Sains Malaysiana 53(11)(2024): 3747-3759
http://doi.org/10.17576/jsm-2024-5311-17

The Effects of Different Solubilizing Agents on the Transport and Pharmacokinetic Profiles of Indomethacin: *in vitro* and *in vivo* Approach

(Kesan Agen Pelarut Berbeza Terhadap Profil Pengangkutan dan Farmakokinetik Indometasin: Pendekatan *in vitro* dan *in vivo*)

SITI AISYAH ZAINUDDIN[1,3], ABDUL LATIP AB HAMID[1], AQILA IMAN RAFANDI[1], MASHANI MOHAMAD[2,4], KHURIAH ABDUL HAMID[1,4,*] & SYED HAROON KHALID[1,4]

[1]*Department of Pharmaceutical Technology, Faculty of Pharmacy, Universiti Teknologi MARA Cawangan Selangor, 42300 Puncak Alam, Selangor, Malaysia*
[2]*Department of Pharmacology and Life Sciences, Faculty of Pharmacy, Universiti Teknologi MARA Cawangan Selangor, 42300 Puncak Alam, Selangor, Malaysia*
[3]*Drug Information Service, Pharmacy Department, Hospital Kulim Lebuh Taman Perindustrian, Kulim Hi-Tech, 09000 Kulim, Kedah, Malaysia*
[4]*Innovative Drug Development and Delivery Research Group, Faculty of Pharmacy, Universiti Teknologi MARA, Cawangan Selangor, Kampus Puncak Alam, 42300 Bandar Puncak Alam, Selangor, Malaysia*

Received: 26 July 2024/Accepted: 27 September 2024

ABSTRACT

The poor water solubility of new chemical entities (NCEs) discovered in pharmaceutical screening programs hampers their development and limits effective treatment delivery. Solubilizing agents offer a direct approach to increase solubility and oral bioavailability. This study employed indomethacin as a model drug from Biopharmaceutical Classification System (BCS) class II. Solubilizing agents, including propylene glycol, Transcutol P, Labrasol, PEG 400, and Tween 80, were separately added at 10% (v/v) to 1 mg/5 mL for *in vitro* diffusion chamber and 10 mg/kg body weight for *in vivo* oral absorption studies of indomethacin. Drug concentrations were analysed using reversed-phase high-performance liquid chromatography (HPLC). *In vitro*, 10% (v/v) labrasol, PEG 400, and Tween 80 significantly ($p<0.01$) increased indomethacin permeability across the rat intestinal layer. However, Transcutol P and PG (propylene glycol 10%v/v) showed no discernible impact on *in vitro* permeability. In the *in vivo* oral absorption study, PG (propylene glycol 10%v/v) and Transcutol P significantly ($p<0.05$) enhanced indomethacin absorption, while 10% (v/v) labrasol, PEG 400, and Tween 80 did not show significant effects. No *in vitro-in vivo* correlation (IVIVC) was observed, likely due to the physicochemical properties of indomethacin and the complex physiological environment in the gastrointestinal (GI) tract. Further investigations are necessary to comprehensively understand the factors affecting indomethacin solubility and absorption, considering both the drug's properties and the GI tract's physiological conditions.

Keywords: Drug transport; indomethacin; pharmacokinetic profiles; solubility; solubilizing agents

ABSTRAK

Keterlarutan air yang rendah bagi entiti kimia baharu (NCEs) yang ditemui dalam program penyaringan farmaseutikal menghalang perkembangan ubatan tersebut dan mengehadkan keberkesanan rawatan. Agen pelarut menawarkan pendekatan langsung untuk meningkatkan keterlarutan dan bioketersediaan oral. Kajian ini menggunakan indometasin sebagai model sebatian ubat berdasarkan Sistem Pengelasan Biofarmaseutikal (BCS) kelas II. Setiap agen pelarut, termasuk propilena glikol, Transcutol P, Labrasol, PEG 400 dan Tween 80 pada kepekatan 10% (v/v) ditambah kepada 1 mg/5 mL sebatian indometasin secara berasingan untuk kajian resapan *in vitro* dan sebanyak 10 mg/kg berat badan diberikan kepada tikus untuk kajian penyerapan sebatian indometasin secara oral. Sebatian indometasin dianalisis menggunakan kaedah kromatografi cecair berprestasi tinggi – fasa terbalik (KCPT-Fasa Terbalik). Dalam kajian *in vitro*, sebanyak 10% (v/v) labrasol, PEG 400 dan Tween 80 telah meningkatkan kebolehtelapan indometasin merentasi lapisan usus tikus dengan signifikan ($p<0.01$). Walau bagaimanapun, 10% (v/v) propilena glikol dan Transcutol P tidak menunjukkan kesan yang signifikan terhadap kebolehtelapan melalui kajian *in vitro*. Dalam kajian penyerapan oral secara *in vivo*, 10% (v/v) propilena glikol dan Transcutol P meningkatkan penyerapan sebatian indometasin secara signifikan ($p<0.05$), manakala

10% (v/v) labrasol, PEG 400 dan Tween 80 tidak menunjukkan kesan yang signifikan. Keputusan kajian menunjukkan tiada korelasi *in vitro-in vivo* (IVIVC), berkemungkinan disebabkan oleh sifat fizikokimia indometasin dan persekitaran fisiologi yang kompleks dalam saluran gastrointestinal (GI). Kajian lanjut diperlukan untuk memahami secara menyeluruh faktor yang mempengaruhi keterlarutan dan penyerapan indometasin dengan mempertimbangkan kedua-dua sifat sebatian dan keadaan fisiologi saluran GI.

Kata kunci: Pengangkutan ubat; indometasin; profil farmakokinetik; keterlarutan; agen pelarut

INTRODUCTION

Solubility, which refers to the process of dissolving a solute in a solvent to create a homogeneous mixture, is a crucial factor in attaining the required concentration of a drug in systemic circulation to achieve the desired pharmacological effect (Savjani, Gajjar & Savjani 2012). More than 40% of new chemical entities (NCEs) produced in the pharmaceutical sector are insoluble in water because their characteristics have shifted toward increased molecular weight and rising lipophilicity (Savjani, Gajjar & Savjani 2012; Shriniwas et al. 2014). For absorption, a drug must dissolve in the gastrointestinal (GI) tract. However, poor solubility results in low bioavailability and frequently causes significant variability in absorption kinetics following oral administration. The physicochemical characteristics of the drug substance (solubility, lipophilicity, and molecular size), physiological factors (GI fluid composition, volume, and hydrodynamics, as well as GI transit), and the dosage form (solution, powder or tablet) after oral administration all have an impact on absorption behavior.

Based on the medications' permeability in the intestinal membrane and solubility in water, the Biopharmaceutics Classification System (BCS) was proposed, which divided the drugs into four types (Sathisaran & Dalvi 2018). The dissolution process is the rate limiting process in the absorption of BCS class II drugs, a significant variation in absorption behavior may be caused by the dissolution process. Pharmaceutical development of drugs with low water solubility requires formulation design employing a variety of techniques to enhance the bioavailability and solubility of a drug in the GI tract (Anuar et al. 2020; Kumar et al. 2013). The ideal formulation approach for any specific drug will be determined by various parameters, including the drug's shelf life, dose, scale-up, and physicochemical qualities. Physical and chemical alterations of the medication and additional procedures such as micronization (Aguiar et al. 2017), crystal engineering (Sathisaran & Dalvi 2018), salt creation (Park et al. 2019), solid dispersion (Xi et al. 2020) and complexation (Ismail et al. 2021) have been applied to enhance the medicines' low solubility (Savjani, Gajjar & Savjani 2012). However, current strategies to enhance the bioavailability of these medications are hampered by a variety of issues. For example, solid dispersion (SD) industrial applications have low reproducibility and consistency in SD quality, which frequently leads to differences in bioavailability. Furthermore, given the inherent properties of SD, the physicochemical instability of the dosage form during manufacturing and storage is perhaps the most pressing issue that has yet to be fully addressed (Huang & William 2017). For the reasons stated earlier, controlling the commercial production of SD-based goods is more difficult than managing the commercial manufacture of traditional products containing pharmaceuticals in their most stable solid form (Liu et al. 2017). Micronization frequently results in medication with poor flowability. Although this method is frequently employed, the end product suffers from particulate agglomeration due to charge formation, resulting in poor flow (Vandana et al. 2014). Furthermore, due to solid-state changes, micronization frequently results in unstable forms or amorphous impurities, presenting stability concerns during processing and storage.

