In Vitro Permeability of Poorly Aqueous Soluble Compounds Using Different Solubilizers in the PAMPA Assay with Liquid Chromatography/ Mass Spectrometry Detection

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Purpose. This study compares the use of UV-VIS detection with liquid chromatography/mass spectrometry (LC/MS) detection for the PAMPA (Parallel Artificial Membrane Permeability Assay) permeability determination of compounds in the drug discovery stage. LC/MS detection offers a selective and sensitive method for the determination of the PAMPA permeability for compounds that do not contain a UV chromophore or possess a low UV extinction coefficient. To enhance the reliability of our permeability measurements for compounds with low aqueous solubility, we demonstrated the use of LC/MS detection as a means for facilitating the study of solubilizing agents to enhance aqueous solubility that normally would interfere with UV-VIS detection. In doing so, the PAMPA assay can be expanded to study the *in vitro* permeability of poorly water soluble compounds and evaluate the effects of solubilizers' on the membrane permeability of different compounds. This might be useful in selecting solubilizers for poorly water soluble compounds to be used for further *in vivo* studies.

Methods. A diverse set of 20 drugs using UV-VIS detection were compared with data using LC/MS detection. A PAMPA screening method was designed which used solubilizers (Brij® 35, Cremophor EL, ethanol, and Tween 80) for compounds with low aqueous solubility. The stability of the artificial membrane was determined using various solubilizer concentrations (0.1-5% w/v) to ensure the phospholipid membrane was not disrupted. Two compounds, amiodarone and miconazole, with low aqueous solubility yielding an undetected response in the PAMPA assay using UV-VIS detection were subjected to the different solubilizing agents and their PAMPA permeability was measured using LC/MS detection.

Results. Most of the compounds showed similar PAMPA permeability using the two detection systems. However, for compounds lacking a UV chromophore or with a low UV extinction coefficient, LC/MS was the detection method of choice for determination of PAMPA permeability values. LC/MS also gave reliable quantification data for compounds containing impurities, as well as compounds that were not stable during the assay. Although many solubilizers were found to interfere with UV-VIS detection, the LC/MS approach was applicable to determine the permeability values of compounds with normally low aqueous solubility.

Conclusions. LC/MS detection offered greater sensitivity and selectivity as compared with UV-VIS detection for the PAMPA assay. With this added versatility in detection, PAMPA can be used in both discovery and pre-formulation applications, which has not been described before.

KEY WORDS: solubility; permeability; parallel artificial membrane permeability assay (PAMPA); liquid chromatography/mass spectrometry (LC/MS); solubilizer.

INTRODUCTION

The 1990s brought drug discovery to a new level. Improvements in chemical synthesis (combinatorial chemistry), active compound identification (high throughput screening), and informatics made drug discovery change from a slow process to a rapid identification procedure affording hundreds to thousands of active compounds to pursue. Although most of these compounds eventually fail later in development it has become necessary to use key developmental studies (physicochemical and ADME) at the discovery level to filter through active compounds (1–11). Decisions need to be made about what to pursue and what may be a false lead. A wrong decision can cost a company millions of dollars. The need to develop decision making tools to assess thousands of compounds with speed, precision and accuracy for ADME, and physico-chemical properties has grown to replace traditional lower throughput methods for assessment that are slower, and require more compound for testing. As a result, many new procedures have been developed explicitly for working on thousands of compounds to provide the required information to make the best decisions about a compound's future (12–20).

One such method is the parallel artificial membrane permeability assay (PAMPA) that was developed as an alternative to the low throughput Caco-2 assay used to correlate passive permeability with *in vivo* oral absorption (13–15). This assay utilizes a simple phospholipid-coated filter disc instead of a monolayer of cells to measure permeability. The technique is rapid, simple, and has higher precision between laboratories than cell culture studies. However, its shortcoming is that it only allows observation of the passive permeability component.

Since the initial description in 1998 most PAMPA publications have been put forth describing new permeability methods focusing on the use of different lipids. Most of this work emphasizes *in vivo*/*in vitro* correlation using commercial drugs and not on problems found when implementing the assay using real discovery-level compounds. In this work we address this latter issue and illustrate that PAMPA can be used with excipients or solubilizers. The use of excipients with PAMPA is important because most compounds coming from early discovery have very low aqueous solubility making both bioassays and ADME work problematic and difficult to interpret. These problems affect the decision process and may lead to costly mistakes in development. Here we report a method for evaluating the *in vitro* permeability of compounds using PAMPA and solubilizers with LC/MS as the detection system. The present work illustrates that previous difficult-to-analyze compounds can be readily assayed using excipients in PAMPA assays.

