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# The Effect of Surfactants and pH Modifying Agents on the Dissolution and Permeation of Pimobendan

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**Abstract**

Solubility and permeability are key parameters for establishing in vitro-in vivo correlation for poorly water-soluble active pharmaceutical ingredients (APIs). Recent studies demonstrate that not only solubility, but also effective permeability of the API may change due to the addition of solubilizing agents, and there is a certain mathematical relation between these physicochemical parameters. The aim of this study was to show the importance of early screening of solubility and permeability in presence of additives in order to achieve the expected bioavailability of the API. In this work, the effect of surfactants and microenvironmental pH modifiers were in focus, and pimobendan was chosen as model drug.

In the case of pH modifiers, the equilibrium solubility of the API increased, while the permeability decreased significantly. No negative effect was observed for two surfactants at low additive levels, but these two additives also exhibited a slightly negative effect on permeability when used at higher concentrations. In the simultaneous dissolution-permeation studies the surfactants-containing formulation was found to have slightly higher flux than the pH-modifier-containing one. It can be due to the phenomenon that the dissolution of the active substance can be enhanced by these surfactants without any significant permeability reducing effect.

The results obtained from the present study clearly demonstrate the importance of studying drug-additive interactions in every step of formulation development and based on these, the selection of the appropriate quality and quantity of additives. In addition, the results also underline the significance of performing simultaneous dissolution-permeation studies to predict bioavailability.

**Keywords**

pimobendan, dissolution, permeation, absorption, flux, bioavailability

## 1 Introduction

Solubility and permeability are the two key parameters for the correlation of *in vitro* dissolution and *in vivo* bioavailability results of pure active pharmaceutical ingredients (APIs). In case of poorly water-soluble APIs, the solubility might be altered when using solubilizing agents or formulating amorphous solid dispersions [1–3]. Recent studies demonstrate that not only solubility, but also effective permeability ($P_{eff}$) of the API may change due to the addition of formulation additives [4], and also there is a certain mathematical relation between the two parameters [5–7].

Dahan et al. [8] and Beig et al. [9] investigated this phenomenon called solubility-permeability interplay quite thoroughly and found that the equilibrium solubility is inversely proportional to the intestinal permeability *in vitro* (measured with lipophilic membrane) and *in vivo* as well.

They categorized the studied formulation additives based on their mechanism of solubilization. Although the effect of natural (bile salts) and artificial surfactants (e.g., sodium lauryl sulfate) on dissolution and permeation has been extensively studied *in vitro* and results showed that in case of

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improved solubility decreased permeability was observed [10–14], the first data proving the existence of solubility-permeability interplay *in vivo* was only published in 2015 [15].

Microenvironmental pH-modification is an exceptional solution for improving the dissolution behavior of APIs with pH-dependent solubility [16, 17]. Although the total concentration of the API in solution might be increased, the ionization of the molecules can hinder permeation. Namely, only the uncharged form of the ionizable molecule is able to permeate by passive diffusion efficiently [18, 19].

For poorly water-soluble drugs a number of examples demonstrate that only dissolution data is not enough to predict the *in vivo* behavior of the API. For that reason, the dissolution test-based biowaivers are not authorized for generic formulations containing poorly water-soluble APIs. Recently, many international research co-operations were established between industry, academia and regulatory agencies to improve the predictive power of *in vitro* tests [20–23]. As a result, different combination of dissolution and permeation assays were developed and optimized. These were shown to be valuable tools in understanding the *in vivo* processes as well as improving the *in vivo* predictive power of *in vitro* tests [24, 25]. Recently, a new approach, called flux-based formulation development has been published: this concept uses not only dissolution and solubility but also simultaneous dissolution-permeation assays in each step of the formulation development from the selection of excipients to the testing of final dosage forms. By using this concept the interplay between solubility and permeability can be monitored constantly during formulation development [26].

Pimobendan (PIMO) is a BCS II classified veterinary drug [27] that was selected as a model poorly water-soluble compound. Fig. 1 shows the structure of PIMO, it is a basic drug with a $pK_a$ of 4.3, and as a free base, it has an *n*-octanol/buffer (pH 7.4) partition coefficient of log*P* = 3.2. [28] PIMO is used in the management of heart failure in dogs. There are several products on the market containing PIMO under the brand names Vetmedin, Cardisure, Safeheart, Pimocard, Pimotab, Zelys and Fortekor Plus out of which Vetmedin is the originator brand. Fortekor Plus is a combination of benazepril and PIMO. Remarkably, PIMO has been shown to have fewer side effects in dogs than its main rival drugs, the ACE inhibitors benazepril (brand names Lotensin, Fortekor) and enalapril maleate (brand names Enacard, Vasotec) [29].