Solubilizing chemicals is one of the simplest ways to increase a drug's water solubility and high bioavailability following oral delivery. In recent years, much interest has been shown in using solubilizing agents with the assumption of high aqueous solubility of the medicine will result in increased bioavailability (Di Cagno & Stein 2019). The development of enabling formulations is now guided by the (simplified) premise: It is possible to establish an absorption profile similar to that of a soluble molecule (BCS class I drug) by transforming a weakly soluble medication (BCS class II drug) into a solubilised form (Buckley et al. 2013). Solubilizing agents have been reported to be a significant factor in controlling the dissolution of lipophilic medicines in the GI tract (Letho et al. 2011). The addition of different solubilizing agents may alter the nature of crystals as well as bonding forces in the crystal lattice, resulting in a change in the drug's solubilization behavior (Despande et al. 2018).

Solubilizing agents are categorised based on their chemical characteristics as well as their primary solubilizing mechanism (Azman et al. 2022). Some solubilizers also have beneficial self-emulsifying capabilities, producing a dispersion of lipid-solubilised medication in the aqueous contents of the GI tract and establishing optimal conditions for absorption. Certain solubilizing agents have the potential to modify the function of the intestinal membrane barrier and cause harm to the intestinal epithelium

(Hamid et al. 2009). Nevertheless, past research has shown that a 10% (v/v) concentration of these solubilizing agents causes no membrane damage. Therefore, a 10% (v/v) concentration has been selected for this study from the standpoint of efficacy and toxicity.

Indomethacin, an acetic acid derivative, emerged as an exceedingly strong nonsteroidal anti-inflammatory drug (NSAID) during a tremendous attempt to identify efficient anti-inflammatory and analgesic drugs over 50 years ago (Lucas 2016). It plays a significant role in treating mild to moderate acute pain (Koonrungsesomboon et al. 2020) and relieving symptoms of arthritis (osteoarthritis and rheumatoid arthritis) or gout, such as inflammation, swelling, stiffness, and joint pain (Abdollahi et al. 2021). Indomethacin has low GI absorption and solubility following oral administration and showed the BCS class II properties, so to enhance the solubility this drug was chosen in this study (Zhang et al. 2018).

Numerous studies have investigated the solubility and absorption characteristics of indomethacin, primarily due to its poor water solubility, which limits its bioavailability. One prominent strategy to enhance solubility involves the use of self-nanoemulsifying drug delivery systems (SNEDDS) to improve both the solubility and dissolution rate of indomethacin (Shakeel et al. 2014, 2013) but no absorption studies were conducted to assess the oral delivery effectiveness of these formulations. In another approach, Hu et al. (2014) explored the use of inorganic mesoporous silica materials combined with alginate through ionic interaction as a potential strategy for oral sustained drug delivery systems. In another study, a four-fold increase in the area under the curve (AUC) compared to indomethacin solution after oral administration at a dosage of 40 mg/kg BW in rats was observed (Zhang et al. 2018) when incorporated into the pores of aminopropyl-functionalized mesoporous silica nanospheres. More recently, research has explored the complexation of indomethacin with β-cyclodextrin encapsulated in electrospun nanofibers for sustained release delivery (Norouzi & Abdouss 2023). Besides that, the first zwitterionic cocrystal of indomethacin with an amino acid was found to exhibit optimized physicochemical properties, including accelerated absorption and slowed elimination *in vivo* (Wang et al. 2020). Moreover, amorphous solid dispersions (ASD) are recognized as one of the most effective supersaturating drug delivery systems (SDDS) for improving the solubility of poorly water-soluble drugs. However, dissolution tests on physical mixtures (PMs) of ASD with PVP-K30 showed precipitation inhibition and dissolution challenges when PVP-K30 exceeded 2% in the PMs (Wang et al. 2020). In contrast, the use of solubilizing agents provides a straightforward and highly effective strategy to significantly enhance both solubility and oral bioavailability (Bertoni et al. 2019). Currently, indomethacin is available in capsules, extended release capsules and injections in the market (Villar et al. 2021). Most of the published studies reported on how to enhance the solubility of indomethacin with different solubilizing agents with limited data of pharmacokinetics. This is the gap we have tried to fill in with the solubility enhancement together with *in vitro* and *in vivo* pharmacokinetic profiles (low dose of indomethacin).

This study is an extension of our previous work, where we investigated the effects of common solubilizing agents on intestinal membrane barrier function and toxicity in rats. Based on the findings, we selected 10% (v/v) concentrations of propylene glycol, Transcutol P, Labrasol, polyethylene glycol (PEG) 400, and Tween 80, as they were shown to be safe and did not compromise the integrity of the intestinal membrane (Hamid et al. 2009). The aim of the current study was to investigate the effects of above solubilizing agents on the drug transport and pharmacokinetic profiles of indomethacin across intestinal layer using *in vitro* diffusion chamber and *in vivo* absorption study *via* oral delivery. The permeability across the intestine is an important element in the overall absorption of orally delivered medicines (Alqahtani et al. 2021).

MATERIALS AND METHODS

MATERIALS

Indomethacin, phosphate buffered saline (PBS) tablet, Tween 80 and anhydrous sodium carbonate were obtained from Sigma Aldrich Co. (St. Louis, MO, U.S.A.). Nacalai Tesque, Inc. provided the propylene glycol and polyethyene glycol (PEG 400) (Kyoto, Japan). Gattefossé Corp. provided Labrasol and Transcutol P (Saint-Priest, France). Acetonitrile (ACN) and glacial acetic acid were bought from Sigma Aldrich Co. (Burlington, MA, USA). Analytical grade solvents and other compounds were utilized.

PREPARATION OF DRUG SOLUTION

For the *in vitro* study, indomethacin was dissolved and stirred in phosphate buffer saline of pH 7.4 for 3 h to produce an end concentration of 1 mg/5 mL (0.02% w/v). For *in vivo* study, anhydrous sodium carbonate was dissolved in PBS solution of pH 7.4 to yield a concentration of 0.53% (w/v). Indomethacin was added to the solution to yield a final concentration of 0.3% (w/v). The solubilizing agents tested were propylene glycol, Transcutol P, Labrasol, polyethylene glycol (PEG) 400, and Tween 80. These solubilizing agents were added to the drug solution, respectively, having concentration of 10% v/v.

In vitro DIFFUSION CHAMBER STUDY

The diffusion chamber study approach was utilized to facilitate the transportation of indomethacin over the rat intestinal barrier (Alqahtani et al. 2021; Anuar et al. 2020). The animal ethics committee of Universiti Teknologi MARA (UiTM) provided approval and guidelines for this study (UiTM CARE:372/2022).

After an overnight fast, male Sprague Dawley rats weighing 350–500 grams were treated with an intraperitoneal (i.p.) dose of Zoletil-50 (0.1 mL/100 g body weight) to induce anesthesia. A midline abdominal incision was made to expose the intestine, which was then removed and cleaned in ice-cold PBS. After that, the intestinal segment was separated and dissected. The intestine sheets were affixed to the diffusion chamber pins after the muscle layer on the segment's outside was removed, and they were then clamped together. The donor compartment received 1 mg/5 mL of the drug solution, whereas the recipient compartment received the same volume of drug-free buffer (PBS at pH 7.4) (Figure 1).