Furthermore, the use of solubilizers in PAMPA allowed us to rapidly examine the effects of various solubilizers on the transport processes of compounds with low solubility, which can be useful for the selection of excipients in the preformulation of drug candidates. This is a new use of PAMPA.

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METHODS

Chemicals

Allopurinol, amiodarone hydrochloride, amoxicillin, atenolol, azithromycin, benzthazide, bendroflumethazide, carbamazepine, cimetidine, ergonivine maleate, erythromycin, guanabenz, imipramine hydrochloride, ketoprofen, lisinopril, metoprolol tartrate, miconazole nitrate, oxyphenbutazone, phenazopyridine, piroxicam, probenecid, promethazine hydrochloride, propranolol hydrochloride, propylparaben, terbutaline, terfenadine, verapamil hydrochloride, Brij® 35 solution (30% w/v), Cremophor EL, polyoxyethylenesorbitan monooleate (Tween 80), and Lucifer Yellow CH were either purchased from Sigma Chemical Co. (St. Louis, MO, USA) or obtained from the in-house compound room. Buffer solutions used for the permeability experiment were purchased from *p*ION INC (Woburn, MA, USA). All other chemicals and organic solvents were obtained from other established suppliers.

PAMPA experiment with UV detection

The parallel artificial membrane permeability assay was carried out in a 96-well format, similar to that described in the literature (13,21). A 96-well microtitre plate and a 96-well filter plate (Millipore, Bedford, MA, USA) were assembled into a "sandwich" such that each composite well was separated by a 125 μm micro-filter disc (0.45 μm pores). The hydrophobic filter material of the 96 well filter plate was coated with 4 μL of a 2% (wt/v) dodecane solution of dioleoylphosphatidyl-choline. The coated filter plate was gently shaken for 5 min to ensure uniform spreading of the lipid solution. Subsequently, the acceptor wells at the top of the sandwich were hydrated with 200 μL of *p*ION buffer solution adjusted to pH 7.4. The donor wells at the bottom of the sandwich were filled with 200 μL of test compound solution. The test compound solution was prepared by diluting ×200 from a 10 mM stock solution in DMSO using *p*ION buffer solution at pH 7.4 followed by filtration through a 0.20 μm polyvinylidene fluoride (PVDF) 96 well filter plate (Corning Costar, Corning, NY, USA). The resulting "sandwich" construct was then incubated at room temperature for approximately 16 h. At the end of ~16 h incubation period, the "sandwich" was dissembled. The solution in the acceptor wells and the donor wells were transferred to a disposable UV-transparent plate (*p*ION INC., Woburn, MA, USA). UV absorptions were measured with a SPECTRAMax 190 microplate spectrophotometer (Molecular Device Corporation, Sunnyvale, CA, USA) at absorption wavelengths between 190 and 500 nm. After the UV absorption was measured, the samples in the donor, acceptor, and reference wells were saved for LC/MS analysis. All the PAMPA experiments were performed on the PSR4p robotic instrument (*p*ION INC., Woburn, MA, USA). PSR4p Command Software (Version 1.6) was used to control the instrument and process the data. For the PAMPA experiment that compared non-filtered vs filtered sample solutions, 5 mg/mL stock solutions in DMSO were used.

LC/MS experiment

The LC/MS analysis was performed using a Hewlett-Packard 1100 MSD (Wilmington, DE, USA) with an electrospray interface, operating in positive ion mode. The mass spectrometer was coupled to a Hewlett-Packard 1100 HPLC system equipped with a binary pumping unit, a vacuum degasser, a photodiode array UV detector, and a 96 well plate autosampler. HP Chemstation software was used to control the instrument and process the data.

The HPLC column used was a 4.6 × 50 mm YMC ProC18 S-5, 120Å (Waters Corp., Milford, MA, USA). The mobile phase consisted of 0.02% formic acid in water (A) and 0.02% formic acid in acetonitrile (B). Two gradient programs with a flow rate of 1.0 mL/min were applied for the LC/MS analysis of the test compounds. One gradient program, 5–30% B from 0 to 4.0 min, 30–5% B from 4.0 to 4.1 min, and 5% B from 4.1 to 5.0 min, was used for LC/MS analysis of the relatively hydrophillic compounds, amoxicillin, atenolol, cimetidine, and terbutaline. The other program, 5–95% B from 0 to 4.0 min, 95–5% B from 4.0 to 4.1 min, and 5% B from 4.1 to 5.0 min, was used for all remaining compounds. The column temperature was maintained at 37°C and an injection volume of 5.0 μL was used for all samples. External standards at 0.1, 1.0, 10.0, and 50.0 μM (in some cases, 75 μM) concentrations were prepared by diluting the 10 mM stock solution with 50% DMSO in water.