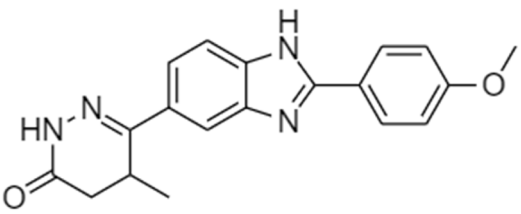



**Fig. 1** Structure of Pimobendan

The aim of this study was to show the importance of early screening of solubility and permeability in presence of additives in order to achieve the expected bioavailability of the API. In this work, the effect of surfactants and microenvironmental pH modifiers were in focus and PIMO as a poorly water-soluble API was chosen as model drug.

## 2 Materials and methods

### 2.1 Materials

Pimobendan (PIMO, 334 g/mol) was received from Lavet Ltd. (Kistarcsa, Hungary). Two PIMO brand tablets (in the following Product-A and Product-B, see list of excipients in Table 1) were obtained from commercial trade. Phosphoric acid (85%), monosodium phosphate, and disodium phosphate were purchased from Molar Chemicals Ltd. (Halásztelek, Hungary) for the dissolution medium, hydrochloric acid (37%) from Sigma-Aldrich Co. Llc. (St. Louis, MO, USA) and sodium chloride from Merck Ltd. (Budapest, Hungary). Monosodium phosphate for the chromatographic measurement was purchased from Molar Chemicals Ltd. (Halásztelek, Hungary), sodium chloride, and sodium hydroxide from Merck Ltd. (Budapest, Hungary), acetonitrile from Sigma-Aldrich Co. Llc. (St. Louis, MO, USA). Buffer components for the dissolution-permeation measurements ($NaH_2PO_4 \cdot H_2O$, $KH_2PO_4$, NaCl, KCl, HCl) were purchased from Sigma-Aldrich Co. Llc. (St. Louis, MO, USA). Formulation additives (SDS, Macrogol 6000, citric acid, malic acid, tartaric acid) were purchased from Sigma-Aldrich Co. Llc. (St. Louis, MO, USA). Gelucire 50/13 pellets were purchased from Gattefossé SAS (Saint-Priest, France). (2-Hydroxypropyl)-$\beta$-cyclodextrin (HP-$\beta$-CD),

**Table 1** List of excipients

| Product-A | Product-B |
|---|---|
| Lactose monohydrate | Lactose monohydrate |
| Microcrystalline cellulose | Microcrystalline cellulose |
| Starch, pregelatinised | Maize starch |
| Dried yeast | Dried yeast |
| Liver powder flavour | Pig liver powder |
| Magnesium stearate | Stearic acid |
| Talc | Silica colloidal anhydrous |
| Sodium starch glycolate (Type A) | Croscarmellose sodium |
| Stearoyl macrogolglycerides (Gelucire) | Copovidone |
| Macrogol 6000 (PEG 6000) | Malic acid |

with a molar substitution degree of 0.64, was provided by Roquette Freres (Lestrem, France). GIT lipid was obtained from Pion Inc. (Billerica, MA, USA).

### 2.2 Solubility measurements of PIMO

The pH-dependent equilibrium solubility of PIMO was studied at 37 °C, pH 1.2, pH 3.0, pH 4.5, pH 6.5, and pH 7.5 phosphate buffer. 1000 mL of the buffers contained 27.8 g monosodium phosphate and 4.00 mL disodium phosphate solution, the pH of the buffer was adjusted with 1M HCl and 1M NaOH. For measuring the equilibrium solubility, crystalline PIMO (10 mg) was added to 10 mL pH 4.5 phosphate buffer in the presence or absence of formulation additives. Three surfactants and three pH-modifying agents were tested as formulation additives, because among the products on the market, Product-A contains surfactants and Product-B comprises a pH modifier (see Table 1). The resulting mixtures were stirred at 37 °C for 24 h (to the solution equilibrium). The concentration of the API in the buffers was determined without filtering the solutions by the Rainbow Dynamic Dissolution Monitor instrument (Pion Inc., Billerica, MA) using UV calibration.