To combine each solution and maintain the vitality of the membrane, 95% Oxygen and 5% Carbon dioxide gas were used to aerate each chamber in the chamber. The experiment was conducted with the temperature kept at 37 °C. At preset intervals of 0, 15, 30, 45, 60, 75, 90, 105, and 120 min, a volume of 0.2 mL aliquot was removed from the receiver compartment. Immediately after, the aliquot was swapped out for an equivalent volume of buffer solution. The following formula was used to get the apparent permeability coefficient:

$$\text{Papp} = \text{Flux X } \frac{1}{60} \text{ X } \frac{1}{\text{Area}} \text{ X } \frac{1}{C_0}$$

The apparent permeability coefficient, or Papp, is measured in cm/s, and the slope of the linear part of the cumulative transit amount to time at the steady-state, or F, is measured in pmol/mL. The area is the 0.29 cm^2 transport area (fix value) of the diffusion chamber, and C_0 is the drug's initial concentration (pmol/mL) prior to transport.

In vivo ORAL ABSORPTION STUDY

Male Sprague Dawley rats weighing between 350 and 500 grams were acquired from UiTM Puncak Alam's Laboratory Animal Facility and Management (LAFAM). A single dosage, head-to-head comparison strategy including six treatment groups, each containing three rats (n = 3), was used for the investigation. There were five groups of indomethacin solution, each with a different solubilizing agent, and one control group consisting of indomethacin. Twelve hours prior to the commencement of the experiment, the animals were given water on demand during an overnight fast, in accordance with previously documented protocols (Jaafar & Hamid 2019; Zhao et al. 2016). The rats were anaesthetised using an intraperitoneal (i.p.) injection of Zoletil-50 (0.1 mL/100 g body weight) before the start of the experiment to collect blood from the jugular vein at 0 min and to ease oral gavage administration (Yeh et al 2021). The rats were then given the appropriate group's worth of doses of indomethacin solution (10 mg/kg) with or without 10% (v/v) solubilizing agents (Figure 2).

Approximately, 0.25 - 0.30 mL of blood was collected at predetermined time intervals of 0 (before oral administration), 15, 30, 60, 90, 120, 180, 240, 300 and 360 min, post-administration. Blood plasma was produced by centrifuging sample of blood at 15,000 rpm for 15 min. The plasma was transferred into a new Eppendorf tube to be kept frozen at -80 °C until further analysis using HPLC. The plasma concentration-time curves were used to directly calculate the peak concentration (Cmax) and the time required to achieve the peak concentration (Tmax). Using the trapezoidal approach, the area under the curve (AUC) was determined from zero to the final sample time (360 min).

DRUG CONTENT DETERMINATION

The concentration of indomethacin was measured using an Agilent LC-10 pump system, autoinjector, RF-10A or LC-10 detector, CR-6A integrator, and 5 µM Cosmosil (4.6 mm × 150 mm) particles in an analytical column from Thermo Electron Corporation. In the case of the *in vitro* study samples, 200 µL of the sample was filtered into the HPLC vial before a final volume of 20 µL sample was injected into the HPLC system. In the case of the *in vivo* study samples, biological components were removed before injection into the HPLC to prevent them from entering the HPLC column. The samples were mixed with acetonitrile (ACN) at a 2:1 (ACN: sample) and vortexed. They were centrifuged for 15 min at 15,000 rpm. Following a vortex, the samples were centrifuged for 15 min at 15,000 rpm. After filtering, the supernatants were moved into a sanitized HPLC vial. Subsequently, a 20 µL sample volume was added to the HPLC system. The mobile phase used for the quantification of indomethacin in both *in vitro* and *in vivo* investigations consisted of a 50:50 v/v combination of acetic acid and acetonitrile (pH 3.5) at a flow rate of 1.5 mL/min. At 210 nm, the UV detector was calibrated. For the *in vitro* investigation, the standard curve demonstrated good linearity over a concentration range of 3.125 µg/mL to 100 µg/mL, and for the *in vivo* study, 1 µg/mL to 100 µg/mL.

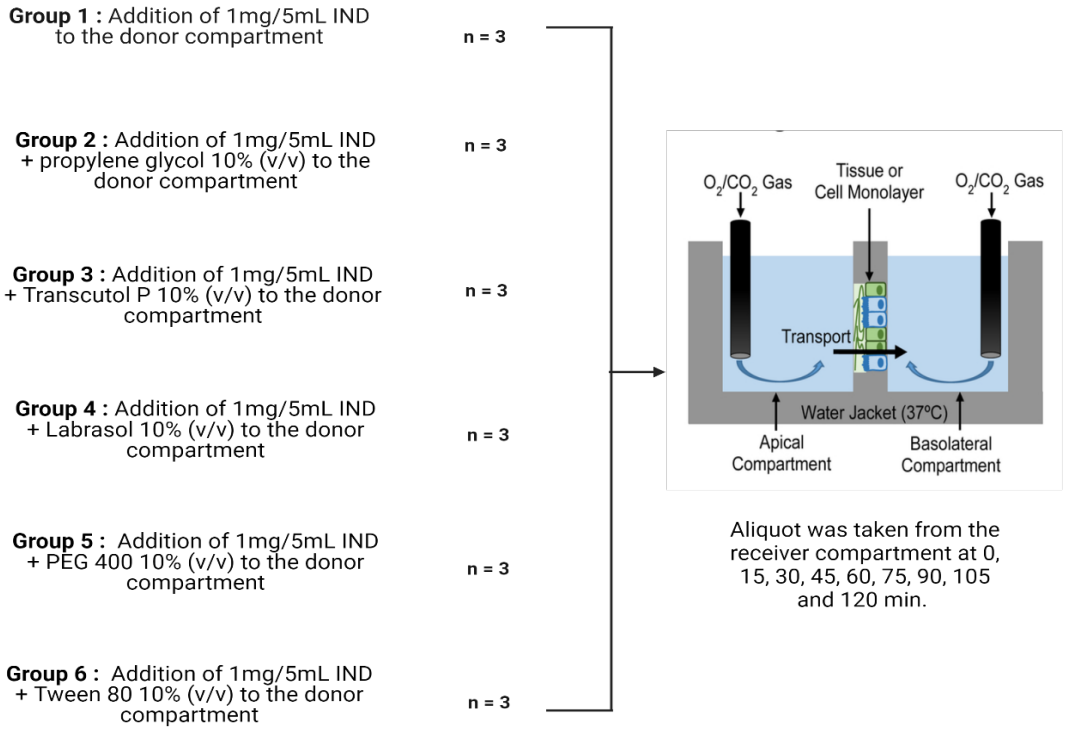


FIGURE 1. Indomethacin formulations with different solubilizing agents that were added to the donor compartment to study the amount of indomethacin transported across rat intestinal cell.

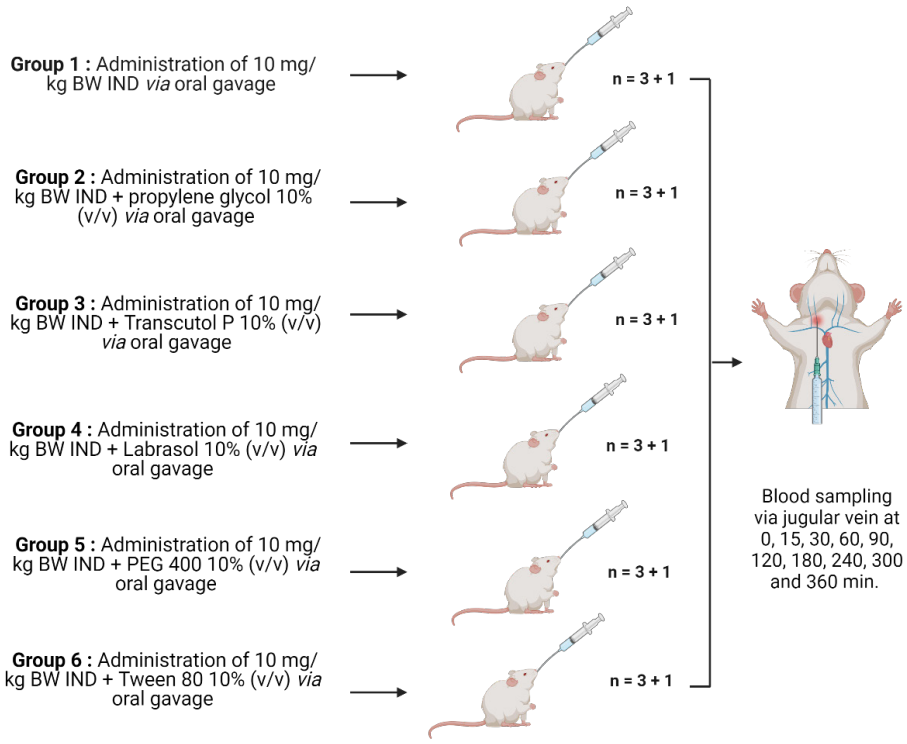


FIGURE 2. Overview of the oral administration study. Each group consisted of 3 male Sprague Dawley rats (n=3).