Prior to the LC/MS experiment, the compounds were analyzed on the mass spectrometer using flow injection in scan mode to determine the optimal fragmentor value for each compound and the m/z of the most abundant molecular ion. The flow rate for the flow injection experiment was 0.2 mL/min. The nebulizing gas was obtained from an in house high purity nitrogen source. The drying gas temperature was set at 350°C. A capillary voltage of 3000 V, gas flow of 13 L/min, and N_2 nebulizer pressure of 60 psi were used. The quadrupole temperature was maintained at 99°C. Other typical values of the MS operating parameters were, a peak width of 0.10 min, and a gain of 1. The MS operating parameters for the LC/MS runs were the same as those used in the flow injection analyses, except that single-ion monitoring (SIM) mode was used instead of a scan mode in order to increase sensitivity.

Calculations

Because the PAMPA experiments were not under sink conditions, the modified "two-way flux" equation was used in deriving the effective permeability constant, P_e, of the test compounds.[21] The Eq. is:

$$P_e = -2.303 \cdot \frac{V_a V_d}{(V_a + V_d) \cdot A \cdot (t - t_o)} \cdot 1\mathrm{g}\left\{1 - \frac{V_a + V_d}{V_d \cdot S} \cdot \frac{C_a(t)}{C_d(0)}\right\}$$

where

$$S = \frac{V_a}{V_d} \cdot \frac{C_a(t)}{C_d(0)} + \frac{C_d(t)}{C_d(0)}$$

V_a is the acceptor well volume (i.e., 0.2 cm^3 in this study); V_d is the donor well volume (i.e., 0.2 cm^3 in this study); A is the filter area (0.3 cm^3 in this study); t_o is the steady-state time to fill the membrane (average: 1140 s); t is the permeation time; $C_a(t)$ is the concentration of the drug in the acceptor well at time t (i.e., permeation hours × 60 min/hour × 60 s/min); $C_d(t)$ is the concentration of the drug in the donor well at time t; and $C_d(0)$ is the concentration

of the drug in the donor well at time zero (equal to the drug concentration in the reference well). The factor S is the fraction of the sample remaining in the donor and the acceptor wells after the permeation time. This modified "two-way flux" Eq. considers the effect of mass loss to the membrane.

Fluorescence Measurement

Fluorescence intensity of Lucifer Yellow CH in the PAMPA experiment was measured on the Model 403 FLUOstar® Galaxy fluorescence microplate reader (BMG Technologies, Durham, NC, USA). The wavelength of the excitation filter was 430 nm. The wavelength of the emission filter was 530 nm. Other general settings, number of cycles, number of flashes, positioning delay, and cycle time were set at 1, 10, 0.5 s, and 1.0 s respectively. Black Microfluor® plates (VWR Scientific, So. Plainfield, NJ, USA) were used for the fluorescence measurement.

RESULTS

The comparison of permeability values for 20 compounds using the UV microtitre plate reader and LC/MS is shown in Table I. The correlation coefficient (r^2) for the values obtained by the two detection methods is 0.958. This indicates good agreement between the two quantitation approaches for most compounds tested. Figure 1 shows the LC/MS ion chromatograms for verapamil, metoprolol, and ergonivine in the donor, acceptor, and reference wells respectively. Verapamil, metoprolol, and ergonivine represent examples of high, moderate, and low permeability compounds respectively. Though similar P_e results were obtained, the UV spectroscopic method obviously allows for much more rapid sample analysis, as compared to the LC/MS method, ~2.0 h vs ~22 h for the 16 compounds tested. However, as shown in Table I, the UV-VIS plate reader was not able to derive P_e values for six of the compounds tested, amiodarone, azithromycin, erythromycin, lisinopril, miconazole, and terfenadine. Neither azithromycin nor erythromycin has a UV absorption above 250 nm, whereas lisinopril has a low UV absorption. Because of these inherent properties, the UV-VIS reader was unable to detect these compounds resulting, therefore, in an undetected P_e. The LC/MS detection method, however, was able to obtain P_e values for these three compounds. The LC/MS result showed that almost all of these compounds stayed within the donor wells (Fig. 2) and only a trace amount of azithromycin actually penetrated through the phospholipid

Table I. Permeability of 20 Compounds as Determined by PAMPA Using Both a UV-VIS Plate Reader and LC/MS

