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Supported bilayer on a nanopatterned membrane as model PAMPA membranes

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ABSTRACT

Parallel-artificial membrane permeation assay (PAMPA) is widely used to rapidly measure drug permeability across a biological membrane. We have prepared model PAMPAs by supporting a lipid bilayer on a hydrated polymeric cushion adsorbed at the surface of a nanoporous alumina filter. In contrast to conventional PAMPAs, the natural fluidity of the bilayer is expected to be conserved in these model PAMPAs. The assembly was characterized by contact angle measurement and atomic force microscopy (AFM) and the supported membranes were mounted in a Franz cell setup to assess the permeability of acetaminophen and famotidine. The permeability profiles for the model PAMPA were compared to those of conventional PAMPA.

1. Introduction

A major bottleneck in drug discovery is the poor intestinal absorption of promising new chemical entities (NCEs) intended for oral administration. Currently, it is estimated that over 80% of orally administered drugs are absorbed via a passive absorption mechanism at the level of epithelial cells (Kv et al., 2007). The NCEs often lack suitable amphiphilic balance required for absorption because the lead optimization effort of high throughput screening (HTS) has mostly been governed by potency rather than the drug-like properties considerations that are required to ensure adequate absorption, stability and safety (Avdeef, 2001). Inappropriate *in vitro* prediction of drug bioavailability is suspected to be one of the reasons explaining the late rejection of many promising drug candidates, even when considerable amounts of money have already been engaged for the research (Hartmann and Schmitt, 2004). A particular challenge is, thus, to provide just-in time information about drug-like properties to orient the medicinal chemistry lead optimization effort and minimize the resources wasted in the synthesis of poor-quality series. Acceptable human intestinal absorption is critical to the successful development of drug products because it is determinant to the bioavailability of an NCE. The passive intestinal absorption of a drug depends on the balance between its permeability, solubility and also pKa (for ionisable compounds). Work by Amidon et al. (Amidon et al., 1995; Takagi et al., 2006), pioneered the establishment of the Biopharmaceutical Classification System (BCS) which facilitates the evaluation of generic drug products bioequivalence. To assess the ability of a compound to cross the intestinal barrier, several tools were developed over the years ranging from empirical ones (Lipinski et al., 2001; Veber et al., 2002) to complex animal studies such as *in situ* perfusion and hepatic portal vein canulation (Lennernäs, 2003). More relevant to the pre-clinical development phase of drug products, different cellular and non-cellular assays were elaborated to rapidly screen the permeability of pre-clinical drug candidates. The Caco-2 cell permeability assay is considered as the gold standard to evaluate intestinal permeability. Even though this test takes into account several active transport proteins that are present in the enterocyte membrane, it comes with all the tedious drawbacks associated with cellular assays: in addition to being time and resource-consuming, low-throughput and a high rate of variability is observed between assay conditions (Kv et al., 2007; Ungell, 2004; Kansy et al., 1998).

Non-cellular *parallel-artificial membrane permeation assays* (PAMPAs) (Kansy et al., 1998, 2004; Avdeef et al., 2007, 2008; Fischer et al., 2007; Flaten et al., 2006, 2007; Li et al., 2008) were designed to be easily and rapidly performed. They enable to obtain preliminary permeability data when compounds need to be analyzed rapidly during the preclinical development and led to the publication of at least 32 primary publications since 1998 (Kansy et al., 2004). Recent research efforts include the development of PAMPAs simulating the blood–brain barrier (Di et al., 2003), the evaluation of the predictive power of the PAMPA assay (Avdeef et al., 2003) as well as developing PAMPA assays suitable for the early discovery phase of drug R&D (Kerns et al., 2004). However, the PAMPA screening suffers from several experimental limitations. First, the phospholipids deposited on the filters are often dissolved

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in *n*-dodecane (Kansy et al., 1998, 2004), thus provoking the formation of multilamellar bilayers inside the filter channels when the membrane is in contact with the aqueous environment of the donor and receptor compartments (Avdeef et al., 2001). As a consequence, the filter pores can be blocked and the drug permeation can be abnormally slow and non-representative of the molecule "true" permeability. Studies have shown that variation in compound permeability depends on the filter coating (Avdeef et al., 2001; Hämäläinen and Frostell-Karlsson, 2004; Seo et al., 2006). Therefore, another issue of the PAMPA membrane is its biological relevance which involves two main parameters, i.e. its fluidity and composition. A study performed by Seo et al. (2006) with membranes made of individual saturated and unsaturated PC, PE and PS showed that permeable compounds are more sensitive to the membrane fluidity. Although "biomimetic PAMPA" is carried out using membranes made of a mixture of phospholipids, the preparation of the filter coating generates an inherent problem of membrane fluidity that will not be improved by using unsaturated lipids. *De facto*, an interaction between the deposited lipid bilayer and the filter is responsible for a decreased mobility of the lipid and of the fluid character of the membrane (Tanaka and Sackmann, 2005). This situation cannot be correlated to the in vivo situation where the drug only penetrates a single well-defined fluid bilayer.

