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In Vitro and *In Vivo* Evaluation of Dark Chocolate as Age-appropriate Oral Matrix

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ABSTRACT

Swallowing difficulties encountered by geriatric patients who undergo polypharmacy represent a significant challenge that hampers patient compliance and therapeutic management. As an appealing and sensory-pleasing, chocolate-based formulations have emerged as a potential alternative oral dosage form suitable for both the elderly and paediatric populations. However, the extent to which the incorporation of drugs into a chocolate matrix affects their oral availability remains unclear. Therefore, the objective of this investigation was to explore the *in vitro* and *in vivo* performance of an ibuprofen-based chocolate dosage form. A matrix based on dark chocolate and the model drug was prepared at two distinct temperatures: 50 and 80 °C. *In vitro* release studies revealed that ibuprofen formulated through co-melting at 80 °C exhibited a statistically significant slower drug release ($p < 0.05$) compared to formulations prepared at 50 °C in both FaSSGF (fasted-state simulated gastric fluid) and lipolysis media. The enzymatic degradation of chocolate in the presence of lipase accelerated *in vitro* ibuprofen release from chocolate matrices. To delve deeper into the bioavailability of ibuprofen within the chocolate formulations, we conducted an *in vivo* assessment, comparing the pharmacokinetic profiles of ibuprofen in its conventional suspension form with our chocolate-based dosage forms. A notable drop ($p < 0.05$) in the maximum serum concentration of ibuprofen when incorporated into co-melted or solid-suspension chocolate matrices. However, no significant differences in plasma exposure were observed between the two formulations. These findings shed a light on the potential of chocolate to extend of ibuprofen when integrated into various chocolate matrices, showcasing the potential held by these innovative formulations.

1. Introduction

The development of formulations that are suitable for geriatric or paediatric populations plays a pivotal role in enhancing drug palatability, treatment adherence, and therapeutic response (Lopez et al., 2015; Tagami et al., 2021). Globally, it has been reported that over 15 % of the elderly population experiences dysphagia, which poses challenges when swallowing solid oral dosage forms (Drumond and Stegemann, 2020). Similarly, a study involving 172 paediatric patients revealed inadequate adherence to oral medication administration, leading to potential therapeutic failures. Notably, the primary reason for medication refusal amongst these patients was taste aversion (Mennella et al., 2015).

The organoleptic properties, including flavour and smell, significantly influence the acceptability of drug products and are critical considerations in the development of patient-friendly dosage forms for the paediatric and elderly populations (Ivanovska et al., 2014). To overcome taste aversion, several strategies have been employed to improve develop palatable dosage forms. These strategies include taste-masking approaches, such as taste-receptor blocking and the incorporation of flavouring and sweetening agents like saccharin sodium, sucrose, and sorbitol (Walsh et al., 2014).

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It is worth noting that approximately > 80 % of new drug candidates are categorised as poorly water-soluble, as reported by Wulff-Pérez et al. It is widely recognised that the bioavailability of such drugs is often enhanced when they are incorporated or co-administered with a lipid-rich meal. The process of lipid digestion in the gastrointestinal tract creates a colloidal lipidic environment that facilitates the dissolution of solid drugs and their subsequent absorption by the intestinal epithelium (Wulff-Perez et al., 2014). The digestion process of lipids in the gastrointestinal tract creates a colloidal lipidic environment where a solid drug can dissolve and subsequently be absorbed by the intestinal epithelium. Lipid digestion begins in the mouth through the action of lingual lipase and continues in the stomach, where gastric lipase breaks down up to 30 % of lipids (Liao et al., 1984). Further digestion takes place in the small intestine primarily by pancreatic lipase, which plays a crucial role in efficiently breaking down triglycerides into free fatty acids and sn-2 monoglycerides (Widmaier et al., 2013). Additionally, the presence of lipids in the small intestine triggers the contraction of the gallbladder, leading to the release of biliary fluid containing phospholipids, cholesterol, and bile salts, which aid in solubilisation and absorption (Salentinig et al., 2013; Kalantzi et al., 2006; Kalepu et al., 2013).

Cocoa-related products, including chocolate, have been previously used to reduce drug bitterness and increase acceptability amongst the paediatric population (Truong et al., 2021). Numerous studies have identified the health benefits of chocolate due to its rich content of antioxidant and polyphenolic compounds, which have positive effects on cognitive function (Tan et al., 2021). A novel chewable chocolate-based tablet delivery system (CDS) has recently been developed using mould-casting and three-dimensional (3D) printing technology (Karavasili et al., 2020). The implementation of a chocolate dosage form serves as a supportive tool to overcome challenges associated with force-feeding medicine and to improve attitudes toward healthcare (Chachlioutaki et al., 2022).

Still, ibuprofen, a commonly prescribed non-steroidal anti-inflammatory drug, is frequently used amongst the elderly and children, particularly for osteoarthritis and fever. It inhibits both isoforms of the cyclooxygenase enzyme, COX-1 and COX-2, and is considered an anti-inflammatory agent. However, it possesses a bitter taste and a short half-life (~1.8–2 h), limiting its clinical benefits in both populations (Sostres et al., 2013). To improve the properties of ibuprofen, several lipid nanoparticles of ibuprofen have been developed to prolong drug release and masque its unpleasant bitter taste (Qin et al., 2019).

