

Poor pharmacokinetics and lack of efficacy are together responsible in most of the cases in which a new drug compound fails to proceed to market. Due to the increase in lead substances in drug discovery, fast and predictive assays are required to attain improved oral drug absorption and brain penetration at a much earlier stage in drug discovery. We report here a novel *in vitro* method based on the amphiphilicity of the drugs. Amphiphilicity can be assessed through surface activity profiling, which includes CMC/solubility, air/water partitioning coefficient, and molecular cross sectional area. However, the measurement of surface activity has been exceedingly slow, consuming large amounts of material. To resolve this bottleneck Kibron Inc. has developed a new platform, the Delta-8 multichannel microtensiometer, which allows surface activity profiling to be performed in a standard 96-well microplate format, while requiring only 5-30 micrograms of compound. Reading one plate takes less than three minutes and yields the surface activity profile for 8 compounds.

INTRODUCTION

Amphiphilic compounds, due to their dualistic nature with polar and non-polar moieties have the affinity for both aqueous and nonaqueous solvents/media. For the same reason they have the tendency to adsorb into biological membranes without requirement of energy. This passive diffusion of drugs into membranes is dependent on the size, shape, and lipophilicity of the compounds. Additional constraints affecting diffusion are set by the inhomogeneous and anisotropic character of the membrane. An air-water interface is capable of reproducing similar anisotropic partitioning/ordering of the amphiphilic molecules. The processes of adsorption and micelle formation are also spontaneous and occur because of hydrophobicity. Parameters such as amphiphilicity and separation of the polar and non-polar parts in the molecules can be derived by measuring physicochemical changes in air-water interfacial tension.

METHOD

In Kibron method amphiphilicity is assessed through surface activity of the drugs. Surface tension changes induced by adsorption and aggregation of amphiphilic molecules are monitored against the concentration, yielding thermodynamic parameters as CMC/solubility, air-water partitioning coefficient, and molecular cross sectional area (Fig. 1). Assays are performed on serial dilutions in 96-well plates and read using the surface tension microplate reader (Fig. 2)

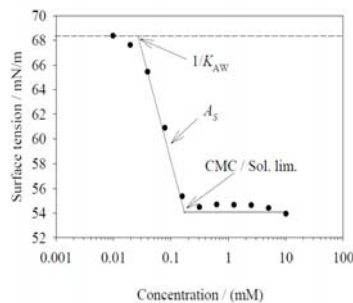


Fig 1. Characteristic parameters obtained from the adsorption isotherm: critical micelle concentration (CMC), air/water partitioning coefficient (K_{AW}), and molecular cross sectional area (A_s).



Fig. 2: A high through-put eight-channel tension microplate reader for measuring surface tension and screening of CMC (Delta 8, Kibron, Inc., Helsinki, Finland).

RESULTS&DISCUSSION

The current poster compiles the results from three different permeability studies using a Kibron Delta-8. The *in vitro* assay based on surface activity profiling was compared with other currently used methods to predict passive intestinal absorption and blood brain barrier permeation.

Figure 3 summarizes the results by Dressman et al.^{1,2} Surface activity approach was shown to best model the human oral absorption (FA, %). The lower correlation using log P is evident as octanol as an isotropic phase can not mimic natural membrane barriers, made of ordered lipids. The relationship between oral absorption and PAMPA is also poor with many outliers, underestimating the permeation.

The use of another common lipophilicity (in silico) descriptor PSA (Polar Surface Area), was recently reviewed against the surface activity assay in the prediction of blood-brain barrier permeability.³ Figure 4 compares those results. The two vertical dotted lines shows the upper thresholds reported by Kelder et al.⁴ ($PSA < 60-70 \text{ \AA}^2$) and van de Waterbeemd et al.⁵ ($PSA < 90 \text{ \AA}^2$) for brain penetration. These limits give 67% correct below 65 \AA^2 and 68% correct below 90 \AA^2 cutoff. The prediction accuracy using surface activity is 75%.

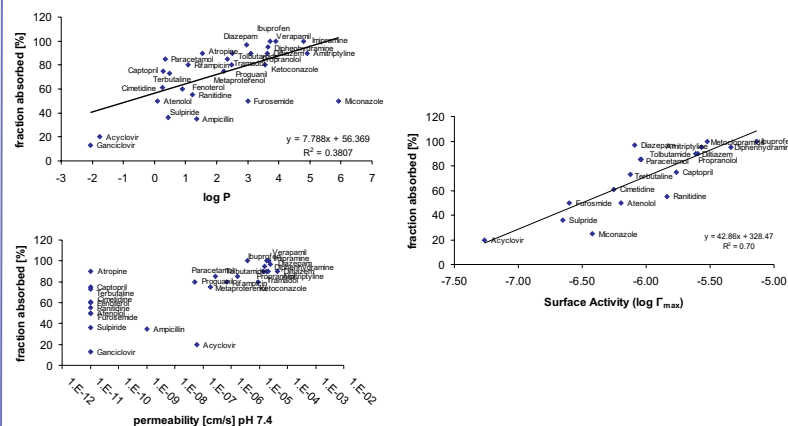


Fig 3. Correlation between fraction absorbed and log P, PAMPA, Surface Activity^{1,2}

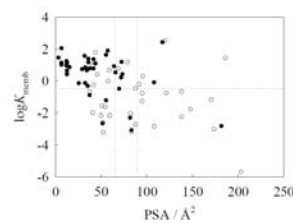


Fig 4. Comparison between surface activity and polar surface area (PSA) for the prediction of blood-brain barrier permeability. • CNS positive drug, ○ CNS negative drug.³

ADVANTAGES OF SURFACE ACTIVITY APPROACH

- Surface activity profiling correlates well with fraction absorbed data
- SAP technique models intestinal absorption better than PAMPA
- Results with SAP approach are superior to log P
- Additionally advantages of the method are:
 - Excellent reproducibility
 - High throughput
 - No need for chemical analysis of the compound

Limitations:

- Only surface active molecules can be estimated
- Cannot be assumed that no surface activity means no absorption

CONCLUSIONS

Surface activity profiling provides a fast, *in vitro* method for assessing membrane partitioning. It is purely a physicochemical measurement and because of this the lab to lab variation is negligible. Furthermore, the values measured can be compared on an absolute basis enabling rational analysis and comparison with *in vivo* data. Importantly, when combined with the PLDscreen the surface activity parameters can be obtained as a spin-off.⁶

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