Formulation Strategies to Improve Drug Absorption: The Role of Lipid-Based Formulations and Permeation Enhancers

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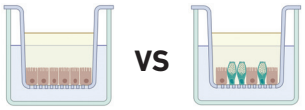
INTRODUCTION

Lipid-based formulations (LBFs) are a key strategy to **enhance oral absorption** of poorly soluble and permeable compounds (**BCS II–IV**), but their development requires **predictive in vitro models** reflecting gastro-intestinal physiology[1]. **Ticagrelor**, a BCS IV antiplatelet drug, has **low solubility and permeability**, making it suitable to evaluate LBFs performance.
Intestinal permeability is usually assessed with **Caco-2 monolayers**[2], yet they **lack physiological features** such as **mucus**, which affects absorption and barrier function. **Caco-2/HT29-MTX co-cultures** introduce a **mucus layer**, improving model relevance[3].
This study formulates LBFs using **excipients with different functionalities** and compares ticagrelor permeability in Caco-2 monolayers versus Caco-2/HT29-MTX (70:30) co-cultures to assess **the impact of mucus** and **how lipid excipients modulate intestinal permeability**.



MATERIALS & METHODS

Table 1. Lipid-based formulations composition.

LBFs	Composition	Functionalities
F1	100% Labrasol® ALF	Permeation enhancer and solubilizer
F2	70% Labrasol® ALF 30% Labrafac™ MC60	Permeation enhancers and solubilizers
F3	70% Tween® 80 30% Maisine® CC	Solubilizer Lymphatic transport enhancer
F4	70% Labrasol® ALF 30% Maisine® CC	Permeation enhancer and solubilizer Lymphatic transport enhancer
F5	100% Transcutol® HP	Solvent
-	Pure API	Reference

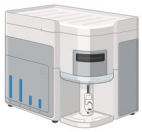
Cytotoxicity: cytotoxicity studies were conducted to assess the toxicity of the lipid-based formulations. Analyses were performed using a **flow cytometer** (Attune™ NxT Flow Cytometer, Thermo Fisher Scientific, USA). Briefly, a cell suspension at a density of **5×10⁴ cells per well** was seeded in 12-well plates. For each condition, cells were treated with the corresponding sample (pure ticagrelor, placebo, or ticagrelor in LBFs) diluted in Hank's Balanced Salt Solution (HBSS) for 4 hours at 37 °C.

Permeability: cells were seeded on 12-well Transwell® inserts at **5×10⁴ cells/mL** and cultured for **21 days**, refreshing medium every two days. Only monolayers with **TEER > 500 Ω·cm² (monoculture)** or **> 300 Ω·cm² (co-culture)** were used. LBFs were formulated at **80 mg/g and diluted 1:8000 in HBSS** before apical addition. Samples were collected at 15, 30, 60, 120, and 180 min and analyzed by **LC/MS**.

Materials: ticagrelor was obtained from Chanyoo Pharmaceutical Co., Ltd. (Nantong, Jiangsu, China). Tween® 80 was purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). Labrasol® ALF, Transcutol® HP, Labrafac™ MC60 and Maisine® CC were kindly provided by Gattefossé (Saint-Priest, France).

RESULTS

Cytotoxicity



0.125 µL/mL placebo LBF maximum concentration

10 µg/mL pure ticagrelor maximum concentration

LBFs 80 mg/g diluted 8000 fold

Permeability monoculture

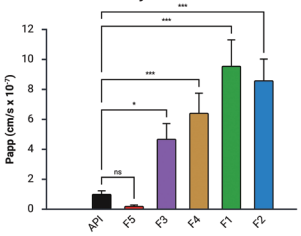


Figure 1. P_{app} of ticagrelor pure or in LBFs in monoculture. Values are expressed as mean ± SD (n=3). ns=not significant, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 (One-way ANOVA).