3D printing has been proposed as a promising approach for personalising age-approrpiate dosage forms for paediatrics (Bracken et al., 2022; Yang et al., 2023). Recently, a novel approach was introduced in the form of a 3D-printed chewable dosage form, utilising a chocolate-based matrix, which exhibited a unique drug release profile for ibuprofen and paracetamol *in vitro* (Karavasili et al., 2020). This innovative dosage form not only provided precise dose adjustment but also incorporated the desirable organoleptic characteristics of chocolate. Moreover, a newly developed pharmaceutical preparation involving the coating of ibuprofen with milk chocolate demonstrated stability over a period of one year, while effectively masking the bitter taste typically associated with ibuprofen (Synaridou et al., 2019). Additionally, ibuprofen microparticles prepared in cocoa butter, assisted by supercritical fluid technology, showed promising results in taste evaluation studies involving human volunteers, indicating a potential innovative method for masking the bitter taste of ibuprofen (Obaidat et al., 2021).

However, to date, no prior studies have investigated or determined the influence of processing temperature and lipolysis digestion on drug release and oral bioavailability in these chocolate-based formulations. The present study aimed to evaluate the pharmacokinetic parameters of ibuprofen in different chocolate-based formulations, in comparison with a conventional formulation. The investigation aimed to shed a light on the impact of these factors on drug release kinetics and oral absorption, contributing to a better understanding of the performance of these chocolate-based formulations as potential drug delivery systems.

2. Materials and methods

2.1. Materials

Ibuprofen, Methocel™ E5 Premium, and mefenamic acid were acquired from Sigma-Aldrich, USA. HPLC-grade acetonitrile, methanol, water, and *ortho*-phosphoric acid were obtained from Tedia, USA. Sodium chloride, sodium hydroxide, trizma maleate, calcium chloride, sodium dodecyl sulfate, and pancreatin were purchased from Sigma-Aldrich, UK. Dark Peruvian chocolate with 72 % cocoa content was procured from Marks and Spencer, UK.

2.2. Preparation of ibuprofen-loaded chocolate discs

A drug-to-dark chocolate weight ratio of 1:4 was employed for the preparation. The weighed ingredients (1 g) were separately placed in compartments of a silicon tray and heated for 2 h in an oven at pre-set temperatures (50 or 80 °C, Fig. 1). The molten ingredients were then manually mixed using a glass rod and then the discs were allowed to cool overnight at room temperature. The resultant dosage forms were separated from the compartment and then wrapped in foil and stored at 2–8 °C for a maximum of 12 weeks. Since ibuprofen has a melting point of 76 °C (Eraga et al., 2015), ibuprofen-chocolate discs were prepared below (drug suspension; at 50 °C) and above (co-melted; at 80 °C) the melting point of ibuprofen. Formulations in this study were labelled based on their preparation temperature and storage conditions, where I50, I50RT, and I50F represented the ibuprofen (I) formulations prepared at 50 °C (50) and used fresh, stored at room temperature (RT), or refrigerated (F), respectively. The same labelling scheme applied to the formulation prepared at 80 °C, denoted as I80, I80RT, and I80F.

To prepare the chocolate for analysis, pre-chilled discs (−20 °C) were ground and sieved (500 μm) using a coffee grinder (Krups F2034251, US). Samples of ibuprofen and chocolate were placed in aluminium pans (ME-51,119,870 Al-crucibles 40 μL without pins), and the melting point of each was determined using a differential scanning calorimeter (DSC) with a heat rate of 10 °C/min, scanning from 25 to 250 °C. The resulting physical mixture with a drug-to-chocolate ratio of 1:4 represented the composition of the chocolate discs. The physical mixtures, as well as the freshly prepared and aged samples, were subjected to testing.

2.3. In vitro dissolution in FaSSGF and lipolysis medium

The FaSSGF (Fasted-State Simulated Gastric Fluid) and lipolysis solution for ibuprofen was prepared as previously reported (Dressman et al., 1998;Ali et al., 2008). FaSSGF (gastric biorelevant media) is the dissolution medium used to simulate the conditions in the stomach, while lipolysis medium pH 6.5 is used to simulate the conditions in the small intestine in the presence of lipase. Briefly, pancreatin (37.5 mg) was thoroughly mixed with 5 mL of deionised water. The resulting suspension was added to the bio-relevant medium 10 min after the start of each lipolysis experiment. In addition, a 6.5 pH buffer was prepared by dissolving $CaCl_2 \cdot 2H_2O$ (5 mM), NaCl (150 mM), tri-maleate (50 mM), and NaOH (39.75 mM) in deionised water. In contrast, to obtain the deactivated enzyme, 37.5 mg of pancreatin was dissolved in deionised water and heated in an oven at 80 °C for 2 h. To maintain the pH of this buffer at pH 6.5, the pH of the medium was checked every 5 min and drops of sodium hydroxide solution (NaOH 1 M) were added as needed to sustain the target pH. A USP II dissolution apparatus was employed with a bath temperature maintained at 37 ± 0.5 °C and a paddle speed of 50 rpm.