### 2.3 Dissolution equipment and method

HANSON Vision® G2 Elite 8™ dissolution tester equipped with an autosampler was used for the dissolution tests. Pall GHP Acrodisc 25 mm Syringe Filter with 0.2 µm GHP Membrane was used during the sampling. The tests were accomplished at 37 °C with 75 rpm paddle speed (apparatus 2). The volume of dissolution media was 1000 mL and the sampling points were at 10, 20, 30 and 45 minutes. The tablets were tested at pH 1.2, 3.0, 4.5, 6.8 and 7.5 with 12 units (the preparation of the dissolution medium is summarized in Table 2). During the dissolution tests, a 1.5 mL sample was directly filled into HPLC vials via autosampler, and the sample was directly injected into the UHPLC without dilution. Purified water was used for the preparation of the dissolution medium.

#### 2.3.1 Determination of dissolution rate

Agilent 1290 UHPLC chromatographic system equipped with Diode Array Detector was used for the detection and quantification of API in the dissolution medium. Phosphate buffer contains 0.60 g monosodium phosphate and 0.81 g sodium chloride dissolved in MilliQ water. The pH of the buffer was adjusted with 5 N sodium hydroxide solution to 7.2. Data acquisition and processing were performed by Agilent OpenLAB 2 CDS (chromatography data system). Table 3 contains the applied chromatographic method.

### 2.4 Permeability measurements with PAMPA

The parallel artificial membrane permeability assay (PAMPA) method was used as previously described by Avdeef and Tsinman [18] by applying the PAMPA Evolution instrument (Pion Inc.). PAMPA "sandwiches" were formed from a donor 96-well microtitre plate and a matching filter plate (Millipore Corp., Bedford, MA, USA) with apparent porosity of 0.76, coated with 4 µL of GIT lipid (Pion Inc. [30]). The initial donor sample concentrations were about 5 µg/mL. Phosphate buffer pH 4.5 was used at the donor side (preparation sees in Table 2) and phosphate-buffered saline pH 7.4 was used at the acceptor side as receiver buffer, which was prepared by mixing 137 mM NaCl, 2.7 mM KCl, 10 mM $Na_2HPO_4$, 1.8 mM $KH_2PO_4$. Receiver buffer containing 20% HPBCD (according to the

**Table 3** Chromatographic method

| | |
|---|---|
| Eluent | 28 v/v % acetonitrile, 72 v/v % phosphate buffer |
| Separation mode | isocratic |
| Analytical column | Kinetex C18 50 mmx3 mm, 1.7 µm particle size |
| Column temperature | 45 °C |
| Detection wavelength | 290 nm |
| Eluent flowrate | 0.4 mL/min |
| Injection volume | 2 µL |
| Run time | 3 min |

**Table 2** Preparation of the different dissolution mediums (for 1000 mL)

| Reagent | pH value | | | | |
|---|---|---|---|---|---|
| | 1.2 | 3.0 | 4.5 | 6.8 | 7.5 |
| Phosphoric acid (85%) | - | 0.74 mL | - | for pH adjustment | - |
| Monosodium phosphate | - | 10.68 g | 27.80 g | 1.20 g | 1.20 g |
| Disodium phosphate solution (0.2 M, self-made from the solid salt) | - | - | 4.00 mL | - | - |
| Disodium phosphate | - | - | - | 0.89 g | 0.89 g |
| Sodium hydroxide solution (5 N, self-made from the solid salt) | - | - | - | - | for pH adjustment |
| Hydrochloric acid (37%) | 8.50 mL | - | - | - | - |
| Sodium chloride | 2.93 g | - | - | - | - |

manufacturer [30] of the PAMPA plate and GIT lipid, this receiver solution is compatible with the GIT lipid solution and does not cause any changes in the integrity of the membrane within 6 hours) was used to ensure sink condition on the acceptor side of the membrane. The solubility of PIMO was measured in this solution and found to be 532.81 ± 32.21 µg/mL, showing that this solution is able to effectively solubilize PIMO and ensure sink condition [31] for this system. The Pion Gut-Box™ was used to effect individual well magnetic stirring. The plate sandwich was allowed to incubate in the Gut-Box at 37±1 °C for 1h with vigorous stirring. Afterwards, sample concentrations in both the acceptor and donor wells were determined by UV plate spectrophotometry. Effective permeability coefficients ($P_e$) were determined by taking into account the apparent filter porosity [32] and sample mass balance [33].

