5 mm (height).

In vitro evaluation

The *in vitro* performance and drug release patterns of the developed pulsatile drug delivery system were evaluated in a phosphate buffer (pH 6.8) at a paddle speed of 50 rpm. Figures 5A and B depict a visual demonstration of the mechanism of action of the delivery system during the *in vitro* dissolution test. Initially, the system floated on the surface of the dissolution medium owing to the presence of an air

Fig. 4 Ventrodorsal (VD, left) and lateral (LAT, right) abdominal X-ray images at 0 h (A), 4 h (B), 8 h (C), and 12 h (D) after oral administration of the pulsatile drug delivery system in beagle dogs. Compartments were labeled with barium sulfate for identification: circle (○) for compartment #1, equal sign (=) for compartment #2, and triangle (Δ) for compartment #3

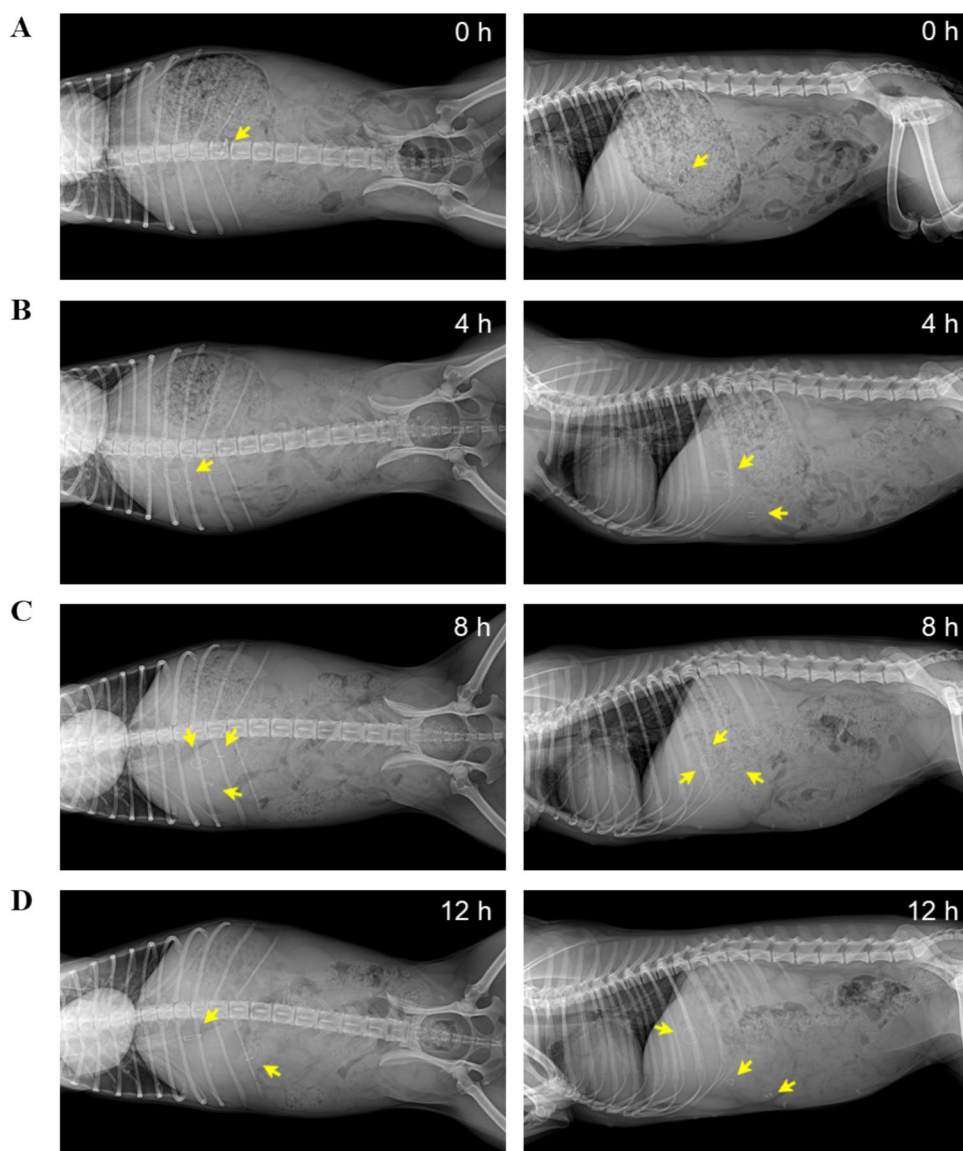