In both Caco-2 monoculture and Caco-2/HT29-MTX co-culture (Figures 1 and 2), ticagrelor LBFs showed a **similar** permeability ranking. **Pure ticagrelor exhibited low P_{app}** (1.02×10^{-7} cm/s in monoculture, 1.25×10^{-7} cm/s in co-culture). The Transcutol® HP-only formulation **(F5)** showed the **lowest permeability**, reflecting its limited ability to maintain solubilization, despite its solvent capacity. **LBFs without permeation** enhancers **(F3)** provided **modest improvement**, while **replacing Tween® 80 with Labrasol® ALF (F4) increased P_{app}** due to **Labrasol® ALF permeation-enhancing** effect.

Permeability co-culture

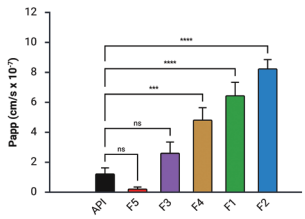


Figure 2. P_{app} of ticagrelor pure or in LBFs in co-culture. Values are expressed as mean ± SD (n=3). ns=not significant, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 (One-way ANOVA).

Formulations containing **only permeation enhancers (F1, F2)** showed the **greatest improvement**: in monoculture, P_{app} increased **9.4- and 8.4-fold**, and in co-culture, **5.2- and 6.6-fold**, respectively.
Overall, **permeability was lower in the co-culture** (Figure 3), however, this difference **was not statistically significant** and should be interpreted only as an **observed trend**. This tendency may be related to the **patchy mucus layer** (Figure 4), which could increase local **viscosity** and partially limit the **diffusion** of ticagrelor and LBFs.

Permeability comparison

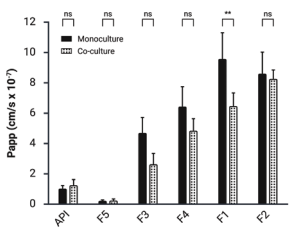


Figure 3. Comparison of P_{app} of ticagrelor pure or in LBFs in monoculture and co-culture. Values are expressed as mean ± SD (n=3). ns=not significant, **P<0.01, (Two-way ANOVA).

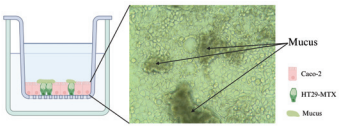


Figure 4. Schematic representation and microscopic image of mucus areas on the Caco-2/HT29 MTX co-culture monolayer.

CONCLUSION

- This study is the **first** to **assess ticagrelor LBFs permeability using a Caco-2/HT29-MTX co-culture**, which better mimics intestinal physiology, and highlights the influence of the mucus layer on apparent permeability.
- Across both models, **LBFs enhanced ticagrelor absorption** even at **low excipient concentration. Conventional solubilizing excipients increased P_{app} versus the pure API, while dual-functional permeation enhancers produced an additional permeability gain.**
- Compared with Caco-2 monocultures, the **co-culture** generally showed **lower permeability**, consistent with the **diffusional barrier** imposed by the **mucus layer**.

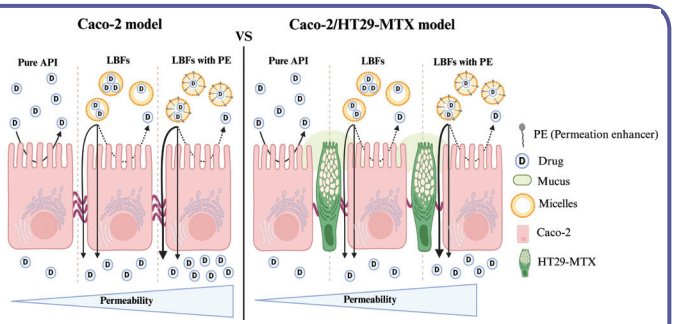


Figure 5. Comparison of P_{app} of ticagrelor pure or in LBFs in monoculture and co-culture.

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