### 2.5 Small volume dissolution-permeation measurements with MicroFLUX apparatus

Final dosage forms of PIMO were tested using µFLUX (Pion Inc., Billerica MA, USA) apparatus which consists of a donor and an acceptor chamber separated by an artificial membrane (PVDF, polyvinylidene-fluoride, 0.45 µm, 1.54 $cm^2$) impregnated with 25 µL GIT lipid to form a lipophilic barrier between the donor and acceptor chambers. 18 mL pH 4.5 buffer was in the donor chamber, which was a discriminative pH in case of dissolution studies. The 18 mL pH 7.4 buffer of the acceptor chamber represents the blood circuit and the pH of the gastrointestinal cells. Preparation of the pH 4.5 buffer is presented in Table 2. Phosphate-buffered saline pH 7.4 was prepared by mixing 137 mM NaCl, 2.7 mM KCl, 10 mM $Na_2HPO_4$, 1.8 mM $KH_2PO_4$ and contains 20% HP-$\beta$-CD. On the donor side, the appropriate amount of the powdered tablet was given to achieve the target concentration of 5 µg/mL. Both chambers were stirred at 250 rpm at 37 °C to keep the thickness of the unstirred aqueous layer at minimum. In both chambers, the API concentration was followed by immersed UV probes connected to the Rainbow instrument (Pion Inc., Billerica MA, USA). In order to compare the absorption of the formulations, the flux across the membrane was calculated from the slope of the lines using the Eq. (1) as follows:

$$J(t) = \frac{\Delta n}{A \times \Delta t}, \quad (1)$$

where is the flux ($J$), the amount of active substance ($n$) per unit area ($A$) per unit time ($t$).

## 3 Results and discussion

The results of the equilibrium solubility measurements were used to determine the pH-solubility profile of PIMO and the solubility of PIMO in the presence of different additives in different quantities. The lower additive concentrations correspond to the amounts of additives released from the tablet in 1000 mL of dissolution medium in dissolution studies. The higher additive concentration is ten times the lower additive concentration that may be closer to the biorelevant conditions in dogs. PAMPA measurements were used to investigate the effects of formulation additives on PIMO's permeability. Two commercially available products containing the active pharmaceutical ingredient PIMO were tested by dissolution measurements at five buffers with different pHs. The formulation of Product-A contains Macrogol 6000 and Gelucire as solubilizing additives to enhance dissolution. Product-B contains malic acid to enhance dissolution with microenvironmental pH modification. A simultaneous dissolution-permeation study was carried out in the most discriminating buffer to investigate the bioavailability of the different formulations.

### 3.1 Solubility-pH profile of PIMO

The solubility of PIMO varies significantly as a function of pH (Fig. 2). It can be observed that at lower pH, called salt range, as expected, the measured solubility values start to deviate slightly from the theoretical Henderson-Hasselbalch curve. The results of equilibrium solubility of PIMO in different dissolution media are shown in Table 4.

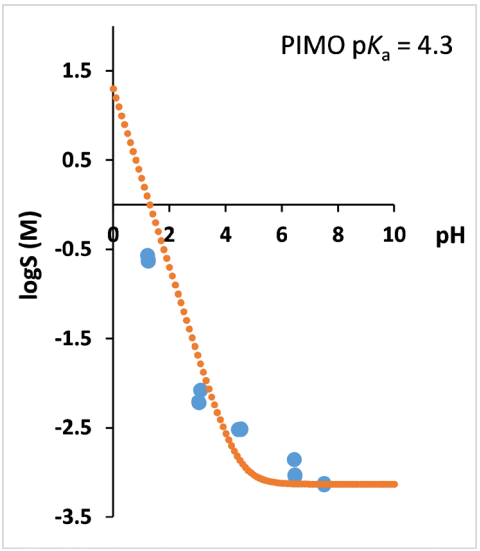



**Fig. 2** Solubility-pH profile of PIMO (logarithm of measured solubility values with blue points, theoretical solubility determined by the Henderson-Hasselbalch equation)

### 3.2 Equilibrium solubility measurements with additives

The results of the equilibrium solubility measurements in different additive systems are shown in Table 5. If the donor medium represents the concentration of additive released from the formulation in the standard dissolution study of tablets on the market, the equilibrium solubility increased significantly in the presence of SDS and tartaric acid, and slightly in the presence of citric acid and malic acid.