STATISTICAL ANALYSIS

Analysis of variance (ANOVA) was used to determine statistical significance for multiple comparisons at a significance level of $p<0.05$ and $p<0.01$, and the findings were presented as the mean ± SE.

RESULTS

CALIBRATION CURVE

Figure 3(a) and 3(b) demonstrate a strong linear relationship between the data points within the utilised concentration range for both the *in vitro* diffusion chamber method and the *in vivo* oral absorption study. The coefficient of determination (R^2) for both methods was calculated to be 0.9999, indicating a high degree of correlation between the obtained data and the response signal. The concentration range chosen for linear fitting of these data displayed high rationality, accuracy, and applicability of the analytical method for highly sensitive detection in the indomethacin samples of low concentration range.

In vitro DIFFUSION CHAMBER STUDY

To achieve effective concentration in the target tissue, ideal drug candidates should have appropriate aqueous solubility and permeability (Kratz et al. 2012). An *in vitro* model of intestinal permeation using an Ussing chamber lined with intestinal sheets was used to evaluate the degree of oral absorption and describe absorption mechanisms across the layers of the rodent intestine. The Ussing chamber consists of two clamped half chambers with developed epithelial sheets on permeable supports, allowing for the separation of the apical and basolateral sides. This model simulates the transport of ions from the apical to the basolateral side and vice versa, mimicking the circulation between these sides and providing an environment similar to the body (Song et al. 2013).

In this study, six different *in vitro* formulations of indomethacin were developed using five different solubilizing agents, along with a control group containing no solubilizing agent. The concentration of indomethacin used in the formulations was 1 mg/5 mL (0.02% w/v). Figure 4 illustrates the average amount of indomethacin transported across the rat intestinal layer for each of the six different *in vitro* formulations. Notably, the indomethacin solution containing 10% (v/v) labrasol exhibited a significant increase ($p<0.01$) in the transported amount of indomethacin compared to the control group. Following labrasol, indomethacin solutions with 10% (v/v) polyethylene glycol 400 and 10% (v/v) Tween 80 showed a moderate increase in the transported amount. Conversely, indomethacin solutions containing 10% (v/v) propylene glycol and 10% (v/v) Transcutol P had no significant effect on the amount of indomethacin transported, as their values were relatively similar to the control group.

The assessment of the transported amount of formulations across intestinal cells was accompanied by the determination of the apparent permeability coefficient (Papp) values, which provide insights into the permeation characteristics of particles in the ileum (Larregieu & Benet 2014). A higher Papp value indicates a greater degree of permeation of the particle through the intestinal cells. In relation to the amount of indomethacin transported, Figure 5 provides insights into the Papp values for different formulations. Notably, the indomethacin solution containing 10% (v/v) labrasol exhibited a significantly increased Papp value (0.2527×10^{-6} cm/s) compared to the control, with a Papp value of 0.0284×10^{-6} cm/s. This finding suggests that the presence of 10% (v/v) labrasol significantly enhanced the transport of indomethacin across the rat intestinal layer. Similarly, the indomethacin solutions containing 10% (v/v) PEG 400 and Tween 80 also showed significantly increased Papp values compared to the control. The Papp value for the solution with 10% (v/v) PEG 400 was 0.2490×10^{-6} cm/s, while the solution with 10% (v/v) Tween 80 had a Papp value of 0.1732×10^{-6} cm/s. These results indicate that both PEG 400 and Tween 80 contributed to the enhanced transport of indomethacin across the intestinal cells.

On the other hand, the indomethacin solution containing 10% (v/v) propylene glycol exhibited the lowest Papp value (0.0282×10^{-6} cm/s), which was even lower than the Papp value of the control group. This implies that the transport of indomethacin throughout the rat intestinal layer was not considerably affected by propylene glycol. Similarly, the presence of 10% (v/v) Transcutol P resulted in a slightly higher Papp value (0.0308×10^{-6} cm/s) compared to the control group. However, this difference was not significant enough to affect the transport of indomethacin across the intestinal layer. The comparison of Papp values and enhancement ratios for the different formulations can be observed in Table 1, providing a comprehensive overview of the relative effects of each solubilizing agent on the transport of indomethacin.

These results indicate that the solubilizing agents labrasol, PEG 400, and Tween 80 significantly enhanced the transport of indomethacin across the rat intestinal layer. In contrast, propylene glycol and Transcutol P did not exert a significant effect on indomethacin transport within the tested concentration range. Overall, the Papp values provide additional evidence supporting the role of solubilizing agents, such as labrasol, PEG 400, and Tween 80, in improving the permeability of indomethacin through intestinal cells. The significant increase in Papp values aligns with the increased transported amounts of indomethacin, indicating a positive impact on the oral

absorption of the drug. However, further investigations are warranted to comprehensively elucidate the mechanisms underlying these permeability enhancements and optimise the formulations for effective drug delivery.

In vivo ORAL ABSORPTION STUDY

Utilizing the *in vivo* oral absorption research, it was investigated how solubilizing substances affected the absorption of indomethacin following oral administration. Figure 6 illustrates how solubilizing agents affect the absorption of indomethacin at a dose of 10 mg/kg. As seen in the figure, the absorption of indomethacin was significantly increased ($p<0.05$) in the presence of Transcutol P and 10% (v/v) propylene glycol. By comparison, the absorption of indomethacin was not significantly affected by 10% (v/v) labrasol, PEG 400, or Tween 80 when compared to the control group. The maximal plasma concentration (Cmax), time to attain Cmax (Tmax), and area under the concentration-time curve (AUC) values of indomethacin when given in the intestine in combination with solubilizing agents are summarized in Table 2. The AUC values of indomethacin in the gut were dramatically raised by using Transcutol P and 10% (v/v) propylene glycol; in the absorption study, propylene glycol had the greatest AUC value.

