Determination of log *D* via Automated Microfluidic Liquid−Liquid Extraction

Muhammad Alimuddin,[†] Daniel Grant,[‡] Daryl Bulloch,[†] Noelle Lee,[†] Martin Peacock,[§] and Russell Dahl*[,‡]

Pfizer Inc., 10770 Science Center Drive, San Diego, California 92121, Burnham Institute for Medical Research, 10901 N. Torrey Pines Road, La Jolla, California 92037, and Syrris Inc., 27 Jarman Way, Royston, U.K.

Received May 6, 2008

A method for log *D* (pH 7.4) measurement was developed using microfluidic liquid−liquid extraction. Values were determined for 26 compounds and compared to results obtained via shake-flask methods. Excellent correlation between the values obtained via both methods was achieved ($R^2 = 0.994$). The developed methodology is amenable to automation, enabling high-throughput determination of large compound collections.

Introduction

In the development of new medicines, less than 20% of drug discovery projects reach the clinic, and of those less than 10% will become drugs.[1] This high level of candidate attrition is mostly a consequence of poor pharmacokinetics and toxicity. This has spurred profound interest in the development of in vitro methods to predict bioavailability of specific chemical entities.[2] Outside of measuring a compound's in vivo pharmacokinetic profile, the most effective means of predicting how a molecule of interest will behave systemically is to assess its ADME[a] (absorption, distribution, metabolism, excretion) parameters, preferably at an early stage in the discovery process. Of these parameters, a molecule's lipophilicity is of primary importance and greatly influences overall bioavailability.[3]

Perhaps the most effective and reliable means of estimating a molecule's lipophilicity is measuring the octanol−water distribution coefficient (log *D*) via the shake-flask method.[4–6] In addition to predicting passive permeation through the gut wall, log *D* has also been shown to correlate to a number of other ADME parameters, including blood−brain barrier permeability[7] and plasma protein binding.[8] While the shake-flask method is an operationally simple method to obtain meaningful compound partition data, it is time-intensive and requires significant amounts of materials and octanol to achieve accurate readings. These limitations preclude the use of this method in testing large libraries or compounds whose availability is limited. Herein, we present the development of a method to assess a compound's partition behavior in an automated format using microfluidic liquid−liquid extraction. This method was performed in parallel with traditional shake-flask methods, and an excellent correlation was achieved ($R^2 = 0.994$, Figure 1).

Results and Discussion

Microfluidic technologies have made an impact in various areas of biomedical research and drug discovery.[9] The major advantage of microfluidics is the ability to manipulate minute volumes of materials, thus allowing for precise control over volume and mixing conditions. This technology is amenable to the development of intricate, multiplexed sequences with the ability to scale for mass production applications.

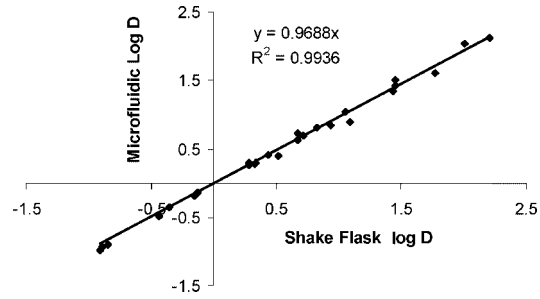

Figure 1. Comparison of log $D_{(7.4)}$ values obtained from the microfluidic method and traditional shake-flask method.

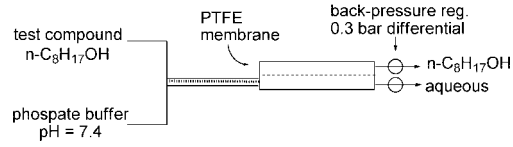

Figure 2. Microfluidic liquid−liquid extraction apparatus.

Continuous flow liquid−liquid extraction routines have been reported in microfluidic systems,[10] enabling the separation of organic−aqueous phases. Microfluidic liquid−liquid extraction involves the separation of immiscible liquids by introducing them to one side of a microfluidic channel that is partitioned along its length by a poly(tetrafluoroethylene) (PTFE) membrane. A pressure differential across this membrane is controlled using back pressure regulators. As the mixture moves through the channel, the differential in pressure drives the organic phase through the membrane while the aqueous phase is retained (Figure 2).

We envisioned the determination of partition values of small molecules via an octanol−water system using a commercially available microfluidic liquid−liquid extraction system. This system comprises a T-fitting, a separation chip, and two back pressure regulators. The separation chip consists of a microchannel divided by a PTFE membrane, an inlet at the beginning of the channel, and two outlets at the end, one on either side of the membrane. The back pressure regulators are used to control the cross membrane pressure and ensure the migration of all the organic phase through the membrane.

Solutions of the test compounds (5.0 mM) were prepared using water-saturated *n*-octanol.[11] Microfluidic log *D* measure-

* To whom correspondence should be addressed. Phone: 858-646-3100, ext. 3327. Fax: 858-646-3193. E-mail: rdahl@burnham.org.
[†] Pfizer Inc.
[‡] Burnham Institute for Medical Research.
[§] Syrris Inc.

[a] Abbreviations: ADME, absorption, distribution, metabolism, excretion; PTFE, poly(tetrafluoroethylene); HPLC, high-performance liquid chromatography; LCMS, liquid chromatography mass spectrometry.