To address these issues, we aimed at preparing a model system which will be amenable to HTS, but where the drug will diffuse through only one single bilayer (Fig. 1). For this purpose, a lipid bilayer was supported on a hydrated polymeric cushion which was itself immobilized at the surface of a filter with controlled porosity. Polymer-supported membranes are excellent models of the cell surface (Tanaka and Sackmann, 2005) because they retain their native fluidity and they exhibit excellent mechanical stability in comparison to free-standing black lipid films. Furthermore, the lipid membrane can rapidly be deposited on a polymeric cushion, even when large areas are to be covered; therefore, it is conceivable that this technique could be implemented in a high throughput procedure. Finally, it is possible to insert membrane proteins in the bilayer, yielding functional lipid membranes. Such membranes proved to be excellent models for the study of various biochemical processes (Watts et al., 1986; Sackmann and Bruinsma, 2002) as they were representative of in vivo membranes.

In this first report, we wish to describe the preparation of such supported bilayer system (Fig. 1). We will also examine the influence of the nature and porosity of the filter and of the polymeric cushion. Ideally, neither the filter nor the cushion should influence the permeability of the drug, as the permeability of drug through the bilayer only should be assessed. This can be achieved by selecting a polymeric layer and a filter which imparts negligible resistance to diffusion in comparison to diffusion through the bilayer. Thus, to assess the influence of the support, permeability profiles have been measured for two model drugs, famotidine and acetaminophen. This proof of concept study will be followed by a more complete assessment of the permeability of a wide of drugs in comparison to Caco-2 and conventional PAMPA diffusion profiles.

2. Materials and methods

2.1. Materials

Membranes S1–S3 were purchased from Synkera (USA), Anodisc A1 from Whatman (USA), amino-PEG (mPEG-NH_2, M_n = 1000 g/mol) from Nanocs (USA), dimyristoyl-sn-glycero-3-phosphocholine (DMPC) from Avanti Polar (Alabaster, AL, USA) and all other chemicals from Aldrich. Water was nanopure grade. Contact angles were measured using a digital camera equipped with a MSN 505 UltraMacro lens from Raynox (32 diopters), and images were analyzed using ImageJ software and Contact Angle plugin, both freely downloadable from internet. Atomic force microscopy pictures taken in air were collected with a Nscriptor™ DPN® System DS006 (NanoInk, USA). Data analysis was performed using the InkCAD software. Pictures taken in water were obtained with a Multimode Dimension 3100 atomic force microscope equipped with a NanoScope IIIa controller (Digital Instruments, Santa Barbara, CA) operated in tapping mode using aluminum-coated silicon tips from NanoWorld (ARROW-NCR-20). Data analysis was performed using the NanoScope III software (version5.30r3). All images were taken at 25 °C.

2.2. Coupling of mPEG-NH2 to S1–S3 filters

The filters (25 mm diameter) were heavily rinsed with acetone (HPLC grade), dried in a dessicator and then immersed in hydrogen peroxide (30%) for 15 min at 55 °C. It was then transferred in 100 mL of water for 15 min at 55 °C. The water was removed under a stream of nitrogen. They were then incubated with a 5% solution of aminopropyl trimethoxysilane (APS) in toluene for 2 h at room temperature. The filters were then rinsed with chloroform, acetone, water and then dried under a gentle stream of nitrogen.

The silanized filters were then incubated in a 25% solution of glutaraldehyde in a pH = 5 buffer at room temperature for 1 h. They were then rinsed six times with water and dried under a gentle stream of nitrogen. The filters were incubated in a solution of mPEG-NH_2 (0.2 g/L) in anhydrous toluene for 1 h at room temperature, then rinsed successively with anhydrous toluene, acetone, water and dried with a gentle stream of nitrogen.

2.3. Coating of the filters by polydopamine

A pH = 8.5 buffer was prepared by mixing 2.62 mL of 0.5 molar solution of NaH_2PO_4 and 98.69 mL of a 1 molar solution of $NaHPO_4$ and 900 mL of water. A dopamine solution (c = 2 g/L) was prepared by dissolving 12 mg of dopamine powder in 6 mL of buffer. Immediately after, one filter was transferred to this solution, and left for 4 h in the solution which gradually turned green. The filter was then extensively rinsed with water. Rinsing was continued until no dopamine could be detected by UV–Vis spectrophotometry at 280 nm. At this point, the filter was green, whereas the rinsing water was colorless.