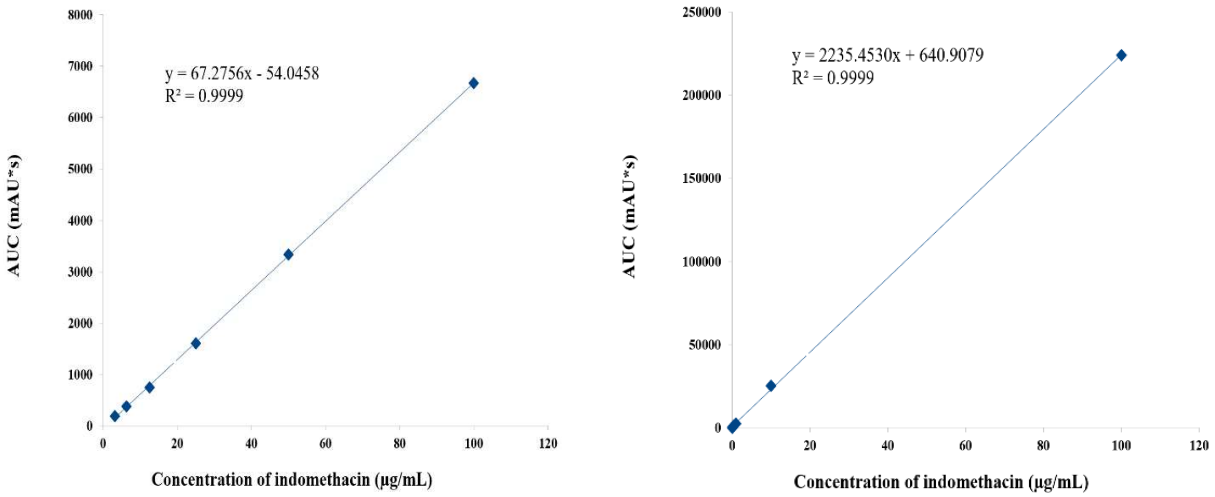
Indomethacin's intestinal absorption in the presence of 10% (v/v) propylene glycol and 10% (v/v) Transcutol P was shown to be extremely permeable based on mass balance measurement; the amount of absorption reached > 90% of the administered dose. However, in the *in vivo* oral absorption investigation, the remaining solubilizing agents did not have a discernible impact on the intestinal absorption of indomethacin. These findings indicate that propylene glycol and Transcutol P have a pronounced impact on enhancing the absorption of indomethacin in the intestine after oral administration. However, the other solubilizing agents, including labrasol, PEG 400, and Tween 80, did not demonstrate a noteworthy impact on indomethacin's intestinal absorption in this specific investigation.

DISCUSSION

In analytical chemistry, establishing a functional relationship between the collected data and the corresponding response signal is crucial for effective results analysis and data processing. One commonly employed approach is to employ function fitting in a coordinate system with two linear axes. This method allows for the creation of a linear curve that can be utilised for experimental method validation and application (Kong et al. 2021). In our study, we assessed the relationship between the data points obtained from the *in vitro* diffusion chamber method and the *in vivo* oral absorption study. Figures 3 and 4 display the data points within the concentration range we utilised for the analysis. The remarkable aspect observed in both cases was the strong linear relationship between the data points, as evidenced by the calculated R^2 value of 0.9999 for both methods. The findings from these analyses suggest that the experimental methods employed in this study are highly reliable and yield consistent results within the tested concentration range. The strong linear relationship observed in the obtained data points enhances our confidence in the accuracy and precision of the experimental techniques utilised. It also provides a solid foundation for further analysis and interpretation of the experimental results.

Overall, the establishment of a robust functional relationship between the obtained data and the response signal is pivotal in analytical chemistry. In this study, the linear curves derived from the *in vitro* diffusion chamber method and *in vivo* oral absorption study demonstrated excellent correlation coefficients (R^2) of 0.9999, highlighting the effectiveness of these experimental approaches. Our findings using the *in vitro* diffusion chamber and *in vivo* oral absorption method had contrary results. An *in vitro - in vivo* correlation (IVIVC) is a predictive mathematical model that describes the relationship between an oral dosage form *in vitro* property and relevant *in vivo* response. IVIVC predicts a drug's *in vivo* performance based on its *in vitro* drug release characteristics (Lu, Kim & Park 2011). In general, the rate or extent of drug dissolution or release is the *in vitro* attribute, whereas the plasma drug concentration or amount absorbed is the *in vivo* response.

Based on our *in vitro* diffusion chamber and *in vivo* oral absorption study data, there was no IVIVC observed. The physicochemical and biological properties of the medicine as well as the physiological environment in the body need to be taken into account to build an effective IVIVC. However, these models do not fully replicate the dynamic processes that occur *in vivo*, such as blood flow, first-pass metabolism, immune responses, and interactions with other cells and tissues, all of which can significantly influence drug behavior *in vivo* (Amaral Silva et al. 2024; Mistry et al. 2016). The controlled conditions *in vitro* (temperature, pH, and nutrient availability) often do not mimic the variability present *in vivo*. For instance, the gastrointestinal tract's fluctuating pH and the presence of enzymes can affect drug solubility and absorption differently than in a controlled *in vitro* environment (Vinarov et al. 2021). Additionally, drug transporters, such as P-glycoprotein (P-gp), play a significant role in drug efflux *in vivo*. They are more actively involved in drug transport within living organisms compared to isolated intestinal segments, which can result in differences in drug absorption and bioavailability between *in vitro* and *in vivo*



a b

FIGURE 3. a) The calibration curve obtained for *in vitro* diffusion chamber study, b) The calibration curve obtained for *in vivo* oral absorption study.

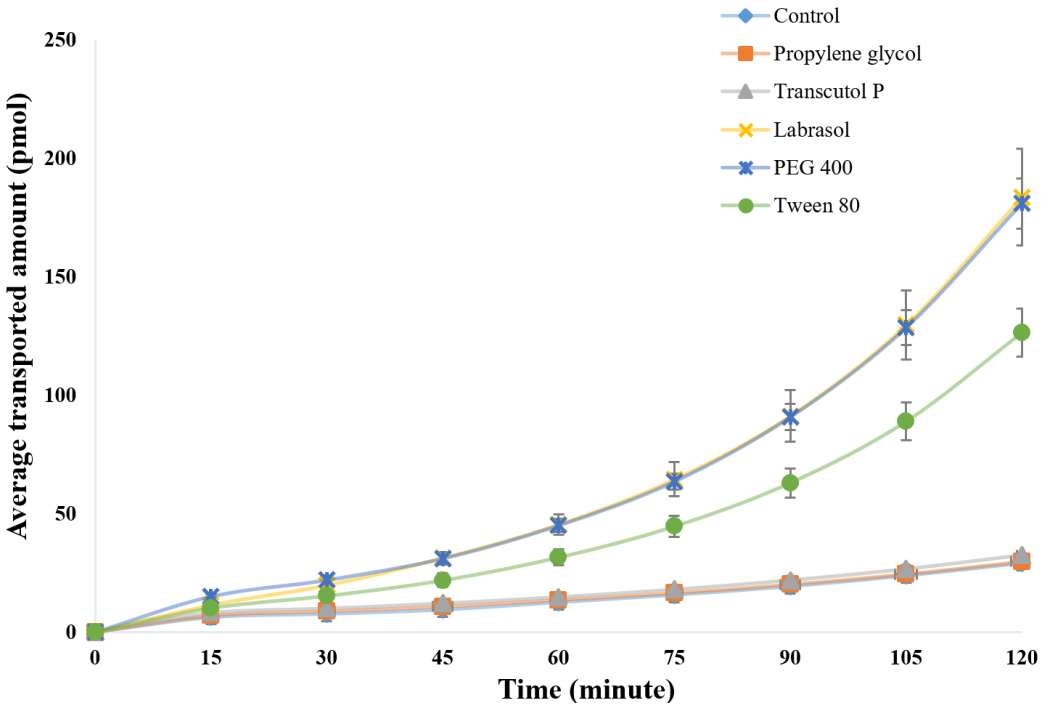


FIGURE 4. Effects of different 10% (v/v) solubilizing agents on the transported amount of indomethacin across the rat intestinal layer. Results are expressed as the mean ± SE of 3 experiments (n=3).

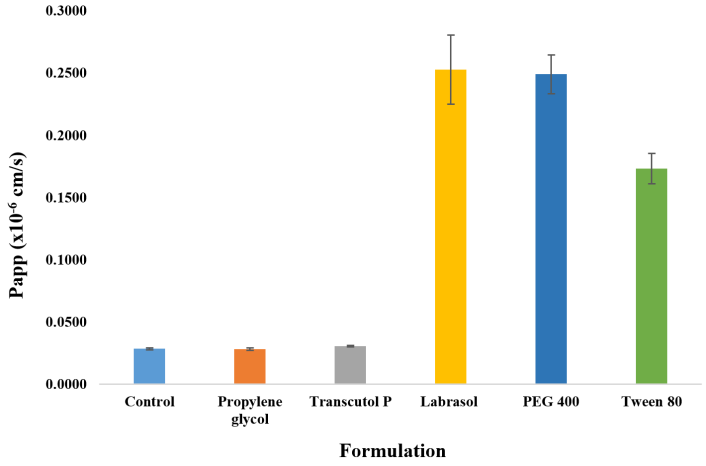


FIGURE 5. Effects of different 10% (v/v) solubilizing agents on the apparent permeability (Papp, × 10^{-6} cm/s) of indomethacin across the rat intestinal layer. The mean ± SE of three experiments (n = 3) is used to express the results.