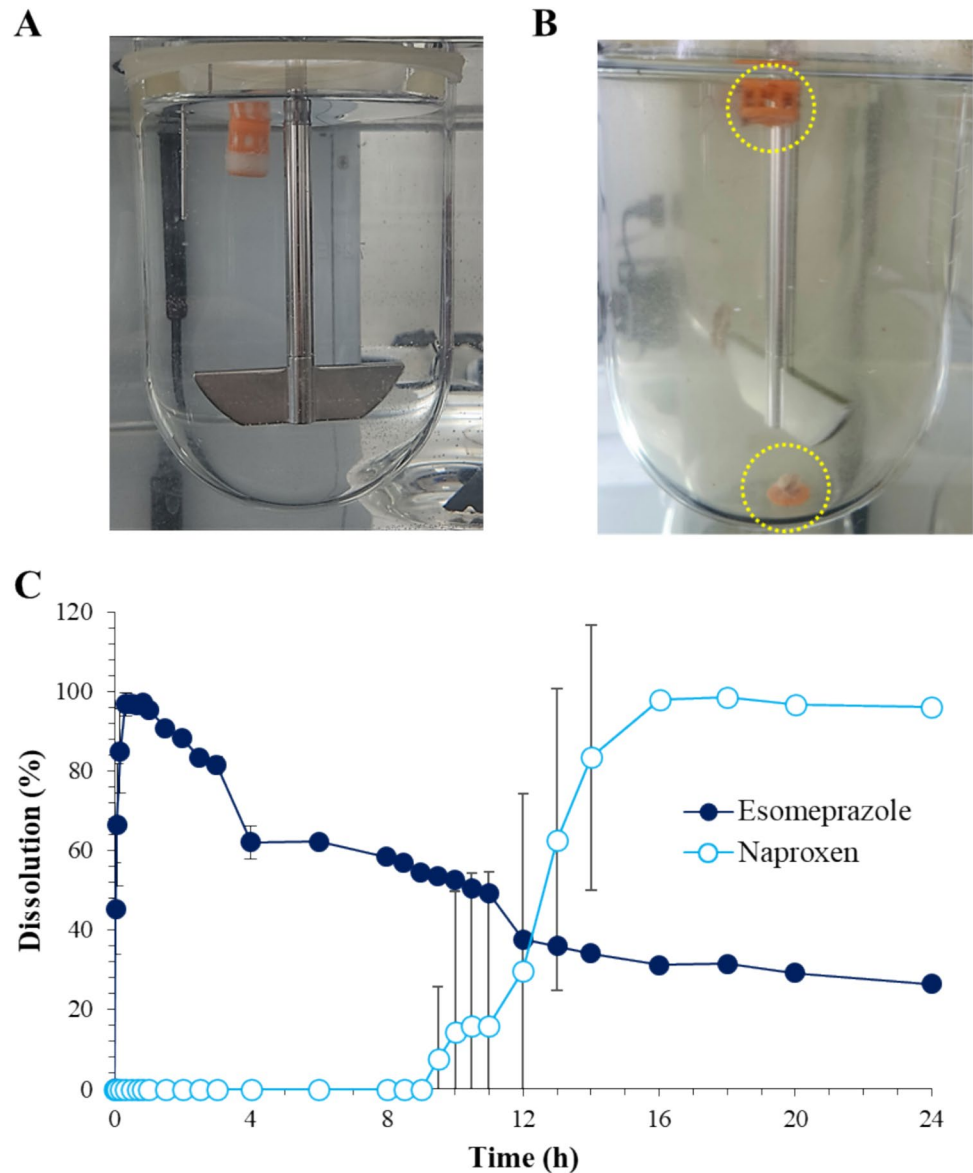


Fig. 5 Visual demonstration of the pulsatile drug delivery system during in vitro dissolution testing at initial stage (**A**), showing the floating system with the esomeprazole tablet in the window compartment, and at later stage (**B**), showing the dissolved PVA barrier (upper yellow circle) and the exposed naproxen tablet (lower yellow circle), and (**C**) the respective in vitro dissolution profiles of esomeprazole and naproxen at pH 6.8 ($n=6$)



pocket (Fig. 5A), and esomeprazole was released rapidly. As the test proceeded, the PVA layer gradually dissolved, eventually exposing the naproxen tablets to the medium. Figure 5B shows complete dissociation of the PVA-protected compartment from the main body after dissolving the PVA layer and exposing the naproxen IR tablet.