Prior to each experiment, each chocolate disc (1 g containing 200 mg ibuprofen) was weighed and divided into eight equal parts. Then, chocolate pieces [8 parts (equivalent to ~1 g) or three parts (total weight ~0.375 g)] were placed into 900 mL of FaSSGF medium or 300 mL of lipolysis medium, respectively. Samples were collected over a 6 h period and filtered using a 0.22 μm filter. In the case of lipolysis

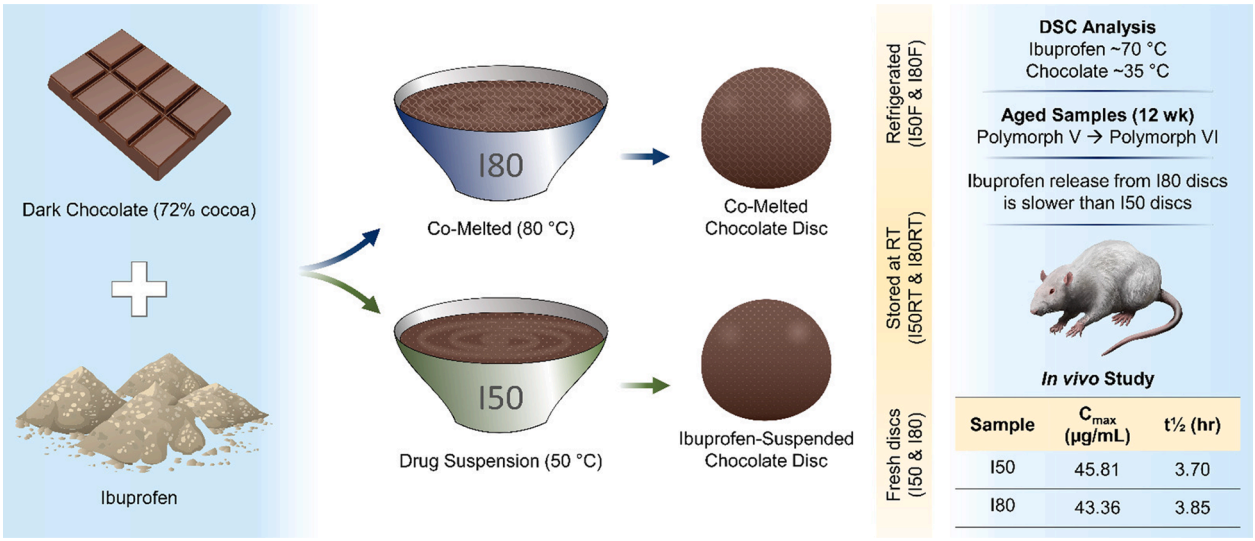

Fig. 1. A schematic graph of the preparation of ibuprofen chocolate loaded discs yielding co-melted and solid dispersions and a summary of thermal analysis and in vivo study outcomes.

experiments, the pH was maintained at 6.5. The dissolution of equivalent dose of ibuprofen 200 mg (Nurofen 200 mg tablets, Reckitt Benckiser, UK) was also assessed for comparison.

For FaSSGF analysis, a mobile phase consisting of 2 mM HCl and acetonitrile in an isocratic ratio of 30:70 was employed. An XTerra (Waters, US) C18, 5 µm particle size, 4.6 × 150 mm column was used with a flow rate of 1 mL/min, an injection volume of 4 µL, and detection at 210 nm. The total run time for each sample was 10 min. On the other hand, a gradient HPLC mobile phase was used for lipolysis medium analysis with HCl solution (2 mM): acetonitrile volume ratios of 3:7, 9:1, 3:7 , and 3:7 at 0, 13, 13.01, and 16 min, respectively.

2.4. *In vivo experiments*

Male Sprague-Dawley (SD) rats (approximate weight of 230 ± 20 g) were obtained from the animal house of the Department of Pharmacy at the University of Petra, Jordan. The rats were housed in a controlled environment with a temperature range of 20–25 °C and 55–65 % humidity. Prior to the experiment, the rats were fasted for 12 h but provided with free access to tap water. They were maintained under a 12 h light/dark cycle. All experimental procedures were reviewed and approved by Al-Ahliyya Amman University Ethical Review Committee (Amman, Jordan). For each experiment, the rats were randomly divided into four groups, each consisting of eight rats.

To administer the drug and chocolate mixture, a 0.25 % Methocel™ E5 Premium suspension was prepared, and the drug was mixed with the solution in a glass mortar. Ibuprofen suspensions were freshly prepared on the day of the experiment, and the ibuprofen content in each dose was adjusted based on the weight of the animals, targeting a dose of 25 mg/kg. The different suspensions were administered using a stainless-steel oral gavage needle syringe, ensuring a constant dose-volume of 5 mL/kg. Blood samples were collected from the eight rats in each group through their tails at different time intervals (0.0, 0.25, 0.5, 1, 2, 3, 4, 6 and 8 h) following the same numbering order.