2.4. Vesicle fusion

The experimental procedure was adapted from the one developed by Johnson et al. (2002). In short, lipids were hydrated in a buffer solution (pH = 8), then the resulting multilamellar vesicles were freezed-thawed five times. The resulting liposomes were sonicated with a sonicating probe (20 W, 15 s pulses for 20 min, each pulse being separated by a dead time of 30 s). Then, they were centrifuged during 30 min at 5000 rpm. The supernatant was collected and was extruded at 46 °C through an Avanti miniextruder equipped with 200 nm polycarbonate membranes, yielding a solution of unilamellar vesicles. In order to induce fusion, a filter, freshly coated by polydopamine was completely immersed in the vesicle solution overnight at 40 °C. In order to wash the excess of lipids, the filter was then immersed in 4 mL of 10 mM Tris buffer solution (pH 8, 300 mOsm/L). After removal from the solution, the filter was immediately used for PAMPA experiments. The membrane was never left to dry.

2.5. Permeation measurement, via PAMPA

For permeation measurements, vertical diffusion cells were used as described by Loftsson et al. (2006). In short, Franz cells

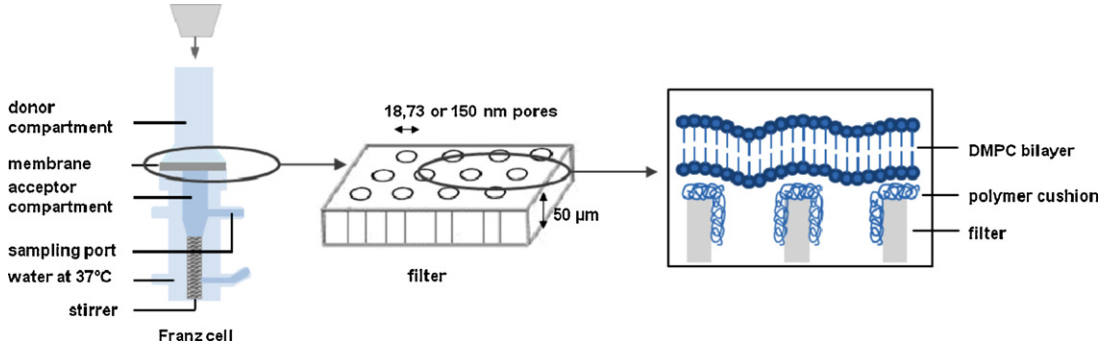

Fig. 1. Model PAMPA membranes. A lipid bilayer is supported on a polymeric cushion (PEG or polydopamine) which is itself covalently anchored or absorbed at the surface of a nanoporous alumina filter. The assembly is mounted in a Franz cell setup (left) for permeability measurements.

(Hanson, 7 mL) were filled with water at 37 °C. The PAMPA membrane was positioned at the interface between the donor and acceptor compartments. Great care was necessary in order to avoid the formation of air bubbles at the lower side of the membrane. Then, the donor compartment was filled with an aqueous solution of acetaminophen (c = 0.4 mg/mL). In the case of famotidine, a 1 mg/mL solution in 10^{-3} mol/L HCl was used instead of pure water (the lower compartment was also filled with 10^{-3} mol/L HCl). Stirring in the lower cell was set at 400 rpm and the cell was maintained at 37 °C during all the experiment (2 h). Every 15 min, 1 mL was aliquoted from the lower compartment and replaced by water (or 10^{-3} mol/L HCl) in order to work at constant volume. The permeated drug concentration was evaluated by UV–Vis spectrophotometric concentration measurement (λ = 243 nm for acetaminophen and λ = 265 nm for famotidine). Permeation of Lucifer Yellow was also assessed by this method (results shown in supplementary content). Since the permeation rate of Lucifer Yellow was negligible, we could conclude that the membrane was integral.

3. Results and discussion

Several filters were selected for this work (Table 1). A first type consists of commercial nanoporous alumina filters from Synkera with uniform pores (18 nm, 73 nm and 150 nm diameter) regularly distributed in a 2D square array. Commercial alumina filters (Anodisc from Whatman) having pores of uniform diameter (200 nm) randomly distributed were also used in this study. Finally, a polysulfone membrane with an average pore diameter of 450 nm was also used in this study. The surface functionalization of these filters by amino ended polyethylene glycol (mPEG-NH_2) was performed using the procedure developed by Popat et al. (2004) and Tanvir et al. (2009). In short, freshly washed filters were first activated with 3-aminopropyl trimethoxysilane (APS) followed by reaction with first glutaraldehyde then amino-PEG (Fig. 2).

Table 1
Filters used to support floating bilayer membranes.

Filter	Nanoporous alumina (Anodisc)	Nanoporous alumina (Synkera)		
	A1	S1	S2	S3
Pore size (nm)	200	18	73	150
Thickness (µm)	60	53	54	52
Contact angle (°)				
Untreated filter	nd	32	97	nd
After H_2O_2 activation	65	61	63	38
After APS treatment	45	52	61	nd
After glutaraldehyde	32	57	73	54
After mPEG coupling	44	51	28	≤25

nd, not determined.

Unsurprisingly, this procedure proved to be inefficient for polysulfone filters (as these filters do not bear any surface hydroxyl groups).