Figure 5C shows the in vitro dissolution profiles of esomeprazole and naproxen from the pulsatile drug delivery system, and Table 2 summarizes the dissolution parameters. The dissolution profiles clearly demonstrated the biphasic drug release behavior of the system, with the immediate release of esomeprazole followed by the delayed release of naproxen after a certain lag time. Esomeprazole, positioned in the immediate-release compartment, showed no lag time ($T_{lag} = 0.00 \pm 0.00$ h) and was rapidly released from the delivery system. Approximately 80% of the esomeprazole

Table 2 In vitro dissolution parameters of the pulsatile drug delivery system containing esomeprazole and naproxen (mean $\pm$ SD, $n=6$)

Parameter*	Esomeprazole	Naproxen
T_{lag} (h)	0.00 ± 0.00	11.33 ± 1.37
T_{80} (h)	0.15 ± 0.06	13.00 ± 1.86

* T_{lag} , lag time; T_{80} , time to release 80% of the drug

was released within 0.15 ± 0.06 h (T_{80}), demonstrating an immediate release profile as intended. After dissolution, esomeprazole concentration in the dissolution medium gradually declined, reflecting its instability at pH 6.8 [21].

In contrast, naproxen, enclosed in the PVA-protected compartment, exhibited a significant lag time (T_{lag}) of 11.33 ± 1.37 h until the drug release. This delay

corresponded to the time required for the dissolution of the PVA barrier, after which the naproxen IR tablet was exposed to the medium. Following the dissolution of the PVA barrier, naproxen was released rapidly, with a T_{80} of 13.00 ± 1.86 h, indicating that once the barrier was dissolved, the enclosed naproxen IR tablet was exposed to the dissolution medium and released its content promptly. Large variability was observed during the 10–14 h transition period, as each device underwent compartment separation at slightly different time points (Supplementary Fig. S1). As naproxen release occurs immediately after compartment separation, small differences in separation timing resulted in apparent variability in the cumulative dissolution curve during this period. The inner tablets were tested separately without the capsular device to confirm that the delayed release was attributable to the PVA barrier rather than to the tablet formulation. Both esomeprazole ($T_{80} = 1.77 \pm 0.08$ min) and naproxen ($T_{80} = 11.01 \pm 1.19$ min) exhibited immediate

release profiles with no lag time (Supplementary Fig. S2), confirming that the PVA barrier controls the delayed release.

In vivo evaluation

The in vivo pharmacokinetics of the pulsatile drug delivery system were evaluated following oral administration in beagle dogs. Plasma concentrations of esomeprazole and naproxen were determined simultaneously using a validated LC–MS/MS method. The LC–MS/MS method was validated according to the FDA guidelines and met all acceptance criteria for specificity, linearity, accuracy, precision, and stability (Supplementary Fig. S3 and Tables S1–S2).

The plasma concentration–time profiles of esomeprazole and naproxen are shown in Fig. 6, and the corresponding pharmacokinetic parameters determined by noncompartmental analysis are summarized in Table 3. The pharmacokinetic parameters of the individual animals are shown

Fig. 6 In vivo pharmacokinetic profiles of esomeprazole and naproxen after oral administration of the pulsatile drug delivery system containing esomeprazole (20 mg) and naproxen (100 mg) in beagle dogs (mean $\pm$ SD, $n = 6$)