### 3.3 Dissolution measurements

Two oral formulations for dogs with the same active pharmaceutical ingredient but different formulation additives (Table 1) were tested with standard dissolution study. There is a significant difference between the dissolution curves (Fig. 3) of the two formulations on the market in buffers of different pH. The dissolution profiles fit in the solubility-pH profile of PIMO, the dissolution is better at lower pH. In the dissolution measurements of PIMO products containing 5 mg API, the dissolution rate of both products was above 90% after 20 min in pH 1.2. For pH 3.0 and pH 4.5 buffers that can represent the pH of the gastric juice and the intestinal content at the proximal end of the small intestine [34], a difference was observed in the dissolution profiles of the two formulations. The release of Product-A containing Macrogol 6000 and Gelucire formulation additives is faster and higher than the release from Product-B formulation containing malic acid. For pH 6.8 and pH 7.5 buffers that represent the pH of the lower parts of the small intestine [34], a lower dissolution rate of about 70–80% dissolution was observed after 45 min.

Based on the results of the dissolution tests, pH 4.5 media was found to be discriminative for the tested formulations. Considering the pKa value (4.3) of PIMO and the pH partitioning hypothesis, it is also a pH where passive diffusion is a fast and efficient way of absorption, therefor this media was selected as a donor solution for permeability and dissolution-permeation tests.

**Table 4** Equilibrium solubility of PIMO in different dissolution media

| buffer pH | equilibrium solubility of PIMO [μg/mL] |
|---|---|
| pH 1.2 | 83.25 ± 6.76 |
| pH 3.0 | 2.29 ± 0.45 |
| pH 4.5 | 1.04 ± 0.06 |
| pH 6.5 | 0.36 ± 0.09 |
| pH 7.5 | 0.25 ± 0.01 |

**Table 5** Equilibrium solubility of PIMO in presence of different additives

| additive | additive concentration [μg/mL] | pH | equilibrium solubility of PIMO [μg/mL] | *t*-test (*p*-value) |
|---|---|---|---|---|
| pure PIMO | - | 4.5 | 1.04 ± 0.06 | - |
| SDS | 84 | 4.5 | 2.97 ± 0.09 | **0.00** |
| Macrogol 6000 | 36 | 4.5 | 1.09 ± 0.09 | 0.43 |
| Gelucire | 12 | 4.5 | 0.98 ± 0.09 | 0.30 |
| citric acid | 100 | 4.3 | 1.17 ± 0.14 | 0.15 |
| malic acid | 100 | 4.3 | 1.12 ± 0.08 | 0.23 |
| tartaric acid | 100 | 4.2 | 1.51 ± 0.16 | **0.00** |

### 3.4 Permeability measurements with PAMPA

The effective permeability values in buffers with different additives obtained from PAMPA measurements are shown in Fig. 4 and Fig. 5, respectively. As can be seen clearly from these figures, different formulation additives reduce the effective permeability to different extents. It is very striking that SDS, even in a small amount, significantly reduces the effective permeability. Student t-test was performed to investigate the effect of different additives on permeability. The results obtained are shown in Table 6. The results suggest that in presence of a lower amount of formulation additives the effect of Macrogol 6000 and Gelucire as solubilizing additives is not significant. However, for the pH modifiers all effects were found to be significant. Malic acid and citric acid exhibited almost the same pattern, whereas tartaric acid was seen to reducing the effective permeability even more expressed. Since permeability measurements are high throughput assays, not only the effect of additive's quality but also its quantity were evaluated. In case of using higher amounts of additives, all the tested additives, except Macrogol 6000, were found to have a significant negative effect on the permeability, and Macrogol 6000 was also on the borderline. The results of the PAMPA correspond well with the results of the equilibrium solubility measurements and it clearly demonstrates the solubility-permeability interplay.