The functionalization reaction was followed by surface contact angle measurement after extensive washing (Table 1). After mPEG-NH_2 coupling, the membrane surface was found to be more hydrophilic, as shown by a significant decrease in contact angle. Interestingly, there is an inverse correlation between pore size and surface hydrophilicity, as measured by contact angle after mPEG treatment (51° for membrane S1 vs less than 25° for membrane S3). This may indicate that the coupling is more successful for the filters with the largest pore sizes; however, it is also important to remember that contact angles are also very dependent upon surface roughness which is obviously not identical for the membranes S1 and S3.

The mPEG-NH_2 grafting was also assessed by atomic force microscopy (AFM) in air. In contact mode, the pores of the filter are clearly apparent (measured with a diameter 124 nm, σ = 23 nm, N = 53) (Fig. 3a). The polymer can be visualized in tapping mode

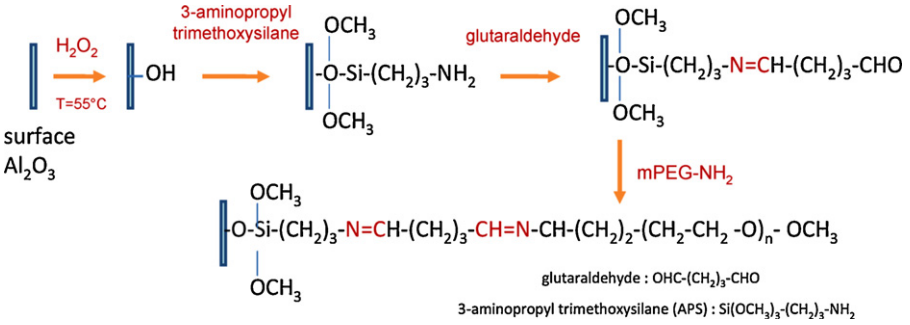

Fig. 2. Surface derivatization of nanoporous alumina filters by mPEG-NH_2.

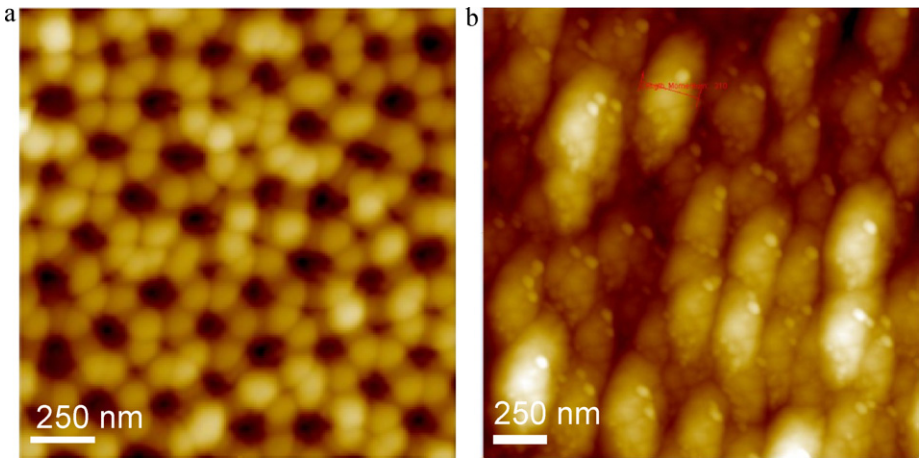

Fig. 3. AFM analysis of membrane S3 derivatized by mPEG-NH_2 in air. (a) Contact mode (scan size 1.78 μm × 1.78 μm). (b) Tapping mode (scan size 2.25 μm × 2.25 μm).

(Fig. 3b) under the form of isolated islands, indicating that the grafting process was not uniform.

Albeit not perfectly covered by mPEG-NH_2, these filters were nonetheless used to support floating bilayer membranes, using the well-known vesicle fusion method (Johnson et al., 2002; Richter et al., 2006). The resulting substrates were characterized by AFM (tapping mode) in water (see Supporting information) indicating that the lipids are adsorbed at the alumina surface and are not supported by the PEG chains in regions with low PEG density. Domains with high PEG density appear to be protruding above the lipid-coated alumina surface. Consequently, the PEG functionalization strategy failed for two reasons. First, the grafting density was not sufficient, leaving bare spots of alumina with which the phospholipid head groups had a strong affinity. Furthermore, PEG chains are not easily amenable to support lipid bilayers due to the lack of sufficiently strong interactions between the lipid and the PEG (Goennenwein et al., 2003; Demé and Marchal, 2005).