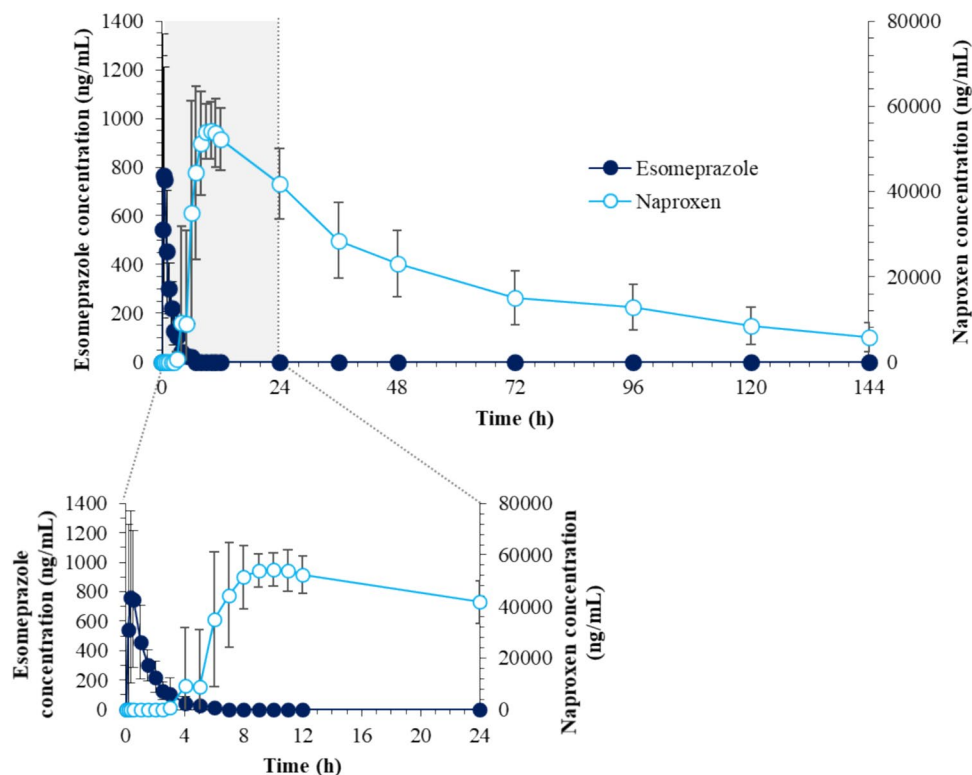


Table 3 Pharmacokinetic parameters of esomeprazole and naproxen following oral administration of the pulsatile drug delivery systems containing esomeprazole (20 mg) and naproxen (100 mg) in beagle dogs (mean $\pm$ SD, $n = 6$)

Parameter	Esomeprazole (20 mg)	Naproxen (100 mg)
$t_{1/2}$ (h)	0.99 ± 0.62	41.16 ± 11.38
T_{lag} (h)	0.00 ± 0.00	4.75 ± 1.17
T_{max} (h)	0.67 ± 0.67	9.83 ± 2.40
C_{max} (ng/mL)	899.23 ± 617.16	58881.98 ± 5622.68
AUC_{last} (ng·h/mL)	1157.12 ± 520.28	2857323.41 ± 763316.90
AUC_{inf} (ng·h/mL)	1180.38 ± 532.43	$3243991.79 \pm 1066739.56$

in Supplementary Table S3. Similar to the *in vitro* results, esomeprazole exhibited no lag time ($T_{\text{lag}} = 0.00 \pm 0.00$ h) in its plasma concentration–time profile, was rapidly absorbed, and reached its maximum concentration (C_{max}) at 0.67 ± 0.67 h. In contrast, naproxen exhibited a clear lag time (T_{lag}) of 4.75 ± 1.17 h in the plasma concentration–time profile. The time to reach C_{max} (T_{max}) for naproxen was 9.83 ± 2.40 h, indicating that the peak plasma concentration was reached approximately 10 h after administration, which aligned with the intended delayed-release profile. The *in vivo* results confirmed the biphasic drug release pattern of the pulsatile drug delivery system, which was characterized by the immediate release and absorption of esomeprazole, followed by the delayed release and absorption of naproxen. No gastric discomfort or adverse gastrointestinal effects were observed throughout the study period. The PLA components were excreted intact in the feces without fragmentation when the PVA barriers were completely dissolved.

Discussion

This study demonstrated the feasibility of a 3D-printed pulsatile drug delivery system capable of the time-controlled, sequential release of multiple drugs by integrating a compartmentalized capsular device with conventional IR tablets. The capsular device consisted of spatially separated compartments enclosed by water-soluble PVA layers of tunable thickness, enabling programmed lag times based on the barrier properties. Following initial development, the system was optimized for the sequential delivery of esomeprazole and naproxen, which were selected because of their clinical relevance in gastroprotection and pain management. *In vitro* and *in vivo* studies demonstrated the immediate release of esomeprazole, followed by the delayed release of naproxen.