TABLE 1. Values of indomethacin in rat intestinal layer and its enhancement ratio when compared to the control

Formulation	Papp (×10^{-6} cm/s)	Enhancement ratio
Control	0.0284 ± 0.0008	-
10% (v/v) Propylene glycol	0.0282 ± 0.0008$^{N.S.}$	1.00
10% (v/v) Transcutol P	0.0308 ± 0.0006$^{N.S.}$	1.08
10% (v/v) Labrasol	0.2527 ± 0.0278*	8.90
10% (v/v) PEG 400	0.2490 ± 0.0155*	8.77
10% (v/v) Tween 80	0.1732 ± 0.0123*	6.10

Results are expressed as the mean ± SE of 3 experiments (n=3). *p< 0.01, N.S. no significant difference compared with the control

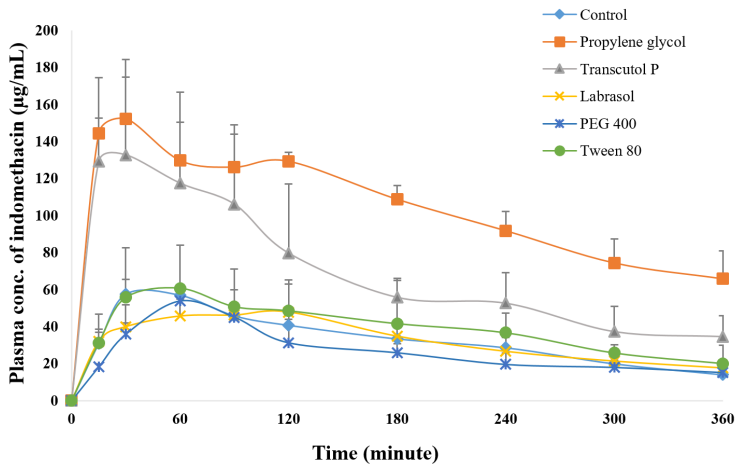


FIGURE 6. Plasma concentrations-time profiles of indomethacin (10 mg/kg) in the presence and absence of 10% (v/v, w/v) solubilizing agents in the small intestine by an in vivo oral absorption study method. Results are expressed as the mean ± SE of 3 experiments (n=3)

TABLE 2. Pharmacokinetic parameters of indomethacin (10 mg/kg) following oral administration in rats.

Formulation	Cmax (μg/mL)	Tmax (min)	AUC_{0-240} (μg/mL · min)
Control	65.5 ± 3.0	40.0 ± 10.0	11973.2 ± 1060.6
10% (v/v) Propylene glycol	184.8 ± 24.2	55.0 ± 21.8	37545.7 ± 2469.3*
10% (v/v) Transcutol P	156.4 ± 46.7	30.0 ± 15.0	24988.3 ± 8736.5*
10% (v/v) Labrasol	64.1 ± 7.4	80.0 ± 26.5	11765.2 ± 1253.9$^{N.S.}$
10% (v/v) PEG 400	63.5 ± 3.9	90.0 ± 17.3	9972.8 ± 1261.6$^{N.S.}$
10% (v/v) Tween 80	76.9 ± 20.4	150.0 ± 62.5	14068.5 ± 3473.4$^{N.S.}$

Results are expressed as the mean ± SE of 3 experiments (n=3). *$p<0.05$, N.S. no significant difference, compared with the control.

studies (Bhoopathy et al. 2021). Metabolic enzymes like cytochrome P450 (CYP), present in the liver and intestinal wall, can also greatly impact a drug's bioavailability *in vivo* by reducing absorption or increasing clearance, which is less prominent in *in vitro* settings (Fedi et al. 2021). These factors highlight the complexity of correlating *in vitro* results with *in vivo* outcomes and underscore the limitations of *in vitro* models in predicting *in vivo* pharmacokinetics. While we aimed to replicate *in vivo* conditions as closely as possible, the limitations of *in vitro* models using excised intestinal tissue should be acknowledged. Further studies using more advanced models that better mimic *in vivo* environments, or a combination of *in vitro* and *in vivo* approaches, may provide a clearer understanding of the mechanisms driving these differences.

The absence of IVIVC in this study may be affected by factors such as drug solubility and permeability, pKa, octanol-water partition coefficient, and environmental pH. The ionisation constant of a substance, or its logarithmic equivalent, the pKa value, impacts its stability, solubility, and absorption under various environmental pH levels. This is significant because the body has natural pH gradients, particularly in the GI system, which result in pH-dependent absorption profiles *in vivo* (Broesder et al. 2020). The particle size is a more evident source of effect on dissolution. It is widely accepted that decreasing particle size increases surface area and speeds up dissolution. Johnson and Swindell (1996) discovered that the influence of particle size on absorption is dependent on drug dose and drug solubility. All of these aspects contribute to the complexity of the model building process. It is recommended for future studies to check for the particle size of the developed formulations as this study did not look at the effect of the particle size on the absorption of poorly soluble drugs.

Another parameter that can be useful in the building of a model is the oil-water partition coefficient. In instance, the ability of a molecule to permeate across membranes for absorption is often determined by measuring its octanol-water partition coefficient (logP) of neutral or unionized species. Hansch and Fujita (1964) used multiple linear regression and a computer to quantify the structure-activity relationships based on lipophilicity. Strong membrane permeability was exhibited by compounds with logP values between 0 and 3, while low membrane permeability was observed in compounds with logP values larger than 4.5 or less than 1.5 (Kramer 1999). This explains why most of the indomethacin (logP = 3.53) solution in our *in vitro* diffusion chamber study showed high Papp values. It is critical to note that, while the octanol-water partition coefficient is a good indicator of membrane permeability, it is insufficient to predict *in vivo* absorption (Lu, Kim & Park 2011).

Because physiological variables can affect drug dissolution as well as the pace and extent of drug absorption, they should be taken into account for the proper development of IVIVC, in addition to physicochemical and biological parameters. For instance, the body's intrinsic pH gradient makes the impact of pH more significant there (Lu, Kim & Park 2011). The GI tract has the most well-known and extensively researched pH gradient, which varies from 1-2 in the stomach to 7-8 in the colon. The bulk of substances ingested orally are absorbed in the small intestine, which has a pH between 5 and 8. Variations in the GI pH profile can impact drug solubility, stability, dissolution, and permeability. The physiological environment is always changing in response to normal bodily functions like eating, which makes matters more complicated. Indomethacin has a pKa of approximately 4.5, indicating that its solubility and absorption are highly pH-dependent (Palanisamy & Khanam 2014). At a pH above 4.5, indomethacin becomes increasingly ionized, which enhances its solubility in aqueous environments. This increase in solubility facilitates better dissolution, particularly in the intestines where the pH is higher (Andrusenko et al. 2021). As the pH rises, indomethacin transitions from its non-ionized form to a more soluble ionized form, leading to improved absorption in the intestines compared to the stomach (Ranjan & Jha 2022). The magnitude of this effect is significant. For instance, indomethacin's solubility at pH 7.2 is markedly

higher than at pH 1.2, resulting in enhanced dissolution and subsequent absorption in the intestinal environment (Palanisamy & Khanam 2014). This pH-dependent solubility is crucial in optimizing the formulation and bioavailability of indomethacin, as the varying pH levels in the gastrointestinal tract can influence the drug's absorption and therapeutic efficacy.

A few things that could be done to make the study better are measuring the pH of the rat's gut and using a solution in the *in vitro* diffusion chamber study that is close to the pH of the small intestine. This will enable a more accurate simulation of the rat's interior environment. Even while solubilizing drugs have attained acceptable solubility rates *in vitro* or may serve as penetration enhancers, adverse variables can still have a negative impact on the absorption process.