In view of the failure of the PEG strategy, we selected polydopamine as a possible candidate to support the bilayers. The rationale for this choice is that dopamine spontaneously forms cross-linked polymeric films onto a wide range of inorganic and organic surfaces when introduced in an aqueous solution at pH 8.5 (Fig. 4) (Lee et al., 2007; Waite, 2008). The film thickness can easily

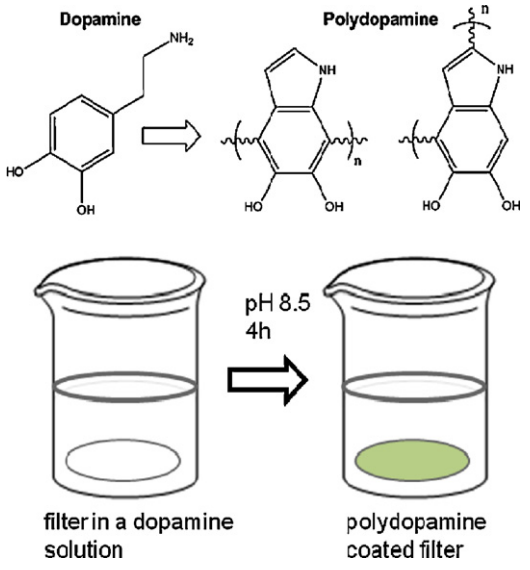

Fig. 4. Polymerization of dopamine onto the surface of a nanoporous alumina filter.

be controlled by adjusting the polymerization time. However, the exact polymerization mechanism as well as the nature of the resulting film is still under scrutiny (Lee et al., 2007; Waite, 2008; Yu et al., 2010). Thus nanoporous alumina surfaces were immerged in aqueous solution of dopamine (c = 2 mg/mL) at pH 8.5 for 4 h, resulting in the formation of 20 nm thick films (the 20 nm thickness is based on literature data for dry films) (Lee et al., 2007). The resulting greenish film was monitored by AFM (tapping mode in water), indicating that most of the surface (contrasted in clear) is uniformly coated by polydopamine, but most pores (contrasted in dark) are unblocked. In several regions, the film of dopamine also covers the pores, making continuous islands of up to 0.6 μm dimension (X in Fig. 5a). Elsewhere, a thin layer of polydopamine, which was measured to be in average 35 nm thick (hydrated thickness), was found to cover the filter. Therefore, polydopamine is more efficient than PEG in making a hydrated cushion over the alumina filter.

The polydopamine covered filters were then used to support DMPC membranes, using the vesicle fusion protocol. To our knowledge, direct fusion of a vesicle on polydopamine has never been reported before. However, we were encouraged by the fact that polydopamine has recently been shown to interact attractively with cellular walls (Kang and Elimelech, 2009; Ku et al., 2010a,b). Additionally, we observed that during the fusion process, the greenish film turned to a brown tinge (and the solution remained colorless), indicating that the vesicles interacted with the absorbed polydopamine film. Fusion of the bilayer was evidenced by AFM (tapping mode in water, Fig. 5b): in stark contrast to the perforated PEGylated surface, the DMPC-dopamine-filter assembly has a very uniform surface. The topography shows only valleys and peaks, all of the same height which are regularly aligned in a square array. The lower zones correspond to the free standing bilayer which is supported on polydopamine pillars (the higher zones). Therefore, the bilayer follows the topography of the cushion covered filter. The roughness parameter increases from 14.6 nm (for the polydopamine only membrane) to 20.1 nm for the DMPC containing film. This increase of roughness is associated to an increase of surface material of approx. 5.5 nm which is consistent with the thickness of a fully hydrated bilayer. Anodisc (A1) filters were also coated with a film of polydopamine which was then used to support the bilayer. We were unable to unambiguously interpret AFM pictures of the resulting films, probably because the pore arrangement is random unlike the S1–S3 filters for which it is periodic. As A1 Anodisc filters and S1–S3 filters are all made of nanoporous alumina, it can be assumed that the cushion is adsorbed at the surface and the bilayer is properly deposited.

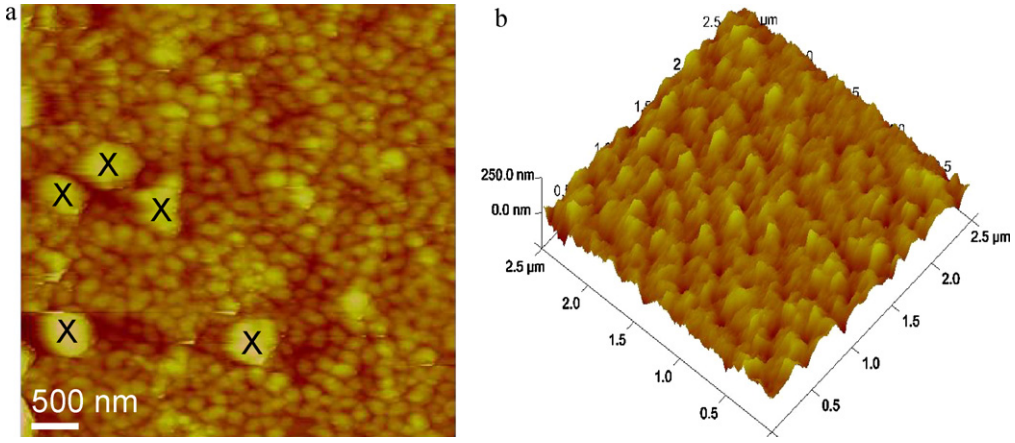

Fig. 5. (a) Surface of S3 covered by a film of polydopamine. X locates the continuous islands of polydopamine. (b) 3D representation of the surface S3 covered by the polydopamine film and the DMPC bilayer.