2.5. *Pharmacokinetic analyses in RAT plasma*

After each blood-pooling time interval, the samples were centrifuged at 6000 rpm for 15 min and then frozen at -20 °C until analysis. Aliquots of 10 µL serum samples were processed and analysed for ibuprofen concentrations using HPLC-UV. The pharmacokinetic parameters were calculated using a non-compartmental model with the aid of Thermo Scientific Kinetica software. Each value was expressed as mean ± standard variation (SD).

Analysis was performed using a UV–VIS plus Detector Finnigan Surveyor HPLC connected to a Hypersil Gold column (4.6 × 250 mm; particle size of 5 µm). The mobile phase consisted of a gradient-grade mixture of acetonitrile, HPLC water, and trimethylamine in a ratio of 600:400:0.25. The pH was adjusted to 2.6 using *ortho*-phosphoric acid. Detection was carried out using the UV detector at a wavelength of 215 nm. The HPLC analysis method was thoroughly validated following the guidelines provided by the European Medicines Agency (EMEA) in their 2011 bioanalytical method validation guideline. The validation process included the assessment of selectivity, sensitivity, linearity, accuracy, precision, recovery, and stability.

To evaluate selectivity, six blank samples, samples without ibuprofen, and samples with the addition of ibuprofen were processed and analysed to ensure that the method accurately identifies and quantifies the target analyte in the presence of other components. The sensitivity of the method was determined using the lower limit of quantification (LLOQ) samples, which contained a concentration of 5 µg/mL of ibuprofen. Additionally, a pooled plasma sample without ibuprofen or the internal standard (IS) was analysed to assess the limit of detection and the method's ability to detect low concentrations of the analyte. For the assessment of accuracy and precision, intra-day and inter-day analyses were conducted. Intra-day accuracy and precision were determined by analysing six samples each of low, middle, and high-quality control (QC) samples, with concentrations of 15, 40, and 65 µg/mL, respectively, as well as six samples of LLOQ concentration. This process was repeated on three different days. Inter-day accuracy and precision were evaluated using the data obtained from measured concentrations on three consecutive days. The coefficient of variation (CV%) values and accuracy% were calculated for all samples analysed within each day.

3. Results and discussion

We investigated the incorporation of ibuprofen as a model drug within the chocolate matrix. The matrix were prepared at two distinct temperatures, above and below the melting point of ibuprofen (50 and 80 °C). Freshly prepared samples (I80 and I50) and aged samples subjected to different storage conditions, including 1 weeks at room temperature (I80RT and I50RT) and refrigeration at 2–8 °C (I80F and I50F), were analysed thermally using DSC, in addition to their *in vitro*

dissolution and lipolysis. Through this meticulous analysis, we aimed to gain profound insights into the intricate interplay between drug release and stability within the chocolate matrices. Additionally, the pharmacokinetic profiles of the formulations were *in vivo* evaluated, enabling us to discern differences and enhance our understanding of their performance. Fig. 1 provides a comprehensive overview, highlighting the experimental details and key *in vitro* and *in vivo* findings.

3.1. Thermal analysis

Thermal analysis was conducted to investigate the melting point and polymorphic forms of ibuprofen and chocolate in the dosage forms. Ibuprofen showed an endothermic peak observed ~70 °C corresponds to its melting point. Physical mixture of the formulation showed two endothermal peak at 35 and ~70 °C corresponding to the melting polymorphic form (VI) of chocolate (Tan and Kerr, 2017) and ibuprofen, respectively, suggesting the formation of solid suspension. When dosage forms were prepared at 50 °C, the two endothermal events appeared in the thermograph of the dosage form (Fig. 2A). However, discs prepared by co-melting the chocolate and ibuprofen at 80 °C, a wider endothermal event complex appeared with an onset of 35 °C (Fig. 2B). Fig. 2 shows a peak at 34–36 °C, which corresponds to the melting polymorphic form (VI) of chocolate (Tan and Kerr, 2017). A shift in the sucrose peak indicates a transition to a different polymorphic form, indicating the presence of a solid suspension. At room temperature, triglycerides in polymorph V have sufficient energy to convert to polymorph VI, allowing realignment of molecules, hence a more stable polymorph of the chocolate matrix, which also explains the aforementioned melting point results (Bakalis et al., 2011).

3.2. Stability of the stored dosage forms

The thermal analysis of ibuprofen illustrated the presence of an endothermic peak at an onset of ~76 °C, indicating the crystallinity of ibuprofen (Fig. 2). Although ibuprofen prepared at 80 °C is expected to exist in an amorphous form due to the preparation conditions exceeding its melting point, the endothermal peak with onset at ~70 °C indicate the presence of crystalline ibuprofen. Following storage, samples exhibit a lower onset than the fresh sample, indicating the presence of different polymorphic forms within the chocolate matrix. The broader peaks observed suggest a large distribution of polymorphic forms, resulting in a lower structure density and a weaker crystal network. It is possible that changes in crystal structure due to the presence of crystal nuclei during solidification, leading to a denser structure, commonly referred as blooming (Smith et al., 2007). The shifting of the co-crystal peak with ageing indicates differences in the co-crystal ratio. The presence of two components can depress the melting point and demonstrates the miscibility of the drug with the chocolate (Yamashita et al., 2013). Another possibility is the variations in the size and shape of ibuprofen crystals within the chocolate matrix, which can further influence the thermal behaviour of the formula (Rasenack and Muller, 2002; Afoakwa et al., 2008).