CONCLUSION

In the *in vitro* investigation, the permeability of indomethacin across the rat intestinal layer was significantly ($p<0.01$) increased by 10% (v/v) labrasol, PEG 400, and Tween 80. Transcutol P and 10% (v/v) propylene glycol, however, had no discernible impact on indomethacin's permeability across the rat intestinal layer. However, 10% (v/v) of propylene glycol and Transcutol P considerably ($p<0.05$) increased the oral absorption of indomethacin, but 10% (v/v) of labrasol, PEG 400, and Tween 80 had no significant effect on the oral absorption of indomethacin. This was shown in an *in vivo* oral absorption study. These data indicate that there was no IVIVC found in this investigation. The limitation of this preliminary study is the small sample size but we believe the data provided has valuable information, which can be further validated in larger studies in future to confirm these findings.

ACKNOWLEDGEMENTS

Acknowledgement is made to Faculty of Pharmacy, Universiti Teknologi MARA (UiTM), Geran Penyelidikan UiTM Cawangan Selangor (DUCS 2.0), 600-UiTMSEL (PI. 5/4 (016/2020) and UiTM CARE (Committee on Animal research and Ethics) (UiTM CARE:372/2022).

REFERENCES

Abdollahi, A.R., Firouzian, F., Haddadi, R. & Nourian, A. 2021. Indomethacin loaded dextran stearate polymeric micelles improve adjuvant-induced arthritis in rats: Design and *in vivo* evaluation. *Inflammopharmacology* 29(1): 107-121.

Aguiar, G.P.S., Arcari, B.D., Chaves, L.M.P.C., Magro, C.D., Boschetto, D.L., Piato, A.L., Lanza, M. & Vladimir Oliveira, J. 2018. Micronization of trans-resveratrol by supercritical fluid: Dissolution, solubility and *in vitro* antioxidant activity. *Ind. Crops Prod*. 112: 1-5.

Alqahtani, M.S., Kazi, M., Alsenaidy, M.A. & Ahmad, M.Z. 2021. Advances in oral drug delivery. *Front Pharmacol*. 12: 618411.

Amaral Silva, D., Le Merdy, M., Alam, K.D., Wang, Y., Bao, Q., Malavia, N., Burgess, D. & Lukacova, V. 2024. Development of mechanistic *in vitro–in vivo* extrapolation to support bioequivalence assessment of long-acting injectables. *Pharmaceutics* 16(4): 522.

Andrusenko, I., Hamilton, V., Lanza, A.E., Hall, C.L., Mugnaioli, E., Potticary, J., Buanz, A., Gaisford, S., Piras, A.M., Zambito, Y., Hall, S.R. & Gemmi, M. 2021. Structure determination, thermal stability and dissolution rate of δ-indomethacin. *International Journal of Pharmaceutics* 608: 121067. https://doi.org/10.1016/j.ijpharm.2021.121067

Anuar, N., Sabri, A.H., Bustami Effendi, T.J. & Hamid, K.A. 2020. Development and characterisation of ibuprofen-loaded nanoemulsion with enhanced oral bioavailability. *Heliyon* 6: e04570.

Azman, M., Sabri, A.H., Anjani, Q.K., Mustaffa, M.F. & Hamid, K.A. 2022. Intestinal absorption study: Challenges and absorption enhancement strategies in improving oral drug delivery. *Pharmaceuticals* 15(8): 975.

Bertoni, S., Albertini, B., Ferraro, L., Beggiato, S., Dalpiaz, A. & Passerini, N. 2019. Exploring the use of spray congealing to produce solid dispersions with enhanced indomethacin bioavailability: *in vitro* characterization and *in vivo* study. *Eur. J. Pharm. Biopharm*. 139: 132-141.

Bhoopathy, S., Bode, C., Naageshwaran, V., Weiskircher-Hildebrant, E., Mukkavilli, V. & Hidalgo, I.J. 2021. Principles and experimental considerations for *in vitro* transporter interaction assays. In *Enzyme Kinetics in Drug Metabolism. Methods in Molecular Biology*, vol 2342, edited by Nagar, S., Argikar, U.A. & Tweedie, D. New York: Humana. pp. 339-365.

Broesder, A., Woerdenbag, H.J., Prins, G.H., Nguyen, D.N., Frijlink, H.W. & Hinrichs, W.L.J. 2020. pH-dependent ileocolonic drug delivery, part I: *In vitro* and clinical evaluation of novel systems. *Drug Discov. Today* 25(8): 1362-1373.

Buckley, S.T., Frank, K.J., Fricker, G. & Brandl, M. 2013. Biopharmaceutical classification of poorly soluble drugs with respect to "enabling formulations". *Eur. J. Pharm. Sci*. 50(1): 8-16.

Deshpande, T.M., Shi, H., Pietryka, J., Hoag, S.W. & Medek, A. 2018. Investigation of polymer/surfactant interactions and their impact on itraconazole solubility and precipitation kinetics for developing spray-dried amorphous solid dispersions. *Mol. Pharm*. 15(3): 962-974.

Di Cagno, M.P. & Stein, P.C. 2019. Studying the effect of solubilizing agents on drug diffusion through the unstirred water layer (UWL) by localized spectroscopy. *Eur. J. Pharm. Biopharm*. 139: 205-212.

Fedi, A., Vitale, C., Ponschin, G., Ayehunie, S., Fato, M. & Scaglione, S. 2021. *In vitro* models replicating the human intestinal epithelium for absorption and metabolism studies: A systematic review. *Journal of Controlled Release* 335: 247-268.

Hamid, K.A., Katsumi, H., Sakane, T. & Yamamoto, A. 2009. The effects of common solubilizing agents on the intestinal membrane barrier functions and membrane toxicity in rats. *Int. J. Pharm*. 379(1-2): 100-108.

Hansch, C. & Fujita, T. 1964. A method for the correlation of biological activity and chemical structure. *J. Am. Chem. Soc*. 86(8): 1616-1626.

Hu, L., Sun, C., Song, A., Chang, D., Zheng, X., Gao, Y., Jiang, T. & Wang, S. 2014. Alginate encapsulated mesoporous silica nanospheres as a sustained drug delivery system for the poorly water-soluble drug indomethacin. *Asian Journal of Pharmaceutical Sciences* 9(4): 183-190.

Huang, S. & Williams, R.O. 2017. Effects of the preparation process on the properties of amorphous solid dispersions. *AAPS PharmSciTech*. 19(5): 1971-1984.

Ismail, A., Kerdpol, K., Rungrotmongkol, T., Tananuwong, K., Ueno, T., Ekasit, S., Muangsin, N. & Krusong, K. 2021. Solubility enhancement of poorly water soluble domperidone by complexation with the large ring cyclodextrin. *Int. J. Pharm*. 606: 120909.

Jaafar, M.H.M. & Hamid, K.A. 2019. Chitosan-coated alginate nanoparticles enhanced absorption profile of insulin via oral administration. *Curr. Drug Deliv*. 16(7): 672-686.

Johnson, K.C. & Swindell, A.C. 1996. Guidance in the setting of drug particle size specifications to minimize variability in absorption. *Pharmaceutical Research* 13(12): 1795-1798.

Kong, D., Zhao, J., Tang, S., Shen, W. & Kee Lee, H. 2021. Logarithmic data processing can be used justifiably in the plotting of a calibration curve. *Anal Chem*. 93(36): 12156-12161.

Koonrungsesomboon, N., Teekachunhatean, S., Chansakaow, S. & Hanprasertpong, N. 2020. Clinical efficacy and safety of yellow oil formulations 3 and 4 versus indomethacin solution in patients with symptomatic osteoarthritis of the knee: A randomized controlled trial. *Evidence-Based Complement. Altern. Med*. 2020: 5782178.