### 3.5 Small volume dissolution-permeation measurements with MicroFLUX apparatus

The two oral formulations investigated with dissolution measurements, Product-A and Product-B were also tested with simultaneous dissolution-permeation measurements in pH 4.5 buffer. The aim of this study was to investigate whether there is any difference in permeation between the two products. Fig. 6 shows that the drug concentration on the donor side did not reach the target concentration of 5 μg/mL for any of the formulations. The Product-A

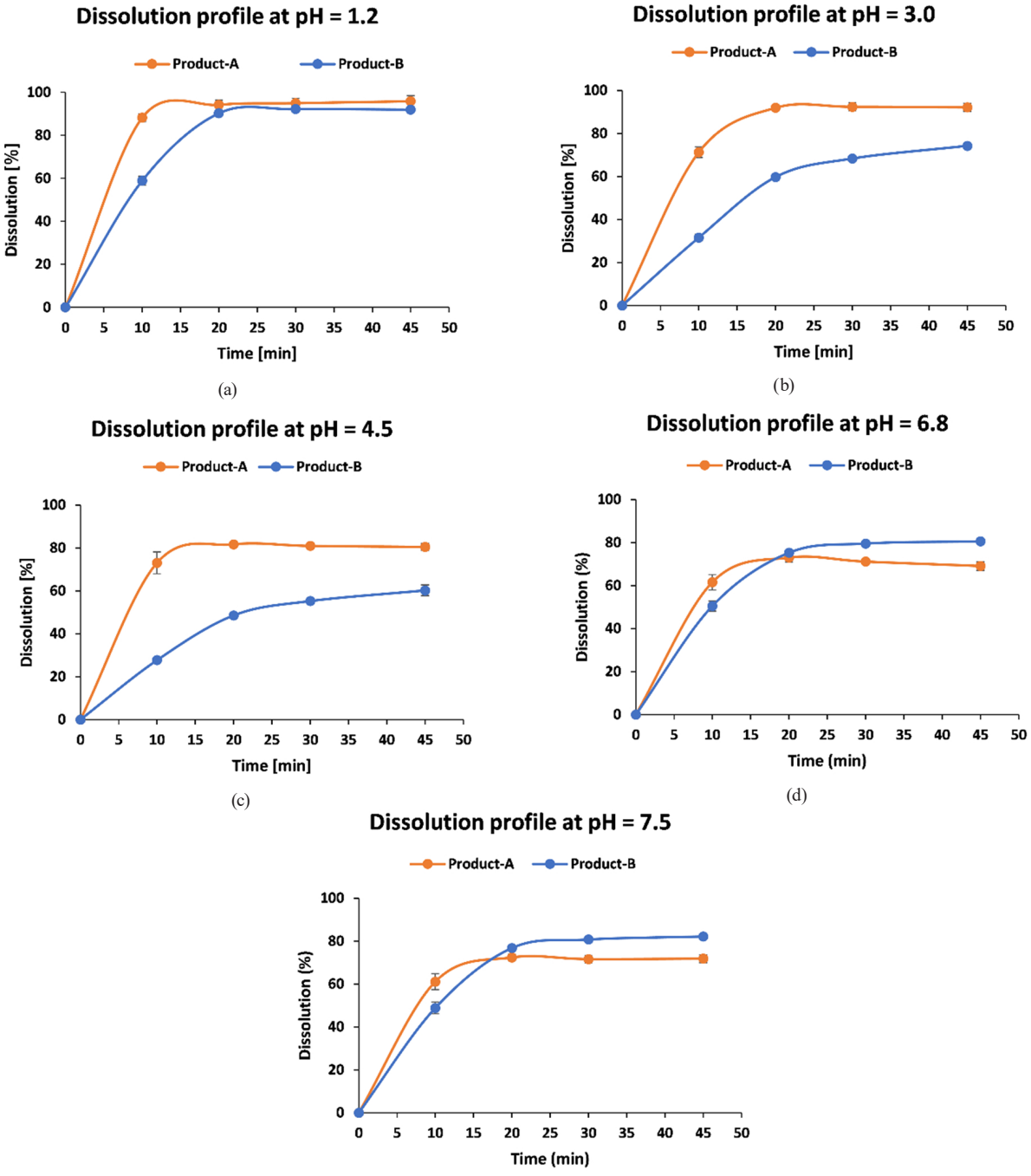



**Fig. 3** Standard dissolution curves of Product-A and Product-B in buffers of different pHs (target concentration = 5 µg/mL)
(a) pH = 1.2; (b) pH = 3.0; (c) pH = 4.5; (d) pH = 6.8; (e) pH = 7.5