3.3. Release of ibuprofen from chocolate matrix in FaSSGF

FaSSGF was selected to assess the chocolate dosage form in the gastric fluids, which is the first medium the dosage form will be exposed to (with exception to brief introduction to saliva in oral cavity). Ibuprofen has a high permeability and its absorption in small intestine is almost complete oral bioavailability (Bermejo et al., 2018). However, it is the poor solubility of ibuprofen in acidic gastric media that is most likely to be the limiting step that affects its pharmacokinetics. In fact, several reports highlighted that accelerating ibuprofen dissolution in gastric fluids via salt formation can directly shorten t_{max} (Martin et al., 1990).

In general, inclusion of ibuprofen within chocolate matrix slowed down drug release in comparison to reference ibuprofen tablet (Fig. 3). The release of ibuprofen from the discs depends on the preparation temperature. Ibuprofen discs prepared at 80 °C showed a slower release compared to those prepared at 50 °C (Fig. 3). It is possible that the ibuprofen crystals remained suspended in the chocolate matrix and drug release in highly soluble medium was mainly dependant on the dissolution of the matrix. While discs prepared at 80 °C exist as co-melted ibuprofen and chocolate matrix, where drug release is dependant on diffusion from the hydrophobic matrix. While the storage seemed to have less significant impact on drug release for discs prepared at 50 °C, while drug release from discs prepared at 80 °C temperatures seemed to slow down upon storage (Molokhia et al., 2008).

To evaluate the impact of lipid digestion on drug release, the drug release was repeated using lipolysis media (Figs. 4 and 5), where lipase

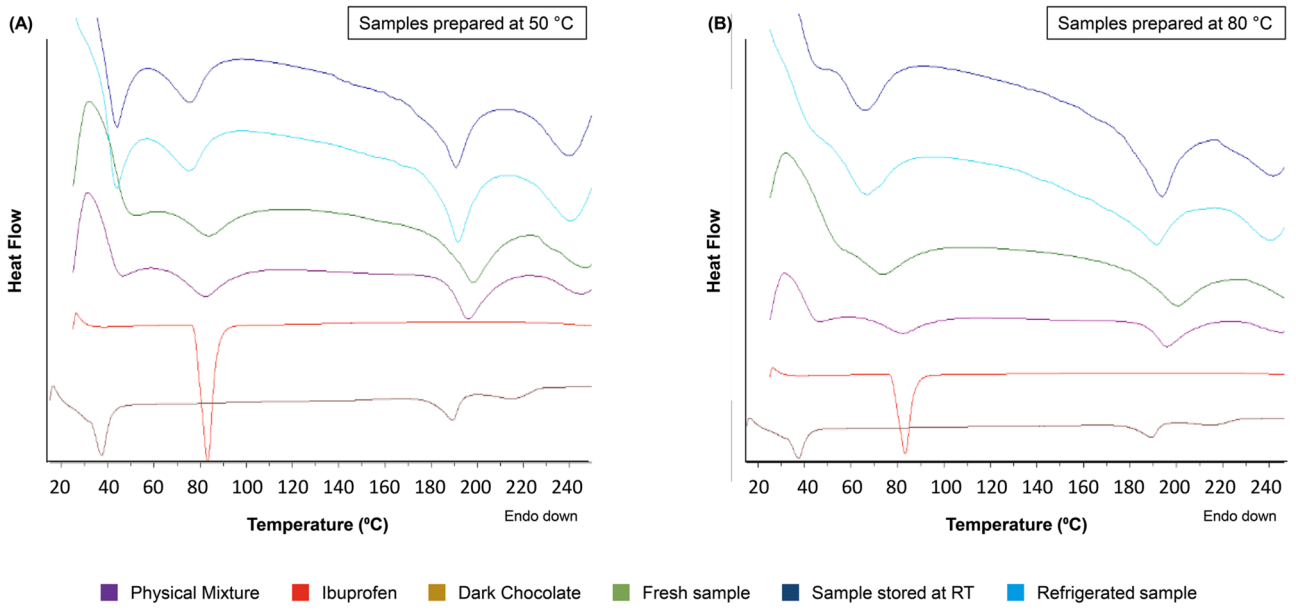

Fig. 2. DSC thermograph of dark chocolate, ibuprofen, ibuprofen chocolate (freshly prepared, and stored at room temperature or fridge. The dosage form was prepared at (A) 50 °C and (B) 80 °C.