The structural design of the capsular device enabled a sequential drug-release profile, that is, the immediate release of a drug followed by a controlled, time-delayed release of the second and third drugs. The immediate-release compartment with a window-like structure provides direct exposure of the inner tablet to the external environment, enabling instantaneous dissolution upon administration. In contrast, the second and third tablets were enclosed within the PVA-walled compartments, where PVA acted as a time-dependent barrier. The inner tablet was exposed only after the PVA layer completely dissolved, and the timing of this exposure was determined based on the thickness of the PVA wall.

Although our previous studies combined 3D-printed capsules with conventional tablets to achieve gastroretention [14] or sustained drug release [15], these systems provided continuous drug release over extended periods. In contrast, the current work introduces a fundamentally different release mechanism, that is, a programmable delayed release with

a defined lag time, through barrier engineering (PVA wall thickness). This enables true sequential delivery, in which the second drug is completely withheld until a predetermined time point, causing temporally separate pharmacokinetic profiles. This delayed-release capability addresses clinical scenarios requiring sequential drug exposure, which cannot be achieved using gastroretentive or sustained-release systems alone.

In vitro studies using ketoprofen as the model drug confirmed that the dissolution timing of each compartment could be modulated by varying the PVA wall thickness. The dissolution of PVA is primarily governed by hydration and subsequent chain disentanglement processes, which are influenced by factors such as the molecular weight, degree of hydrolysis, and surrounding environment [22]. Increasing the barrier thickness proportionally extended the lag time (Fig. 3), demonstrating a clear correlation between PVA wall thickness and the onset of drug release. These findings were further supported by *in vivo* X-ray imaging in beagle dogs, where compartment separation occurred at time points comparable to the *in vitro* lag times.

The system was optimized for a clinically meaningful drug combination of esomeprazole and naproxen. The immediate release of esomeprazole ensured rapid acid suppression, creating a gastroprotective environment before naproxen exposure. Previous studies have demonstrated that pre-administration of PPIs significantly reduces NSAID-induced gastric damage by increasing intragastric pH and mitigating the topical irritant effects of NSAIDs on the gastric mucosa [23, 24]. Thus, esomeprazole and naproxen served as suitable model drugs to demonstrate the programmable delayed-release behavior of our platform. Given the clear clinical rationale for requiring temporally separated exposure, this drug combination represents an effective proof-of-concept for validating the system's capability to achieve time-controlled drug release.

Our data indicated that the present pulsatile drug delivery system can achieve these therapeutic goals, as evidenced by both *in vitro* dissolution and *in vivo* pharmacokinetic data. The *in vitro* dissolution profiles demonstrated the biphasic drug release behavior of the system, with the immediate release of esomeprazole followed by the delayed release of naproxen after a lag time (Fig. 5). The immediate release of esomeprazole can be attributed to its direct exposure to the dissolution medium through the window-type compartment. The gradual decrease in esomeprazole concentration in the dissolution medium was a result of esomeprazole degradation at pH 6.8 [21]. Dissolution tests were performed at pH 6.8, which is the standard condition for PPI formulations, owing to the acidic instability of esomeprazole. PVA exhibits consistent dissolution kinetics across a pH range of 1.2–6.8 [19]. In contrast, naproxen was enclosed within a 3.6 mm-thick PVA compartment, leading to a delayed

release pattern observed in vitro ($T_{lag} = 11.33 \pm 1.37$ h). The in vitro lag time of naproxen release was comparable to that of ketoprofen from the system with the same PVA wall thickness (Fig. 3B). This consistency was attributed to the fact that the release timing was controlled primarily by the PVA wall thickness rather than by the physicochemical properties of the drug or formulation. Thus, the same structural design used for ketoprofen was also effective for naproxen, despite its differing solubility and stability profiles. Compared with conventional delayed-release formulations, which often require customized formulation strategies for each drug, our system allows for a unified design approach. Notably, Fig. 3B includes drug delivery systems from multiple printing batches, showing batch-to-batch reproducibility (T_{lag} CV = 5.6–23.7%). Together with the in vitro (CV = 12.1%) and in vivo (CV = 24.6%) lag time variabilities, the data demonstrated acceptable manufacturing consistency, although clinical translation requires systematic validation of dimensional tolerance. In addition, the system dimensions (15.0 mm $\times$ 18.5 mm, Fig. 2B) are comparable to standard size 00 gelatin capsules (8.53 mm $\times$ 23.3 mm) and Vimovo® tablets (approximately 20 mm $\times$ 10 mm), which are routinely prescribed and well tolerated, suggesting potential for patient acceptability.