Compound	P_e (10^{-6} cm/s) determined by UV-vis plate reader	P_e (10^{-6} cm/s) determined by LC/MS
Amiodarone	ND	ND
Amoxicillin	0.00 ± 0.00	0.04 ± 0.00
Atenolol	0.00 ± 0.00	0.00 ± 0.00
Azithromycin	ND	0.00 ± 0.00
Carbamazepine	7.38 ± 0.38	6.03 ± 0.27
Cimetidine	0.00 ± 0.00	0.00 ± 0.00
Ergonivine maleate	0.04 ± 0.04	0.00 ± 0.00
Erythromycin	ND	0.00 ± 0.00
Guanabenz	0.14 ± 0.04	0.06 ± 0.02
Imipramine	21.54 ± 5.65	15.48 ± 4.23
Ketoprofen	0.15 ± 0.01	0.00 ± 0.00
Lisinopril	ND	0.00 ± 0.00
Metoprolol	0.43 ± 0.04	0.56 ± 0.00
Miconazole	ND	ND
Piroxicam	1.97 ± 0.06	4.51 ± 0.81
Promethazine	9.04 ± 0.20	8.55 ± 1.03
Propranolol	9.81 ± 1.42	9.37 ± 1.49
Terbutaline	0.00 ± 0.00	0.00 ± 0.00
Terfenadine	ND	ND
Verapamil	11.8 ± 0.95	8.63 ± 0.64

ND: not detected.

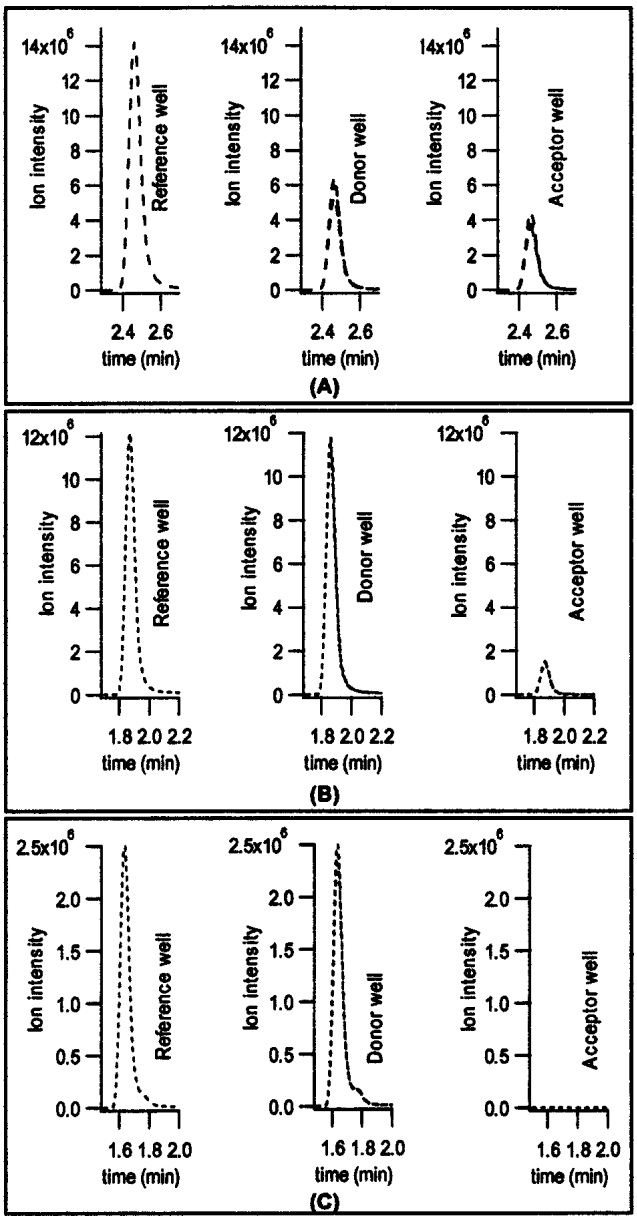

Fig. 1. LC-MS chromatograms of verapamil (A), metoprolol (B), and ergonivine (C) samples collected from the reference, donor, and acceptor wells of the PAMPA experiment. The three ion chromatograms of the test samples from the acceptor and donor wells were overlaid as each test compound was run in triplicate except metoprolol, which was run in duplicate.