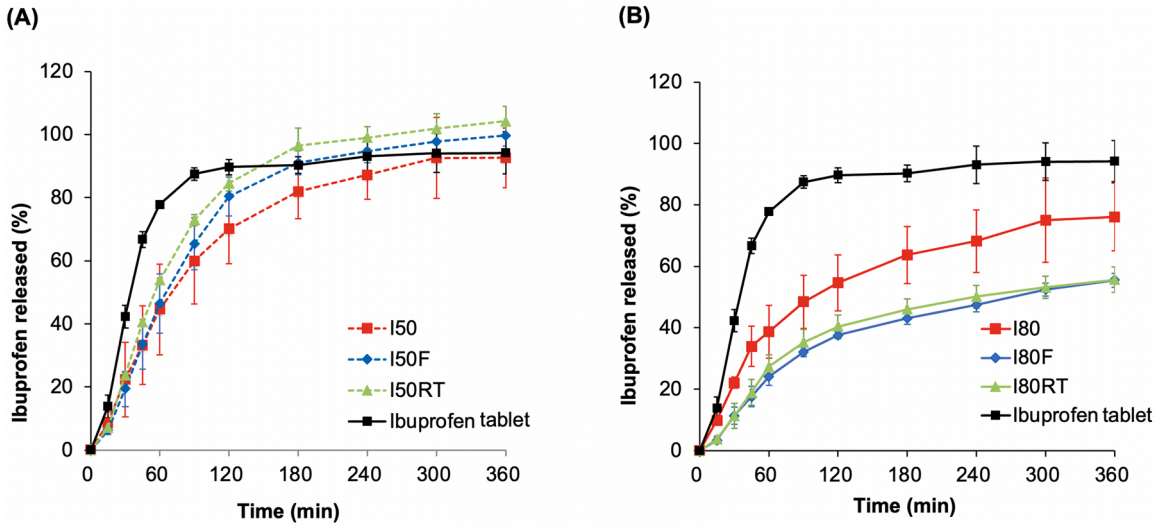

Fig. 3. *In vitro* ibuprofen released from ibuprofen tablet and chocolate dosage from prepared at (A) 50 °C and (B) 80 °C (freshly prepared or following storage in fridge or room temperature).

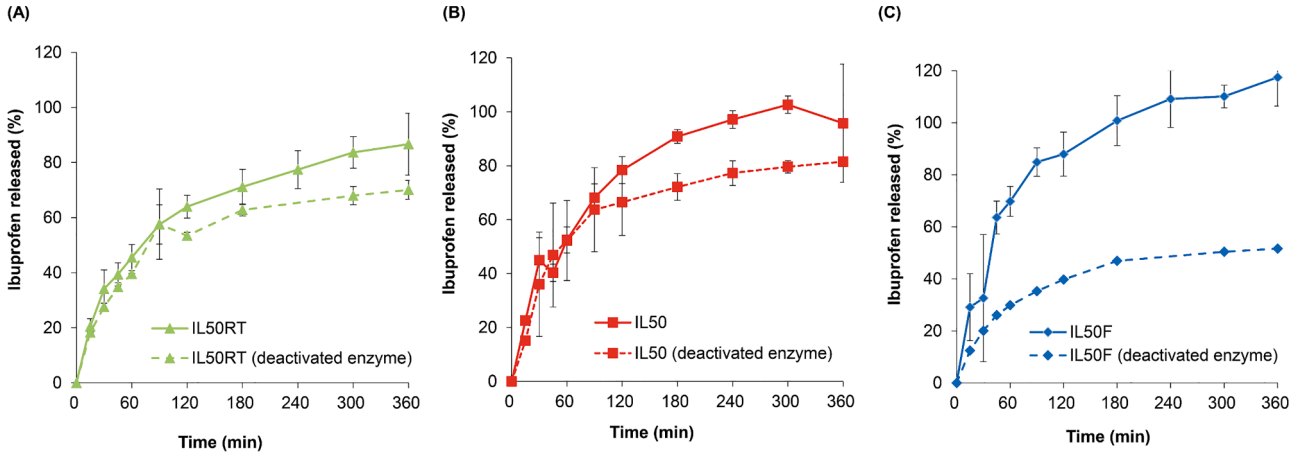

Fig. 4. Impact of lipolysis on ibuprofen release in the presence of activated and deactivated pancreatin for fresh discs prepared at 50 °C (A) and after storage; fridge (B) and room temperature (C).