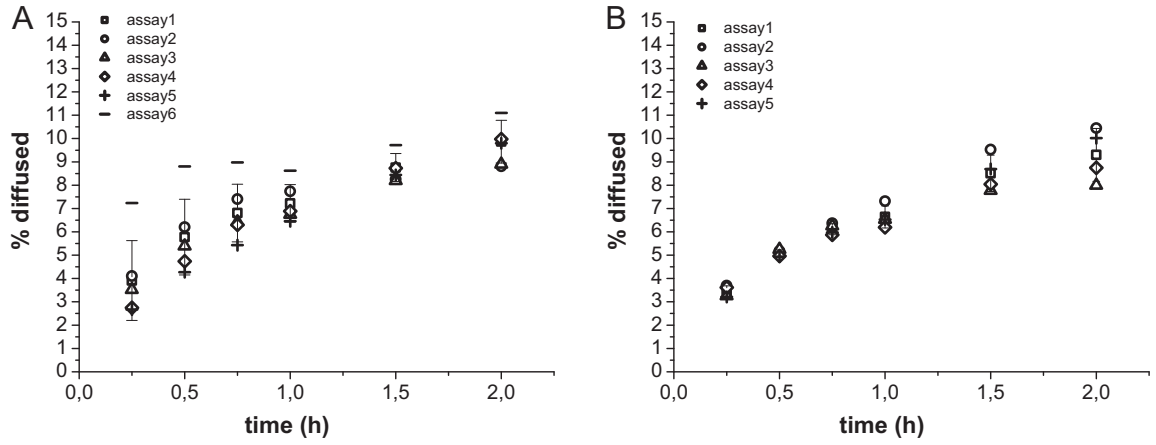

Fig. 6. Diffusion profile of famotidine: (a) classical PAMPA ($N = 6$) and (b) DMPC bilayer on A1 filter with polydopamine hydrated cushion ($N = 5$).

Permeation tests were performed using two model drugs, famotidine and acetaminophen, both of which contain chromophores easily detected by UV–Vis spectrophotometry. Acetaminophen is included in group 1 of the biopharmaceutical classification system, whereas famotidine is in group 2. Diffusion profiles, collected with Franz static diffusion cells, are shown in Figs. 6 and 7. With filters S1 and S2 (with nominal pores of respective diameter of 18 and 73 nm), neither famotidine nor acetaminophen diffused. These results suggest that the pores were clogged by polydopamine, in agreement with the 35 nm polydopamine layer thickness. Furthermore, filters S1–S3 were found to be extremely mechanically fragile (rapid formation of holes through the membrane). Therefore, the S1–S3 membranes were not further employed for these tests.

While Fig. 6a (resp. Fig. 7a) represents the diffusion profile using a classical PAMPA setup, Fig. 6b (Fig. 7b) shows the diffusion of famotidine (resp acetaminophen) on supported DMPC bilayers.

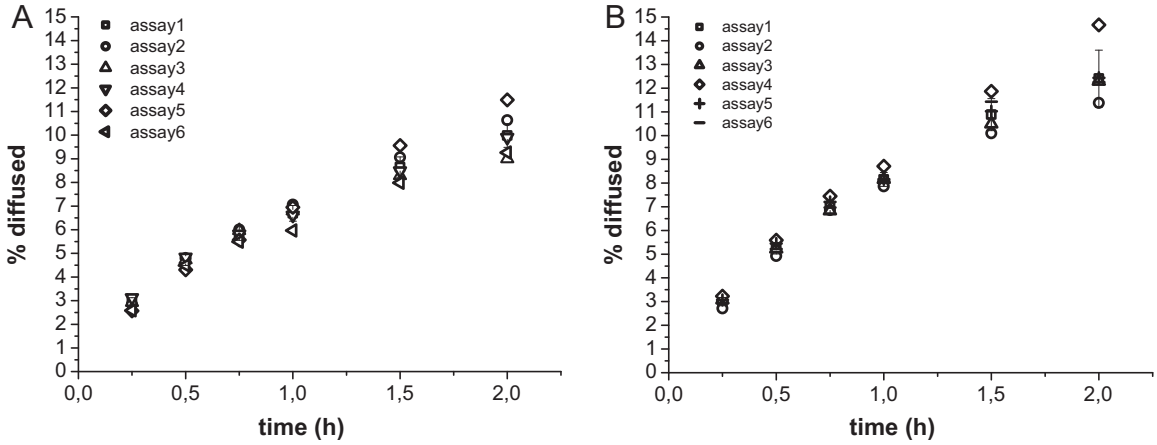

Fig. 7. Diffusion profile of acetaminophen: (a) classical PAMPA ($N = 6$) and (b) DMPC bilayer on A1 filter with polydopamine hydrated cushion ($N = 6$).