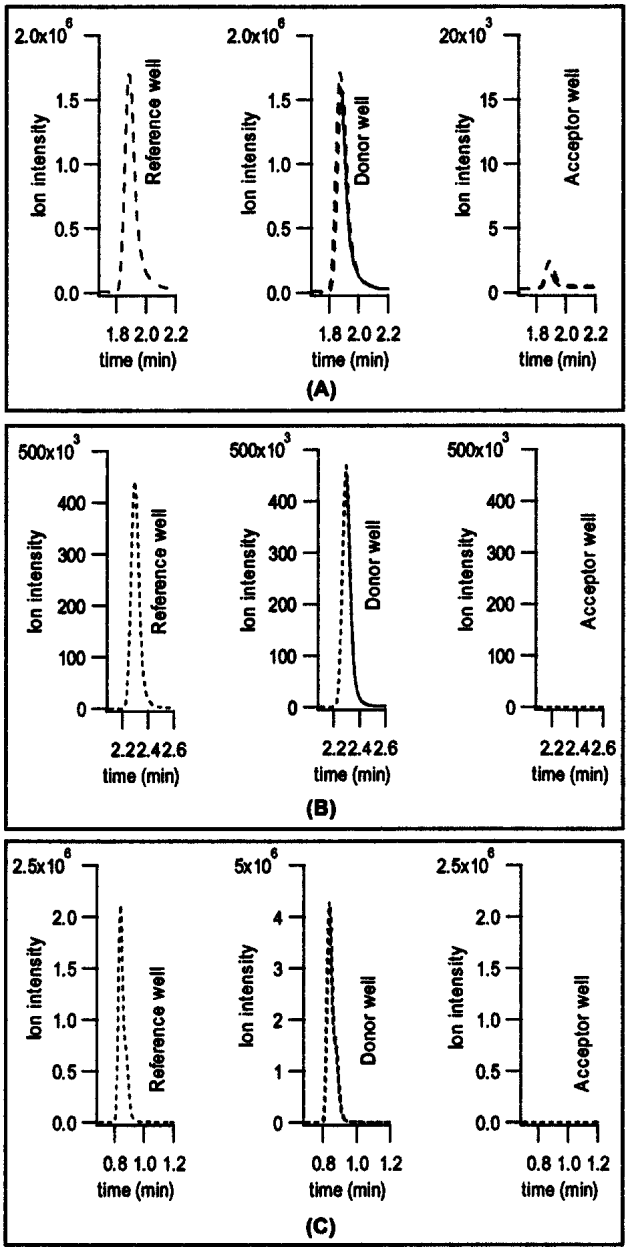

Fig. 2. LC-MS chromatograms of azithromycin (A), erythromycin (B), and lisinopril (C) samples collected from the reference, donor, and acceptor wells of the PAMPA experiment. The three ion chromatograms of the test samples from the acceptor and donor wells were overlaid as each test compound was run in triplicate. To better view the trace of the azithromycin peak in the acceptor wells, the scale of the y-axis is 100x less than in the other graphs. The lisinopril reference sample was diluted 1:1 before running LC/MS.

membrane (see Fig. 2A). Thus, the permeability values for these three compounds were zero as measured by LC/MS. Amiodarone, miconazole, and terfenadine have poor aqueous solubility. The addition of 10 μL of the 10 mM stock solutions of compound to 2 mL of the aqueous buffer caused immediate precipitation, giving rise to haziness in the buffer solutions. After mixing and filtration, the resulting levels of compound in solution was well below the detection limit of the UV-VIS plate reader, therefore, yielding undetectable permeability results for these three compounds. LC/MS revealed that less than 0.2% of miconazole and none of amiodarone and terfenadine remained in the solution after filtration resulting in undetectable levels in the acceptor and donor wells after the ~16-h period for permeation (data not shown). These experimental results demonstrate the limitations of using an aqueous buffer alone in the PAMPA experiment for measuring *in vitro* permeability for compounds with low aqueous solubility.

While conducting the previously discussed experiment, a question was raised as to whether the filter could retain compounds that would cause an interference with the PAMPA assay result. To investigate this, PAMPA experiments for eight water-soluble compounds were conducted where both filtered and non-filtered sample solutions were used. The filtered vs. non-filtered results were compared and the data are summarized in Table II. The UV absorption values of the samples in all three wells (reference, donor, and acceptor) were almost identical for the non-filtered and filtered solutions. This indicates that when compounds are soluble in the assay buffer, filtration does not interfere with the PAMPA assay. However, for compounds that are not soluble in the assay buffer, filtration removes the precipitate, which will usually result in an "undetected" permeability value. However, not filtering the sample solutions during the PAMPA experiment results in large variations and poor reproducibility for compounds with low aqueous solubility (data not shown) as the suspension is not homogeneous.