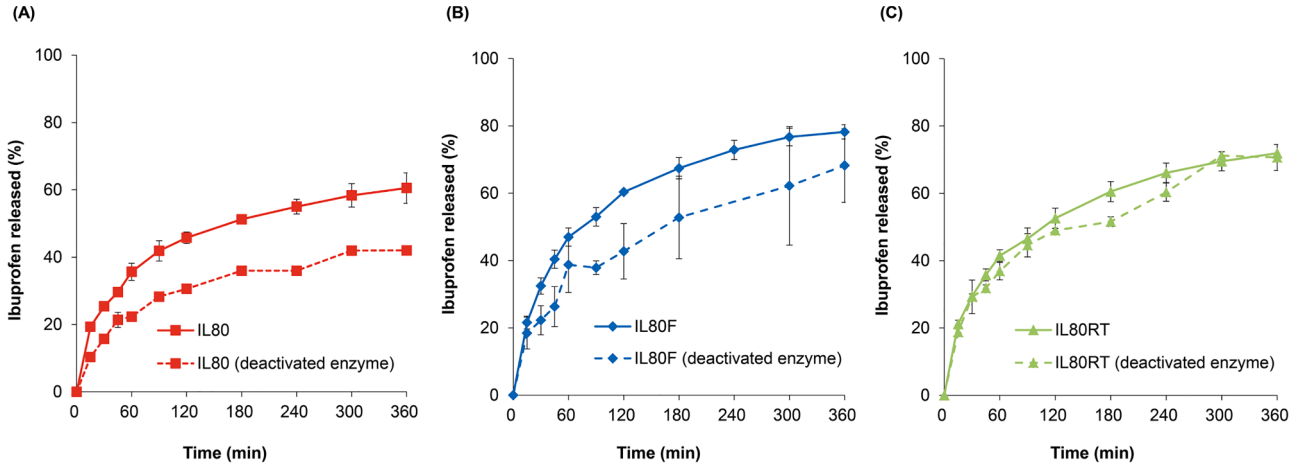

Fig. 5. Impact of lipolysis on ibuprofen release in the presence of activated and deactivated pancreatin for fresh discs prepared at 80 °C (A) and after storage; fridge (B) and room temperature (C).

digests the glycerides into free fatty acids. As expected, ibuprofen from chocolate matrix was accelerated in the presence of active lipase. The *in vitro* release of ibuprofen accelerated in the presence active lipase compared to identical medium with a deactivated enzyme. Pancreatin digests the triglycerides into more soluble fatty acids leading faster dissolution of the lipidic matrix, hence it could accelerate ibuprofen from chocolate discs (Murshed et al., 2022; Thomas et al., 2012).

3.4. *In vivo evaluation of chocolate dosage forms*

The methodology outlined in the previous section was employed to conduct a pharmacokinetic study on rats. The average serum concentration-time profile following the oral administration of ibuprofen chocolate formulations at a dosage of 25 mg/kg is presented in Fig. 6, while the key pharmacokinetic parameters are summarised in Table 1. Notably, the maximum observed concentration (C_{max}) of ibuprofen in rats was found to be 17.6 μg/mL, with a corresponding t_{max} of 4 h. Statistical analysis revealed a significant difference ($p < 0.05$) in the C_{max} of ibuprofen between the solid suspension of ibuprofen and the chocolate formulation compared to the administration of the drug alone. Furthermore, the C_{max} of ibuprofen was determined to be significantly different ($p < 0.01$) between the co-melted ibuprofen and chocolate formulation (I80) and the administration of ibuprofen alone.

Interestingly, when ibuprofen was co-administered with chocolate in a physical mixture, the resulting C_{max} was comparable to that observed when ibuprofen was administered alone ($p > 0.05$). This finding contrasts with the previous chocolate formulations. In terms of therapeutic concentrations, when ibuprofen was administered alone, the mean concentration achieved a value of 65.5 ± 6.7 μg/mL at 0.5 h (t_{max}). However, when given as a solid suspension of ibuprofen and chocolate or as co-melted ibuprofen and chocolate, the mean concentrations decreased to 45.8 ± 7.6 μg/mL and 43.4 ± 9.6 μg/mL, respectively. In contrast to the C_{max} results, the average estimates of the area under the plasma concentration-time curve ($AUC_{0-\infty}$) did not exhibit a significant difference between the different formulations at the 95 % confidence level ($p = 0.1573$). Specifically, the mean $AUC_{0-\infty}$ values for ibuprofen were 217.5 ± 18.3, 269.2 ± 65.6, 224.4 ± 13.7, and 192.5 ± 29.6 μg/mL.hr for ibuprofen alone, the physical mixture of ibuprofen and chocolate, the solid suspension of ibuprofen and chocolate formulation, and the co-melted ibuprofen and chocolate formulation, respectively (Fig. 6).

The observed significant drop in C_{max} between the chocolate-based ibuprofen formulations and the drug alone may be attributed to slower ibuprofen release from chocolate dosage forms as predicted from *in vitro* studies.

Table 1
Pharmacokinetic parameters of the four formulations of ibuprofen. Data are presented as means±SD ($n = 8$).

Formulation type	C_{max} (μg/mL)	t_{max} (hr)	$t_{½}$ (hr)	AUC∞ (μg/mL.hr)	AUC_{last} (μg/mL.hr)
Ibuprofen suspension	65.54 ±6.67	0.5 ±0.0	2.59 ±0.48	217.46 ±18.31	191.40 ±5.01
Physical mixture of ibuprofen and chocolate	65.97 ±10.30	0.70 ±0.41	3.52 ±0.99	269.21 ±65.55	219.27 ±7.30
Solid Suspension of ibuprofen and chocolate	45.81 ±7.62	0.5 ±0.28	3.70 ±1.01	224.374 ±13.74	170.06 ±7.48
Co-melted ibuprofen and chocolate	43.36 ±9.58	0.55 ±0.27	3.85 ±2.52	192.51 ±29.58	145.60 ±18.13

As indicated in Table 1, the co-melted ibuprofen and chocolate exhibited the lowest C_{max} value. This can be attributed to the co-crystallisation of ibuprofen with the triglycerides present in chocolate, leading to the formation of non-covalent bonds that trap ibuprofen and reduce its C_{max}. The results of this study revealed that the different ibuprofen formulations exhibited a t_{max} of 0.5 h, with no significant differences observed between the formulations ($p > 0.05$), indicating no delay in gastric emptying rate. However, although not statistically significant ($p > 0.05$), the $t_{½}$ of ibuprofen was noticeably higher in the chocolate formulations compared to ibuprofen alone. The $t_{½}$ values were 2.59±0.48 h for the ibuprofen suspension and 3.52 ± 0.99, 3.70 ± 1.01, and 3.85 ± 2.52 h for the physical mixture of ibuprofen and chocolate, the solid suspension of ibuprofen and chocolate formulation, and the co-melted ibuprofen and chocolate formulation, respectively. This observation suggests a possible sustained action of ibuprofen in the presence of chocolate.