First, it is clear that the supported bilayers yield reproducible results, as seen by the dispersion of the results which is comparable to conventional PAMPA. Furthermore, it is clear that the model PAMPA yields diffusion profiles which are different from the classical PAMPA one (at least in the case of acetaminophen), indicating that the drug diffuses through different environments, a legacy of the change of fluidity between the supported bilayer vs adsorbed lipid on the filter. Last, for the conventional PAMPA, the diffusion profile of famotidine is similar to the one of acetaminophen (comparing Figs. 6a and 7a). This is not the case for the model PAMPA. Therefore, it seems that the model PAMPAs might be more discriminative than conventional ones, although this will only be proved after testing a very large number of drugs.

Clearly, this very first report on freely supported PAMPA seemingly indicates that supporting the lipid bilayer is a promising strategy to quickly assess the passive permeability of drugs. In order to further assess this phenomenon, we will need to examine longer permeation profiles (up to 8 h) for a wider range of drugs. Our approach will conceptually allow us to study the effect of bilayer composition, bilayer fluidity and elasticity on drug permeability without interference from the filter, since the bilayer is not in contact with it. This is currently not possible with conventional PAMPA as the lipid is adsorbed at the filter surface. We will also be in measure to assess the influence of the nature of the hydrated layer and of the filter porosity on permeation. Ideally, neither the cushion nor the filter should influence the drug permeability. Finally, our approach could be used to insert membrane proteins in the bilayer, which we envision should yield a higher absorption predictability compared to conventional PAMPA approaches.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijpharm.2011.09.013.