To overcome the limitations of PAMPA for poorly aqueous soluble compounds, the use of solubilizers in the PAMPA experiment was examined. Four solubilizers were chosen, Brij® 35, Cremophor EL, ethanol, and Tween 80, all of which are commonly used as formulation excipients to enhance solubility of poorly aqueous soluble compounds. Before investigating the effects of the solubilizers on membrane permeability by PAMPA, concerns were raised about the stability of the phospholipid membranes in the presence of these solubilizers. Previous studies have shown that non-ionic surfactants are distributed between the solution and the membrane to a certain point where the membrane becomes saturated with surfactants. Any further addition of surfactant can then result in the formation of mixed micelles until the membrane bilayer is completely solubilized (22). Among the four solubilizers selected for this study, Brij® 35, Cremophor EL, and Tween 80 are non-ionic surfactants. Studies by Thompson *et al.*, showed evidence that phospholipids form a bilayer lipid membrane on the surface of a hydrophobic polycarbonate filter support (23). Therefore, before adding the four solubilizers to the PAMPA experiment for permeability measurement, it was necessary to study whether the lipid membranes on the filter support in PAMPA would remain stable in the presence of these solubilizers. Lucifer Yellow CH, a fluorescence dye was selected to study membrane leakage in PAMPA with the addition of solubilizers. Studies in the literature have shown that Lucifer Yellow CH does not cross the cell membrane as long as the cell lipid membrane remains intact (24). Thus, in order to measure the stability of the lipid bilayer, the amount of Lucifer Yellow CH found in the acceptor well was measured at various solubilizer concentrations. Figure 3 shows the plots of the fluorescence intensity of Lucifer Yellow CH in the acceptor well (represented as the percentage of Lucifer Yellow CH in the acceptor well) vs. the concentration of the solubilizers. Among the four solubilizers

Table II. UV Absorbance and Permeability Data of 8 Compounds from Non-Filtered and Filtered Sample Solutions at pH 7.4

Compound	UV absorbance of the solutions in the reference well		UV absorbance of the solutions in the donor well after permeation[a]		UV absorbance of the solutions in the acceptor well after permeation[a]		P_e[b] (10^{-6} cm/s)	
	Nonfiltered solution	Filtered solution						
Phenazopyridine	0.79	0.78	0.14	0.14	0.12	0.12	15.53	15.58
Oxyphenbutazone	0.36	0.34	0.28	0.27	0.00	0.00	0.00	0.03
Probenecid	0.37	0.39	0.38	0.40	0.00	0.00	0.00	0.00
Allopurinol	0.61	0.58	0.63	0.64	0.00	0.00	0.00	0.00
Benzthazide	0.22	0.22	0.22	0.22	0.00	0.00	0.16	0.13
Bendroflumethazide	0.33	0.33	0.33	0.33	0.00	0.00	0.00	0.00
Propylparaben	1.61	1.69	0.60	0.64	0.56	0.59	18.14	17.39
Piroxicam	0.99	1.00	0.76	0.77	0.21	0.23	3.05	3.22

[a] UV absorbance values on the left side of the column were determined from the experiment where the non-filtered sample solutions were used to fill the donor compartments. UV absorbance values on the right side of the column were determined from the experiment where the filtered sample solutions were used to fill the donor compartments.

[b] P_e values on left side of the column were obtained from the experiment where the non-filtered sample solutions were used to fill the donor compartments. P_e values on the right side of the column were obtained from the experiment where the filtered sample solutions were used to fill the donor compartments.

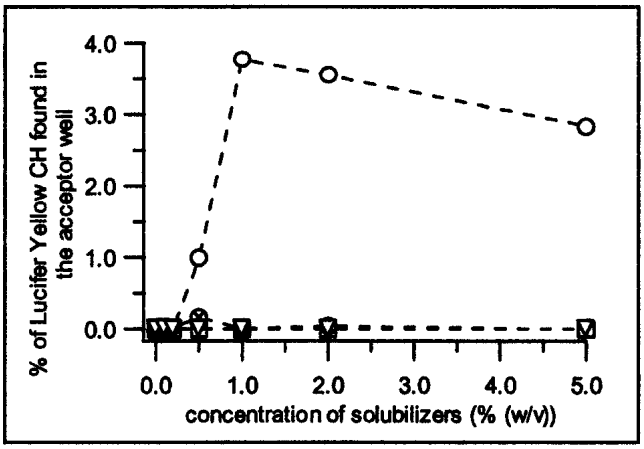

Fig. 3. Fluorescence intensity of Lucifer yellow CH in the acceptor well in the presence of solubilizers, Brij® 35(∇), Cremophor EL (-○-), ethanol (-□-), and Tween 80 (-⊗-). The % of Lucifer Yellow CH in the acceptor well was determined by comparing the fluorescence intensity of the acceptor well to the fluorescence intensity of the reference well.

studied, Brij® 35, ethanol, and Tween 80 appeared to be non-disruptive of the phospholipid membrane in the concentration range studied (i.e., 0.1–5% (w/v)). In contrast, Cremophor EL showed leakage of the lipid membranes at 0.5% (w/v) where Lucifer Yellow CH was first detected in the acceptor well.