Kramer, S.D. 1999. Absorption prediction from physicochemical parameters. *Pharm. Sci. Technol. Today* 2(9): 373-380.

Kratz, J.M., Teixeira, M.R., Ferronato, K., Teixeira, H.F., Koester, L.S. & Simões, C.M.O. 2012. Preparation, characterization, and *in vitro* intestinal permeability evaluation of thalidomide-hydroxypropyl-β-cyclodextrin complexes. *AAPS PharmSciTech*. 13(1): 118-124.

Kumar, S., Bhargava, D., Thakkar, A. & Arora, S. 2013. Drug carrier systems for solubility enhancement of BCS class II drugs: A critical review. *Crit. Rev. Ther. Drug Carrier Syst*. 30(3): 217-256.

Larregieu, C.A. & Benet, L.Z. 2014. Distinguishing between the permeability relationships with absorption and metabolism to improve BCS and BDDCS predictions in early drug discovery. *Mol. Pharm*. 11(4): 1335-1344.

Lehto, P., Kortejärvi, H., Liimatainen, A., Ojala, K., Kangas, H., Hirvonen, J., Tanninen, V.P. & Peltonen, L. 2011. Use of conventional surfactant media as surrogates for FaSSIF in simulating *in vivo* dissolution of BCS class II drugs. *Eur. J. Pharm. Biopharm*. 78(3): 531-538.

Liu, X., Feng, X., Williams, R.O. & Zhang, F. 2017. Characterization of amorphous solid dispersions. *J. Pharm. Investig*. 48(1): 19-41.

Lu, Y., Kim, S. & Park, K. 2011. *In vitro-in vivo* correlation: Perspectives on model development. *Int. J. Pharm*. 418(1): 142-148.

Lucas, S. 2016. The pharmacology of indomethacin. *Headache* 56(2): 436-446.

Mistry, B., Patel, N., Jamei, M., Rostami-Hodjegan, A. & Martinez, M.N. 2016. Examining the use of a mechanistic model to generate an *in vivo/in vitro* correlation: Journey through a thought process. *AAPS Journal* 18(5): 1144-1158.

Norouzi Zahra & Abdouss Majid. 2023. Electrospun nanofibers using β-cyclodextrin grafted chitosan macromolecules loaded with indomethacin as an innovative drug delivery system. *International Journal of Biological Macromolecules* 233: 123518.

Palanisamy, M. & Khanam, J. 2014. Effect of physiochemical variables on phase solubility and dissolution behavior of indomethacin solid dispersion system. *Journal of Pharmaceutical Investigation* 44(3): 147-162.

Park, C., Meghani, N.M., Shin, Y., Oh, E., Park, J.B., Cui, J.H., Cao, Q.R., Tran, T.T-D., Tran, P.H-L. & Lee, B-J. 2019. Investigation of crystallization and salt formation of poorly water-soluble telmisartan for enhanced solubility. *Pharmaceutics* 11(3): 102.

Ranjan, A. & Jha, P.K. 2022. Recent advances in dissolution testing and their use to improve *in vitro–in vivo* correlations in oral drug formulations. *Journal of Pharmaceutical Innovation* 17(3): 1011-1026.

Sathisaran, I. & Dalvi, S.V. 2018. Engineering cocrystals of poorly water-soluble drugs to enhance dissolution in aqueous medium. *Pharmaceutics* 10(3): 108.

Savjani, K.T., Gajjar, A.K. & Savjani, J.K. 2012. Drug solubility: Importance and enhancement techniques. *ISRN Pharm*. 2012: 195727.

Shakeel, F., Haq, N., Alanazi, F.K. & Alsarra, I.A. 2014. Thermodynamic modeling for solubility prediction of indomethacin in self-nanoemulsifying drug delivery system (SNEDDS) and its individual components. *Drug Development and Industrial Pharmacy* 40(9): 1240-1245.

Shakeel, F., Haq, N., El-Badry, M., Alanazi, F.K. & Alsarra, I.A. 2013. Ultra fine super self-nanoemulsifying drug delivery system (SNEDDS) enhanced solubility and dissolution of indomethacin. *Journal of Molecular Liquids* 180: 89-94.

Shriniwas, Y., Sumit, P., Pankaj, S. & Anurag, B. 2014. Design & development of solid self micro-emulsifying osmotic drug delivery system for isradipine. *J. Drug Deliv. Ther*. 4(3-s): 28-41.

Song, D., Guo, J., Han, F., Zhang, W., Wang, Y. & Wang, Y. 2013. Establishment of an *in vitro* model of the human placental barrier by placenta slice culture and ussing chamber. *Biosci. Biotechnol. Biochem*. 77(5): 1030-1034.

Vandana, K.R., Raju, Y.P., Chowdary, V.H., Sushma, M. & Kumar, N.V. 2014. An overview on *in situ* micronization technique - An emerging novel concept in advanced drug delivery. *Saudi Pharm J*. 22(4): 283-289.

Villar-Martínez, M.D., Moreno-Ajona, D., Chan, C. & Goadsby, P.J. 2021. Indomethacin-responsive headaches - A narrative review. *Headache* 61(5): 700-714.

Vinarov, Z., Abdallah, M., Agundez, J.A.G., Allegaert, K., Basit, A.W., Braeckmans, M., Ceulemans, J., Corsetti, M., Griffin, B.T., Grimm, M., Keszthelyi, D., Koziolek, M., Madla, C.M., Matthys, C., McCoubrey, L.E., Mitra, A., Reppas, C., Stappaerts, J., Steenackers, N., Trevaskis, N.L., Vanuytsel, T., Vertzoni, M., Weitschies, W., Wilson, C. & Augustijns, P. 2021. Impact of gastrointestinal tract variability on oral drug absorption and pharmacokinetics: An UNGAP review. *European Journal of Pharmaceutical Sciences* 162: 105812.

Wang, L.Y., Yu Y.M.,, Jiang, F.B., Li, Y.T., Wu, Z.Y. & Yan, C.W. 2020. The first zwitterionic cocrystal of indomethacin with amino acid showing optimized physicochemical properties as well as accelerated absorption and slowed elimination: *in vivo*. *New Journal of Chemistry* 44(10): 3930-3939. https://doi.org/10.1039/c9nj06180k

Wang, W., Li, M., Yang, Q., Liu, Q., Ye, M. & Yang, G. 2020. The opposed effects of polyvinylpyrrolidone K30 on dissolution and precipitation for indomethacin supersaturating drug delivery systems. *AAPS PharmSciTech*. 21(3): 107. https://doi.org/10.1208/s12249-020-01647-7

Xi, Z., Zhang, W., Fei, Y., Cui, M., Xie, L., Chen, L. & Xu, L. 2020. Evaluation of the solid dispersion system engineered from mesoporous silica and polymers for the poorly water soluble drug indomethacin: *in vitro* and *in vivo*. *Pharmaceutics* 12(2): 144.

Yeh, K.C., Yeh, T.K., Huang, C.Y., Hu, C.B., Wang, M.H., Huang, Y.W., Chou, L.H., Ho, H.H., Song, J.S., Hsu, T., Jiaang, W.T., Chao, Y.S. & Chen, C.T. 2021. DBPR108, a novel dipeptidyl peptidase-4 inhibitor with antihyperglycemic activity. *Life Sci*. 278: 119574.

Zhang, W., Zheng, N., Chen, L., Xie, L., Cui, M., Li, S. & Xu, L. 2018. Effect of shape on mesoporous silica nanoparticles for oral delivery of indomethacin. *Pharmaceutics* 11(1): 4.

Zhao, W., Uehera, S., Tanaka, K., Tadokoro, S., Kusamori, K., Katsumi, H., Sakane, T. & Yamamoto, A. 2016. Effects of polyoxyethylene alkyl ethers on the intestinal transport and absorption of rhodamine 123 : A P-glycoprotein substrate by *in vitro* and *in vivo* studies. *J. Pharm. Sci*. 105(4): 1526-1534.

*Corresponding author; email: khuriah@uitm.edu.my