Liposomal Fluorescence Assay: Influence of the Ionization States on the Permeation

Dominik Schmid[1], Stefanie D. Krämer[1]
[1]Institute of Pharmaceutical Sciences, Department of Chemistry and Applied Bioscience, ETH Zurich, Switzerland

1 Introduction

Our *in vitro* liposomal fluorescence assay [1] provides a simple, well-defined and inexpensive method for the prediction of passive lipid bilayer permeation. The liposomal lumen is loaded with a pH-sensitive fluorescent dye 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS) and the outer phase is buffered using 2-(N-morpholino)ethanesulfonic acid (MES) for pH 6.0 or 3-(N-morpholino)propanesulfonic acid (MOPS) for pH 7.4. The MES buffer mimics condition of the duodenal lumen, whereas MOPS buffer allows to investigate extravasation and blood-brain barrier permeation of drug-like compounds. The permeation of an acidic or basic drug-like compound into the vesicle changes the luminal pH, which can be observed by the changes in fluorescence over time using a stopped-flow apparatus.

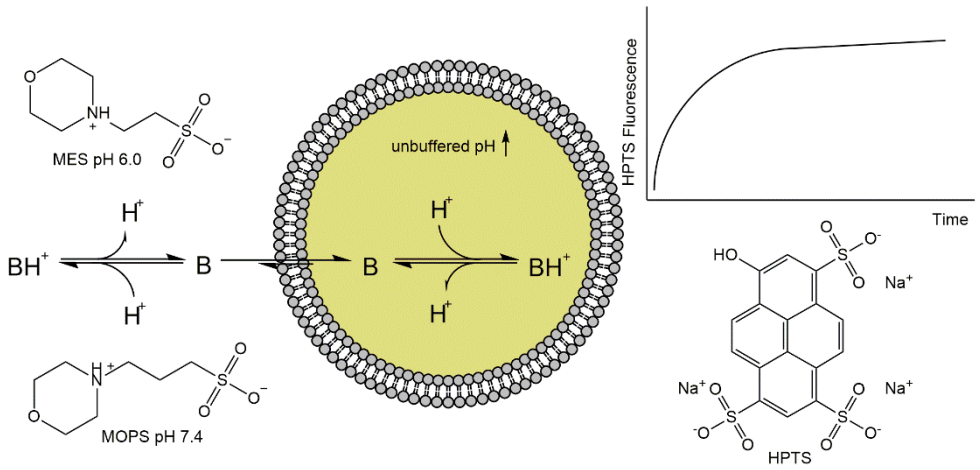

Figure 1. Principle of liposomal permeation assay for a base B. The graphic is adapted from Eyer K, et al. [2].

2 Goal

Our assay was challenged by the hypothesis that drug permeation is not the rate determining process by Sezer D, et al. [3]. In response, we investigated whether permeation kinetics follow a Henderson-Hasselbalch function for individual drugs. This would be further evidence that the observed kinetics are determined by drug permeation.

3 Methods

Unilamellar 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) liposomes containing HPTS were prepared by lipid film hydration followed by extrusion. After exchanging extra-liposomal HPTS with buffer pH 6 or 7.4 by size exclusion, the liposomes were mixed in a stopped-flow apparatus with basic or acidic drugs and the pH depending change in fluorescence was recorded.

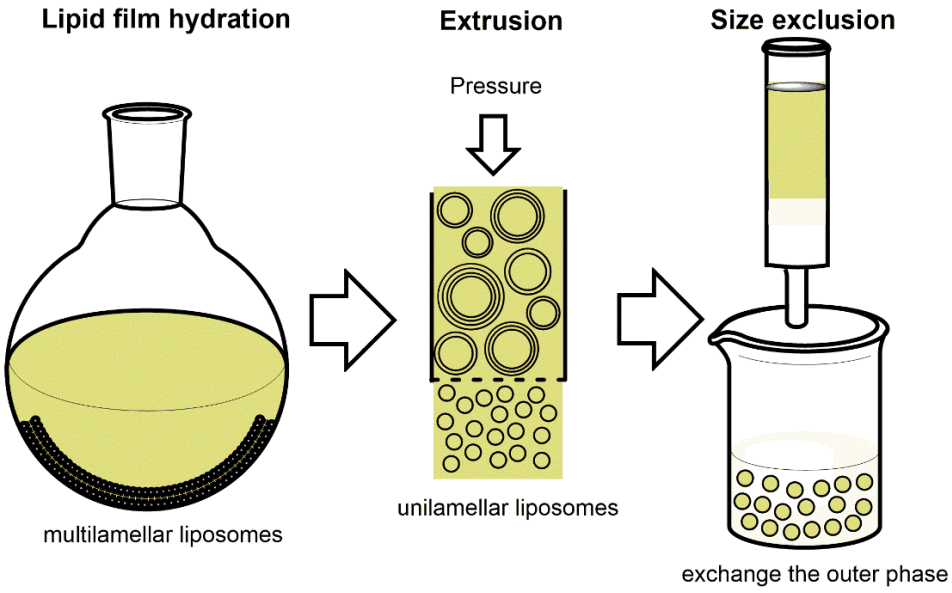

Figure 2. Preparation of unilamellar liposomes containing HPTS with a desirable size and size distribution.