References

Amidon, G.L., Lennernäs, H., Shah, Vinod P., Crison, J.R., 1995. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm. Res. 12, 413–420.
Avdeef, A., 2001. Physicochemical profiling (solubility, permeability and charge state). Curr. Top. Med. Chem. 1, 277–351.
Avdeef, A., Strafford, M., Block, E., Balogh, M.P., Chambliss, W., Khan, I., 2001. Filter-immobilized artificial membranes. Studies of the permeability properties of lactones in Piper methysticum Forst. Eur. J. Pharm. Sci. 14, 271–280.
Avdeef, A., Artursson, P., Neuhoff, S., Lazarova, L., Gråsjö, J., Tavelin, S., 2003. Caco II permeability of weakly basic drugs predicted with the double-sink PAMPA pK_a^{flux} method. Eur. J. Pharm. Sci. 24, 333–349.
Avdeef, A., Bendels, S., Di, L., Faller, B., Kansy, M., Sugano, K., Yamauchi, Y., 2007. Parallel Artificial Membrane Permeability Assay (PAMPA) – critical factors for better predictions of absorption. J. Pharm. Sci. 96, 2893–2909.
Avdeef, A., Kansy, M., Bendels, S., Tsinman, K., 2008. Absorption-excipient-pH classification gradient maps: sparingly soluble drugs and the pH partition hypothesis. Eur. J. Pharm. Sci. 33, 29–41.
Demé, B., Marchal, D., 2005. Polymer cushioned lipid bilayers in porous alumina. Eur. J. Biophys. 34, 170–179.
Di, L., Kerns, E.H., McConnell, O.J., Carter, G.T., 2003. High throughput artificial membrane permeability assay for blood–brain barrier. Eur. J. Med. Chem. 38, 223–232.
Fischer, H., Kansy, M., Avdeef, A., Senner, F., 2007. Advances in screening for membrane permeability: high-resolution PAMPA for medicinal chemists. Eur. J. Pharm. Sci. 31, 32–42.
Flaten, G.E., Bunjes, H., Luthman, K., Brandl, M., 2006. Drug permeability across a phospholipid vesicle-based barrier: characterization of barrier structure, storage stability and stability towards pH changes. Eur. J. Pharm. Sci. 2830, 336–343.
Flaten, G.E., Skar, M., Luthman, K., Brandl, M., 2007. Drug permeability across a phospholipid vesicle based barrier: characterization of drug–membrane interactions and the effect of agitation on the barrier integrity and on the permeability. Eur. J. Pharm. Sci. 30, 324–332.
Goennenwein, S., Tanaka, M., Hu, B., Moroder, L., Sackmann, E., 2003. Functional incorporation of integrins into solid supported membranes on ultrathin films of cellulose: impact on adhesion. Biophys. J. 85, 646–655.
Hämäläinen, M.D., Frostell-Karlsson, A., 2004. Predicting the intestinal absorption potential of hits and leads. Drug Discov. Today Technol 1, 397–405.
Hartmann, T., Schmitt, J., 2004. Lipophilicity – beyond octanol/water: a short comparison of modern technologies. Drug Discov. Today 1, 431–439.
Johnson, J.M., Ha, T., Chu, S., Boxer, S.G., 2002. Early steps of supported bilayer formation probed by single vesicle formation assays. Biophys. J., 3371–3379.
Kang, S., Elimelech, M., 2009. Bioinspired single bacterial cell force spectroscopy. Langmuir 25, 9656–9659.
Kansy, M., Senner, F., Gubernator, K., 1998. Physicochemical high throughput screening: parallel artificial membrane permeation assay in the description of passive absorption processes. J. Med. Chem. 41, 1007–1010.
Kansy, M., Avdeef, A., Fischer, H., 2004. Advances in screening for membrane permeability: high-resolution PAMPA for medicinal chemists. Drug Discov. Today 1, 349–355.
Kerns, E., Di, L., Petusky, S., Farris, M., Ley, R., Jupp, P., 2004. Combined application of parallel artificial membrane permeability assay and Caco-2 permeability assays in drug discovery. J. Pharm. Sci. 93, 1440–1453.
Ku, S.H., Lee, J.S., Park, C.B., 2010a. Spatial control of cell adhesion and patterning through mussel-inspired surface modification by polydopamine. Langmuir 26, 15104–15108.
Ku, S.H., Ryu, J., Hong, S.K., Lee, H., Park, C.B., 2010b. General functionalization route for cell adhesion on non-wetting surfaces. Biomaterials 31, 2535–2541.
Kv, S., Devi, G.S., Mathew, S.T., 2007. Liposomal formulations of serratiopeptidase: in vitro studies using PAMPA and Caco-2 models. Mol. Pharm. 5, 92–97.
Lee, H., Delllatore, S.M., Miller, W.M., Messersmith, P.B., 2007. Mussel-inspired surface chemistry for multifunctional coatings. Science 318, 426–430.
Lennernäs, H., 2003. Intestinal drug absorption and bioavailability: beyond involvement of single transport function. J. Pharm. Pharmacol. 55, 429–433.
Li, C., Wainhaus, S., Uss, A.S., Cheng, K.C., 2008. High-throughput screening using Caco-2 cell and PAMPA systems. Biotechnol. Pharm. Aspects 7, 418–429.
Lipinski, C.A., Lombardo, F., Dominy, B.W., Feeney, P.J., 2001. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug. Deliv. Rev. 46, 3–26.
Loftsson, T., Konrádsdóttir, F., Másson, M., 2006. Development and evaluation of an artificial membrane for determination of drug availability. Int. J. Pharmaceut. 326, 60–68.
Popat, K.C., Mor, G., Grimes, C.A., Desai, T.A., 2004. Surface modification of nanoporous alumina surfaces with poly(ethylene glycol). Langmuir 20, 8035–8041.
Richter, R.P., Bérat, R., Brisson, A.R., 2006. Formation of solid-supported lipid bilayers: an integrated view. Langmuir 22, 3497–3505.
Sackmann, E., Bruinsma, R.F., 2002. Cell adhesion as wetting transition? Chem. Phys. Chem. 3, 262–269.
Seo, P.R., Teksina, Z.S., Kaoc, J.P.Y., Polli, J.E., 2006. Lipid composition effect on permeability across PAMPA. Eur. J. Pharm. Sci. 29, 259–268.
Takagi, T., Ramachandran, C., Bermejo, M., Yamashita, S., Yu, L.X., Amidon, G.L., 2006. A provisional biopharmaceutical classification of the top 200 oral drug products in the United States, Great Britain, Spain, and Japan. Mol. Pharm. 3, 631–643.
Tanaka, M., Sackmann, E., 2005. Polymer-supported membranes as models of the cell surface. Nature 437, 656–663.
Tanvir, S., Pantigny, J., Boulnois, P., Pulvin, S., 2009. Covalent immobilization of recombinant human cytochrome CYP2E1 and glucose-6-phosphate dehydrogenase in alumina membrane for drug screening applications. J. Membr. Sci. 329, 85–90.
Ungell, A.L.B., 2004. Caco-2 replace or refine? Drug Discov. Today 1, 423–430.
Veber, D.F., Johnson, S.R., Cheng, H.-Y., Smith, B.R., Ward, K.W., Kopple, K.D., 2002. Molecular properties that influence the oral bioavailability of drug candidates. J. Med. Chem. 45, 2615–2623.
Waite, J.H., 2008. Mussel power. Nature Mater. 7, 8–9.
Watts, T.H., Gaub, H.E., McConnell, H.M., 1986. T-cell-mediated association of peptide antigen and major histocompatibility complex protein detected by energy-transfer in an evanescent wave-field. Nature 320, 179–181.
Yu, F., Chen, S., Chen, Y., Li, H., Yang, L., Chen, Y., Yin, Y., 2010. Experimental and theoretical analysis of polymerization reaction process on the polydopamine membranes and its corrosion protection properties for 304 Stainless Steel. J. Mol. Struct. 982, 152–161.