After determining the concentration range of solubilizers in which the lipid membrane was stable, the permeability of two poorly aqueous soluble compounds, amiodarone and miconazole, were measured by PAMPA in the presence of the two solubilizers, Brij® 35 and Tween 80. Table III summarizes the solubility and *in vitro* permeability values of amiodarone and miconazole measured by PAMPA using 0.2% (w/v) Brij® 35 or 0.2% (w/v) Tween 80 as solubilizers and LC/MS for detection. Both excipients completely solubilized the compound added to the buffer solution (i.e., 50 μM) enhancing the aqueous solubility of the two compounds by more than 500 fold. As shown in Table I, both amiodarone and miconazole resulted in "undetected" permeability values when no solubilizers were added. However, in the presence of either 0.2% (w/v) Brij® 35 or Tween 80, miconazole became highly permeable (i.e, $P_e > 1.0 \times 10^{-6}$ cm/s). Conversely, amiodarone showed low permeability ($P_e < 0.1 \times 10^{-6}$ cm/s) in 0.2% Tween 80 and only moderate permeability (i.e., 0.1×10^{-6} cm/s $< P_e < 1.0 \times 10^{-6}$ cm/s) in 0.2% Brij® 35. This data suggests that use of solubilizers in PAMPA can be effectively applied to screen the membrane permeability of poorly aque-

Table III. Aqueous Solubility and In Vitro Permeability (P_e) of Amiodarone and Miconazole in the Presence of Solubilizers as Measured by LC/MS

Compound	Solution with 0.2% Brij® 35		Solution with 0.2% Tween 80	
	P_e (10^{-6} cm/s)	Solubility (μM)	P_e (10^{-6} cm/s)	Solubility (μM)
Amiodarone	0.28 ± 0.06	>50	0.05 ± 0.01	>50
Miconazole	2.45 ± 1.42	>50	1.16 ± 0.32	>50

ous soluble compounds and, therefore they assist in the selection of compounds that possess the desired permeability. The study also suggests that we could use a set of commonly used solubilizers at low concentration (e.g., 0.2% Brij® 35 and 0.2% Tween 80) in the PAMPA to screen *in vitro* permeability of compounds. This is particularly valuable for poorly water-soluble compounds, which often precipitate in the high-throughput screening of discovery compounds in aqueous buffer. Another interesting finding was that Brij® 35 and Tween 80 had different effects on the membrane permeability of amiodarone. Brij® 35 gave a permeability value that was almost five times higher than that observed with Tween 80. The simplicity of the PAMPA assay system, however, cannot describe the interaction of solubilizers with the gastrointestinal milieu which involves a much more complex biologic system. The assay can help us rapidly evaluate the permeability of poorly water-soluble compounds in the presence of a various different solubilizers that could, in part, help us select solubilizer to assist formulation of poorly water-soluble compounds directed toward *in vivo* studies.

Figures 4 and 5 show respectively, LC/MS ion chromatograms, of amiodarone and miconazole in the reference, donor, and acceptor wells of the PAMPA experiment in the presence of 0.2% (w/v) Brij® 35 and 0.2% (w/v) Tween 80. As shown in Fig. 4 and 5, significant amounts of amiodarone and miconazole were found in the acceptor wells once solubilizers were added in the PAMPA. LC/MS was able to differentiate the amiodarone peak from an impurity peak, which was introduced by the Brij® 35 solution used (see Fig. 4A). This further demonstrates the advantage of the LC/MS detection method vs. the UV-VIS detection method because there is no separation or specificity in the latter.