4 Results and Discussion

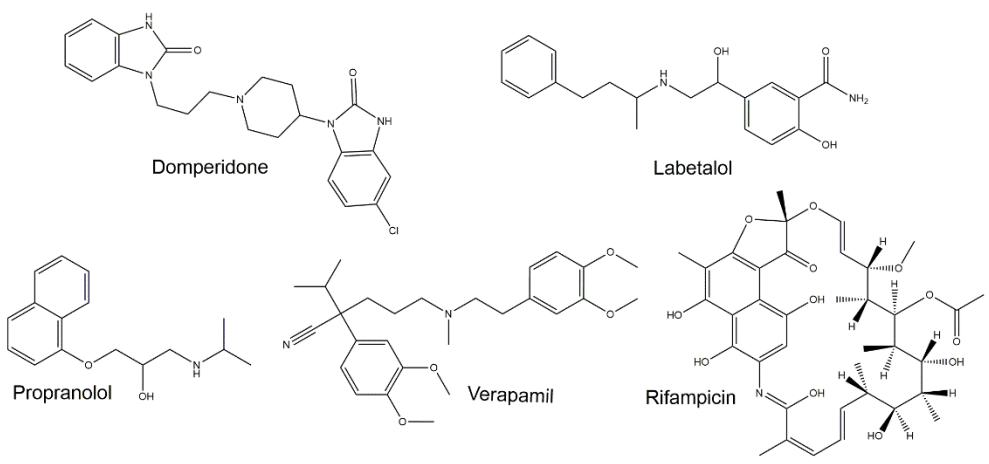

Figure 3. Chemical structures of the test compounds domperidone, labetalol, propranolol, rifampicin and verapamil.

Table 1. Liposomal permeation coefficient of the investigated drugs in MES and MOPS buffer.

Drug	pK_a [1]	$logP_{FLipP}$ (cm/s) in MES pH 6.0 [1]	$logP_{FLipP}$ (cm/s) in MOPS pH 7.4
Domperidone	7.9B	-5.19 ± 0.04	-4.81 ± 0.12 (5)
Labetalol	7.4A, 9.4B	-6.34 ± 0.15	-5.51 ± 0.02 (7)
Propranolol	9.5B	-3.87 ± 0.09	-2.97 ± 0.16 (4)
Rifampicin	1.7A, 7.9B	-4.04 ± 0.16	-4.65 ± 0.02 (7)
Verapamil	8.9B	-3.30 ± 0.10	-3.69 ± 0.34 (7)

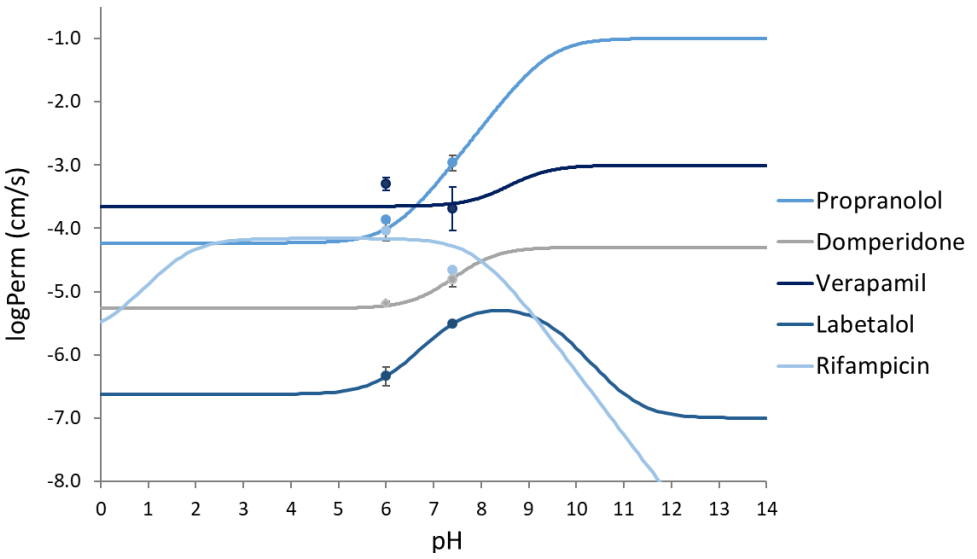

Figure 4. pH-dependent permeability. Symbols (●) indicate the experimental permeability coefficient at pH 6.0 [1] and pH 7.4. The lines show the simulated permeability according to the ionization states of the respective drugs.

5 Conclusion and Outlook

- With exception of verapamil, the measured permeation coefficient confirm the hypothesis, that the degree of ionization contribute strongly to the permeation behaviour, which is mainly mediated by neutral species.
- The observed permeation behaviours verify that drug permeation is the rate determining process and contradict the hypothesis of Sezer D, et al. [3]

6 References

[1] Hermann K, Neuhaus C, et al. (2017) Eur. J. Pharm. Sci. 104: 150-61
[2] Eyer K, Paech F, et al. (2014) Control Release, 173: 102-9
[3] Sezer D, et al. (2017) J Phys Chem B, 121: 5218–5227