Consistent with the in vitro results, the in vivo data confirmed the biphasic drug release profile of the pulsatile drug delivery system, which was characterized by immediate release and absorption of esomeprazole, followed by delayed release and absorption of naproxen (Fig. 6). Although the in vivo lag time for naproxen ($T_{lag} = 4.75 \pm 1.17$ h) was shorter than in vitro, the extended delay is notably longer than typical enteric-coated formulations, which typically release the drug within 2–3 h after gastric emptying. This extended delay may offer clinical benefits for patients requiring prolonged acid suppression. The shorter in vivo T_{lag} was possibly due to physiological variables, including peristalsis, gastric content, enzymatic activity, and variable pH environments. The observed in vivo T_{lag} (4.75 ± 1.17 h), although longer than conventional enteric coatings, demonstrates the platform's capability for extended temporal control. As shown in Fig. 3B ($R^2 = 0.943$), the lag time can be adjusted by varying the PVA wall thickness, enabling customization for different therapeutic applications. Aside from the delayed absorption of naproxen, the remaining pharmacokinetic parameters, including the extended half-life (41.16 ± 11.38 h), are consistent with previous pharmacokinetic reports in dogs [25–28]. The notably longer half-life of naproxen in dogs than in humans is primarily due to species differences in metabolism and elimination, particularly the greater extent of protein binding and slower clearance in dogs [27]. The pharmacokinetic parameters of esomeprazole were comparable with those reported for immediate-release esomeprazole formulations in dogs [29]. The intersubject variability

(CV%) for the naproxen T_{lag} was 24.6%. The consistency of the lag phase across all animals (100% showed delayed naproxen release) demonstrated reproducible device performance. The PLA component, which is slowly degraded under physiological conditions, remains intact during gastrointestinal transit and is excreted in the feces within 2–4 days after administration. This indicates that the residual component passes naturally through the gastrointestinal tract after drug release and should be considered for clinical use.

These findings collectively confirmed the reproducible in vivo functionality of the pulsatile system. Although this study did not include a concurrent control group, the biphasic profile was possibly attributable to the capsular device rather than to the tablet formulation. In vitro testing confirmed that both drug tablets exhibited immediate release without the device (Supplementary Fig. S2), demonstrating that the PVA barrier controlled delayed release. In addition, IR formulations of esomeprazole and naproxen do not exhibit extended lag times in vivo, as their pharmacokinetics are well-documented in the literature [30].

This platform has several advantages in terms of formulation and manufacturing. Traditional approaches to pulsatile drug delivery systems often involve complex multilayer coating processes that are difficult to scale and personalize. In contrast, the present strategy, which combines a 3D-printed capsular structure with conventional immediate-release tablets, enables programmable drug delivery without extensive drug reformulation. Drug release timing can be customized by modulating the thickness and placement of PVA barriers, enabling flexible application to several drug combinations with minimal optimization and offering a structurally driven alternative to conventional coating-based approaches.

For example, VIMOVO® employs a pH-sensitive coating to delay the release of naproxen. However, repeated administration of esomeprazole may elevate the gastric pH and compromise the integrity of the pH-sensitive coating, causing premature dissolution of naproxen in the stomach and an increased risk of local gastrointestinal toxicity [31, 32]. Moreover, the lag time (T_{lag}) for naproxen release has been reported to vary widely, from 1.5 to 11 h, depending on food intake [31, 32]. In contrast, the present system provides pH-independent structural control through the structural design and PVA barrier thickness. As the system remains in the stomach, drug release is delayed until the PVA layer fully dissolves in the gastric fluid, enabling more consistent and programmable lag times regardless of physiological pH variations.

Overall, this study highlighted that programmable and sequential drug delivery can be achieved through the structural design of a capsular device in combination with conventional tablets. This modular approach allows for the adaptable and efficient development of time-controlled drug delivery systems using existing drug products, thereby

supporting personalized and programmable therapeutic strategies.

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Data availability All data generated or analyzed during this study are included in this published article and its supplementary material files.

Declarations

Ethics approval and consent to participate This study does not involve human subjects. All institutional and national guidelines for the care and use of laboratory animals were followed. The animal studies were approved by the Institutional Animal Care and Use Committee of HLB Biostep (KNOTUS IACUC 22-KE-0043, KNOTUS IACUC 23-KE-0207).

Consent for publication This study does not involve human subjects.

Competing interests The authors declare no relevant financial or non-financial interests.

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