In recent studies, the dispersion of ibuprofen in cocoa butter using various methods and technologies has shown promise as an innovative approach to masque the bitter taste of ibuprofen (Obaidat et al., 2021; Karavasili et al., 2020). Hence, the chocolate-based dosage form, designed to reduce the bitter taste, may slow down the onset of ibuprofen without significantly compromising its oral bioavailability.

Additionally, the anhydrous nature of chocolate provides resistance against microbial growth and improves the mineralisation of tooth enamel (Pribadi et al., 2019; Harman and Akleyin, 2023). However, it is crucial to consider the drawbacks of the chocolate-based delivery system, including the potential issue in the possibility of children mistaking

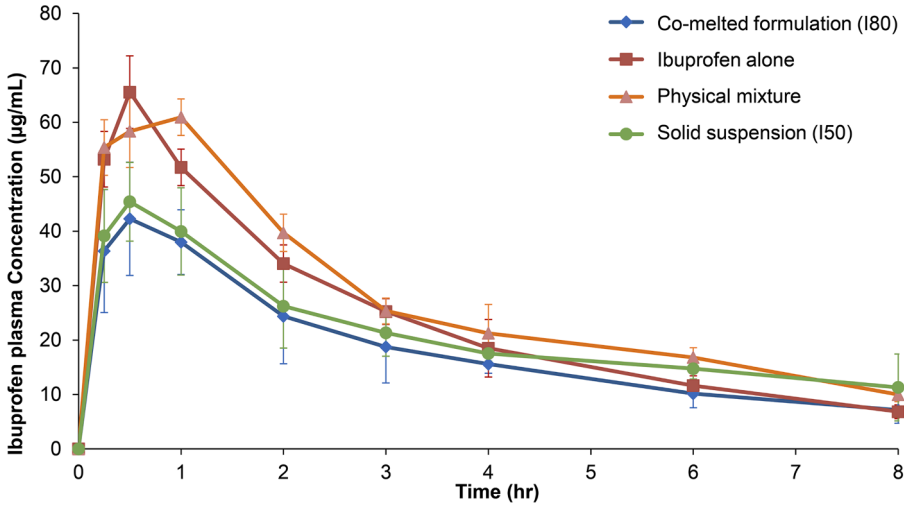

Fig. 6. Mean serum ibuprofen concentrations in rats over 8 h time intervals following the administration of a physical mixture of ibuprofen and chocolate compared to ibuprofen alone and chocolate dosage forms prepared at 50 or 80 °C ($n = 8$).

these medicines for candies and their appropriateness for diabetic patients.

4. Conclusions

In conclusion, preparation temperature had an impact physical form of the drug within the lipid matrix and ibuprofen release from chocolate matrix. The release of ibuprofen also appeared to be accelerated by enzymatic digestion of the constituents of chocolate. While initial stability studies indicated that chocolate dosage forms prepared at 50 °C showed more reproducible drug release, more in-depth stability studies are needed to establish the viability of this concept dosage form. *In vivo* experiments showed that dark chocolate ibuprofen formulations significantly reduced the serum concentration of ibuprofen compared to when the drug was administered alone ($p < 0.05$). However, the extent of drug absorption was found to be similar amongst all the formulations ($p > 0.05$). Notably, despite the reduction in the C_{max} of ibuprofen when embedded in chocolate matrices, the area under the curve for the last measured concentration (AUC_{last}) was also similar for all the formulations. This suggests a potential sustained effect of ibuprofen when incorporated into chocolate matrices. These findings have important implications for the pharmacokinetics of novel dosage forms based on chocolate or other lipid-based formulations.

CRediT authorship contribution statement

Aya Y. Al-Kabariti: Investigation, Methodology, Formal analysis, Writing – original draft, Visualization. **Basel T. Arafat:** Investigation, Methodology, Formal analysis, Writing – original draft, Visualization. **Ghaleb Ali Oriquat:** Conceptualization, Methodology, Writing – original draft. **Petra Možná:** Investigation. **Hadeal Jaidy:** Investigation. **Asma Rehmani:** Investigation. **Kausar Patel:** Investigation. **Nidal Al-Qinna:** Methodology, Formal analysis, Writing – review & editing. **Mohamed A. Alhnan:** Conceptualization, Formal analysis, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare no competing financial interests or personal relationships that may influence this work.

Data availability

The authors are unable or have chosen not to specify which data has been used.

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