formulation achieves a higher concentration (1.9 µg/mL) in 2 hours at the donor side than Product-B (1.4 µg/mL). On the acceptor side, it is observed that the slope of the line corresponding to Product-A is greater than Product-B. The flux results are given in Table 7. As can be seen, between 60 and 120 min, where the substantial absorption occurs, a significant difference in flux values was obtained between the two formulations. This result is in agreement with the results obtained from the effective permeability measurements. Namely, the negative effect observed in the PAMPA measurements for the formulation Product-B containing malic acid could also be detected in the flux measurements. While no negative effect on absorption was observed for the formulation containing Macrogol 6000 and Gelucire, higher flux values were obtained for the Product-A formulation.

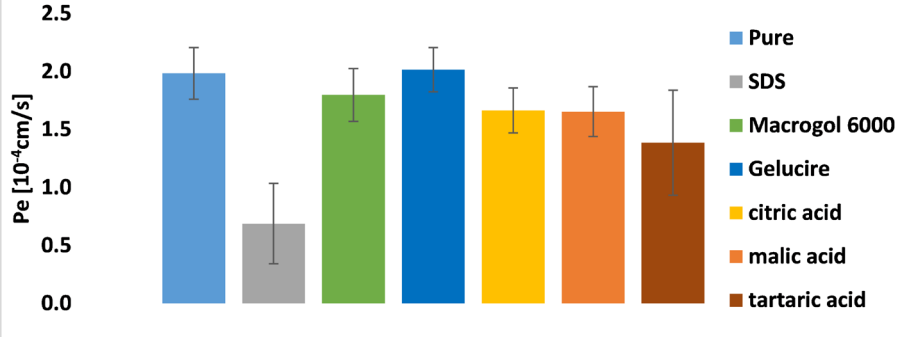



**Fig. 4** Effective permeability values of PIMO at lower additive concentrations

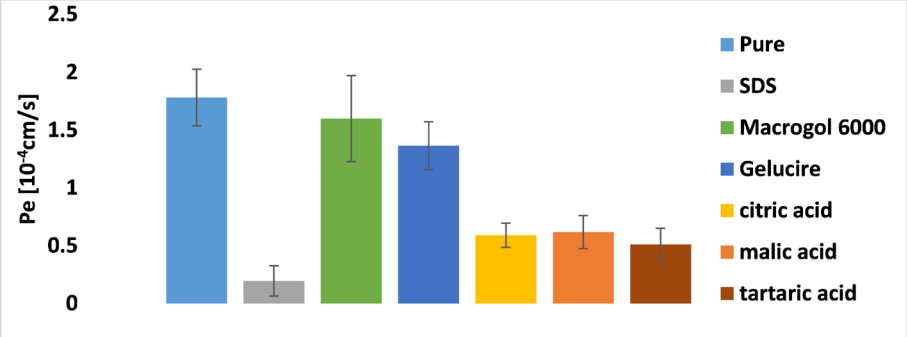


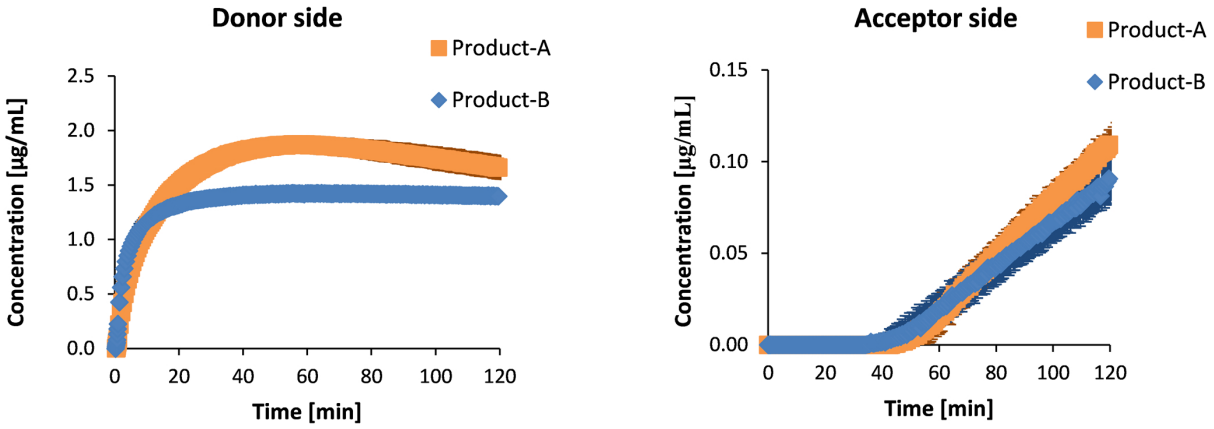

**Fig. 5** Effective permeability values of PIMO at higher additive concentrations

**Table 6** Results of PAMPA for different additives

| Type of additive | Additive | Additive concentration (μg/mL) | pH | $P_e$ | $P_e$ SD | Reference $P_e$ | Reference $P_e$ SD | *t*-test (*p*-value) |
|---|---|---|---|---|---|---|---|---|
| Surfactants | **SDS** | **84** | **4.54** | **0.90** | **0.22** | **1.98** | **0.22** | **0.00** |
| | | **840** | **4.55** | **0.20** | **0.13** | **1.78** | **0.25** | **0.00** |
| | Macrogol 6000 | 36 | 4.54 | 1.80 | 0.23 | 1.98 | 0.22 | 0.06 |
| | | 360 | 4.55 | 1.60 | 0.37 | 1.78 | 0.25 | 0.05 |
| | Gelucire | 12 | 4.54 | 2.01 | 0.19 | 1.98 | 0.22 | 0.71 |
| | | **120** | **4.55** | **1.36** | **0.21** | **1.78** | **0.25** | **0.00** |
| pH modifiers | **citric acid** | **100** | **4.39** | **1.66** | **0.19** | **1.98** | **0.22** | **0.00** |
| | | **1000** | **3.72** | **0.59** | **0.11** | **1.78** | **0.25** | **0.00** |
| | **malic acid** | **100** | **4.37** | **1.65** | **0.21** | **1.98** | **0.22** | **0.00** |
| | | **1000** | **3.71** | **0.62** | **0.14** | **1.78** | **0.25** | **0.00** |
| | **tartaric acid** | **100** | **4.32** | **1.55** | **0.18** | **1.98** | **0.22** | **0.00** |
| | | **1000** | **3.58** | **0.51** | **0.14** | **1.78** | **0.25** | **0.00** |