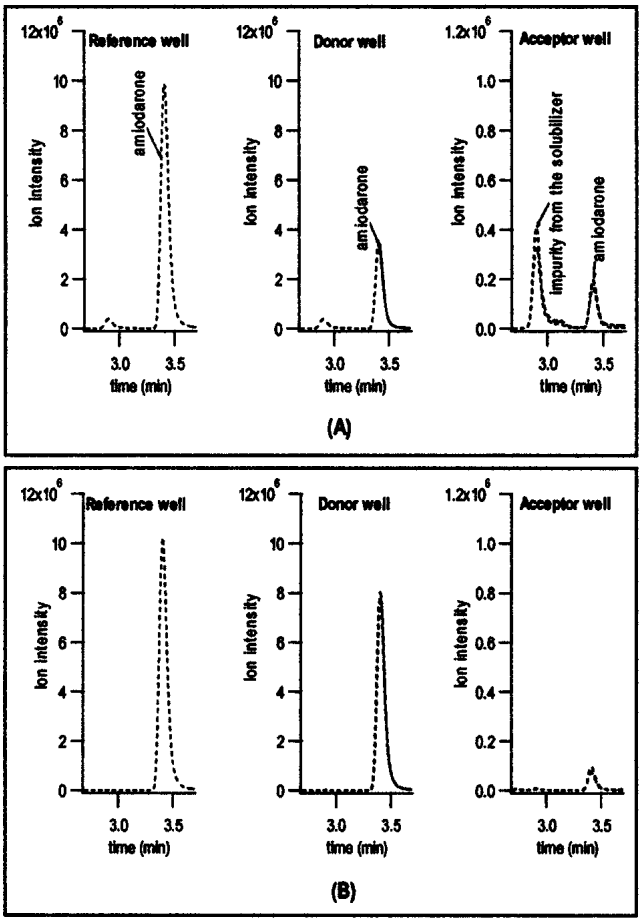

Fig. 4. LC-MS chromatograms of amiodarone samples collected from the reference, donor, and acceptor wells of the PAMPA experiment with 0.2% Brij® 35 (A) and 0.2% Tween 80 (B). The three ion chromatograms of the test sample from the acceptor and donor wells were overlaid as the sample was run in triplicate. To better view the impurity and the amiodarone peak in the acceptor wells, the scale of the y-axis is 10x less than in the other graphs.

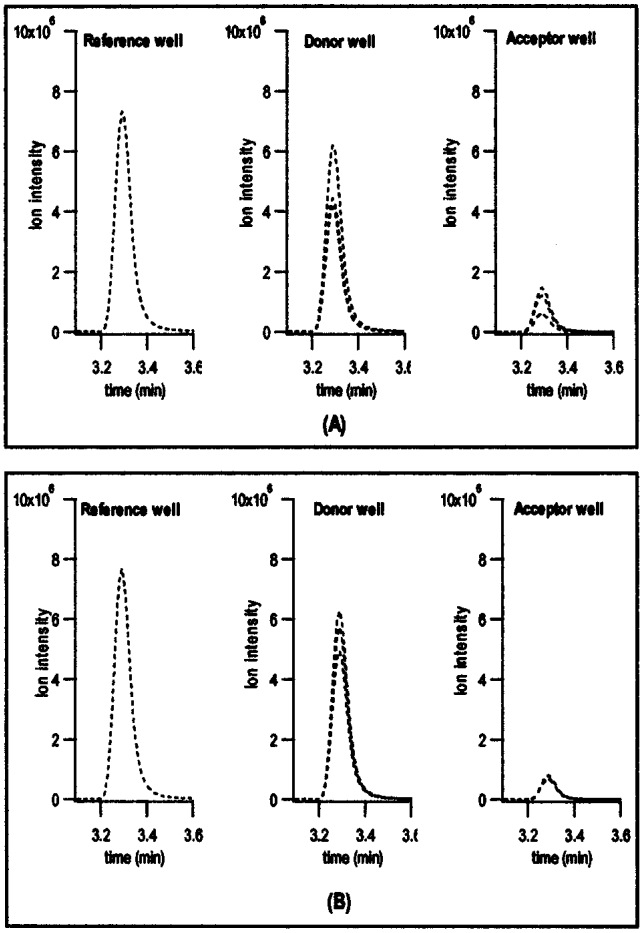

Fig. 5. LC-MS chromatograms of miconazole samples collected from the reference, donor, and acceptor wells of the PAMPA experiment with 0.2% Brij® 35 (A) and 0.2% Tween 80 (B). The three ion chromatograms of the test sample from the acceptor and donor wells were overlaid as the sample was run in triplicate.

CONCLUSIONS

This work successfully applied solubilizers to the PAMPA experiment for measuring *in vitro* membrane permeability of compounds with low aqueous solubility. The PAMPA system coupled with LC/MS detection allows for a rapid examination of the effects of various surfactants and potentially different formulations that will effect the *in vitro* membrane permeability of compounds.

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