## 4 Conclusions

The development of the final dosage form for APIs with poor water solubility but good permeability poses significant challenges. These challenges include investigating the simultaneous effects of dissolution- enhancing additives on dissolution and absorption. In our work, two formulations containing the same API but different formulation additives were tested for the potential effects of additives on solubility and permeability. In equilibrium solubility measurements with a lower concentration of formulation additives, an increase in solubility was observed in the presence of SDS and pH modifiers. Consistent with these measurements, PAMPA studies showed that the tested pH modifiers: citric acid, malic acid and tartaric acid significantly reduced the effective permeability of PIMO. SDS, an additive belonging to the group of surfactants, had the greatest negative effect on the effective permeability. No negative effect was observed for Macrogol 6000 and Gelucire, also belonging to this group, at low additive levels, but these two additives also exhibited a slightly




(a) (b)

**Fig. 6** Concentration curves at donor and acceptor side in case of Product-A and Product-B formulation; (a) Donor side; (b) Acceptor side

**Table 7** Flux values

| | Flux (60–120 min) | SD | *p*-value |
|---|---|---|---|
| Product-A | 0.01792 | 0.00067 | **0.008** |
| Product-B | 0.01364 | 0.00135 | |

negative effect on permeability when used at higher concentrations. In line with the results of the solubility and permeability measurements, in the dissolution-permeation studies, the Macrogol 6000 and Gelucire-containing formulation (Product-A) was found to be slightly better than the pH-modifier-containing one (Product-B). It can be due to the phenomenon that in the tablets of Product-A, the dissolution of the active substance is enhanced by the surfactants without any significant permeability reducing effect at the additive concentrations used in the formulation.

In conclusion, the results obtained from the present study clearly demonstrate the importance of studying drug-additive interactions in formulation development and based on these, the selection of the appropriate quality and quantity of additives. In addition, the results also underline the significance of performing simultaneous dissolution-permeation studies to predict bioavailability.

## Acknowledgement

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