10.1021/jm8005228 CCC: $40.75
Published on Web 07/31/2008

Table 1. Comparison of log $D_{(7.4)}$ Values of Representative Compounds Obtained via Shake-Flask and Microfluidic Methods, $n = 3$

	measured log $D_{(7.4)}$		
compd	shake flask	microfluidic	microfluidic standard deviation
acebutolol	−0.44	−0.48	0.051
acetaminophen	0.33	0.27	0.018
caffeine	−0.13	−0.14	0.017
chloramphenicol	1.05	1.04	0.015
atropine	−0.35	−0.36	0.013
diphenhydramine	0.67	0.72	0.015
fluconazole	0.44	0.41	0.008
hydrocortisone	1.45	1.42	0.073
hydrocortisone 21-acetate	2.21	2.11	0.104
cimetidine	0.34	0.29	0.012
desipramine	1.09	0.89	0.140
diclofenac	0.83	0.81	0.015
procainamide	−0.89	−0.94	0.131
propafenone	1.77	1.60	0.069
propranolol	0.72	0.69	0.010
quinidine	1.45	1.50	0.065
ranitidine	−0.91	−0.99	0.028
scopolamine	0.28	0.30	0.031
tiapride	−0.85	−0.90	0.026
trimethoprim	0.67	0.63	0.033
atenolol	−0.16	−0.19	0.033
3-chlorophenol	2.01	2.04	0.074
chlorpheniramine	0.28	0.26	0.027
chlorthalidone	0.94	0.85	0.099
disopyramide	0.52	0.39	0.010
imipramine	1.43	1.33	0.283

ments were obtained by combination of the octanol solution and the aqueous phosphate buffer (pH 7.4) in the T-fitting. The two immiscible solutions formed a continuously flowing stream of alternating microdroplets. The high surface to volume ratio in the microdroplets allowed for rapid partitioning between the two phases. A flow rate of 35 μL/min for the octanol and aqueous streams proved to be the most effective for complete separation of the phases. The admixture was passed through a capillary (10 mm × 0.25 mm i.d.), allowing for a contact time of roughly 6 s before reaching the separation membrane. The separated streams were collected for analysis, and the concentrations of the organic and aqueous phases were determined by high-performance liquid chromatography (HPLC) using area under the curve methods for quantification. The log D values were calculated via the method of Scherrer and Howard.[12] Shake-flask measurements for comparison were obtained using the EPA Product Properties Test Guidelines (OPPTS 830.7550).

The correlation of the measured log D values in Table 1 validates the utility of this method for the routine profiling of drug candidates. The range of lipophilicity values (−0.89 to 2.21) and variety of chemotypes tested demonstrate that the microfluidic testing method produces reliable partition coefficients over a broad range. It should also be noted that apart from the means of bringing the two phases together (shake flask or microfluidic), all other parameters (e.g., analytical methods, stock solutions) were identical, limiting the potential for additional sources of error and further establishing the interchangeable nature of these two methods.

Interestingly, there appears to be a minor "membrane effect" imposed by the interaction of the PTFE membrane with the compounds tested. This phenomenon is evident in the slight depression of the slope obtained via regression analysis (Figure 1) and in an average difference of −0.05 in microfluidic log D values across the entire data set. This result is likely caused by the actual retention of lipophilic compounds within the membrane itself.

Conclusion

A microfluidic method of log D measurement was developed that generated values showing remarkable correlation to the established shake-flask methods. This method is operationally simpler than prior methods of log D determination and provides values in very short time frames. In addition, the developed methodology is amenable to robotic automation, enabling the determination of log D values for large collections of compounds in an efficient manner.

Experimental Section

General Procedure for Microfluidic log D Measurement. A solution of the compound to be analyzed was prepared in water-saturated *n*-octanol (0.5 mM). Phosphate buffer and water saturated *n*-octanol were pumped through the microfluidic system via a dual channel Syrris reagent pump module at a rate of 35 μL/min each. The compound solution in *n*-octanol (0.5 mM) was introduced to the flow stream by way of a 1.0 mL sample injection loop. The flow steams were attached to the inlets of a Syrris FLLEX extraction module. A back pressure of 3.0 bar was maintained on the aqueous outlet of the FLLEX, while the organic outlet's back pressure was maintained at a 300 mbar deficit to the aqueous outlet by way of the FLLEX module's internal pressure differential control valve. The separated phases were collected in triplicate and analyzed via LCMS. The log $D_{(7.4)}$ values were calculated using eq 1.

$$\log D = \log(\text{response ratio}) \tag{1}$$

$$\text{response ratio} = \frac{(\text{peak area compd in } n\text{-octanol})}{(\text{peak area of compd in buffer (pH 7.4)})}$$

Analytical. All LCMS data were gathered on an Agilent 1100 LC with MSD (Agilent model G1946B upgraded to D model) single-quadrupole mass spectrometry detector with electrospray spray ionization source. The LC instrument includes a binary pump (Agilent model G1312A) with an upper pressure limit of 400 bar attached to an autosampler (Agilent model G1313A) that uses external try for sample submission. The column compartment (Agilent model G1316A) is attached to a diode-array detector (Agilent model G1315A). The instrument acquisition and data handling were done with ChemStation, revision B.02.01. Column was a Xbridge C18, 2.1 mm × 30 mm, 2.5 μm particle size with column temperature of 60 °C. Solvent A was water (0.1% formic acid and 0.05% ammonium formate), and solvent B was acetonitrile (5% water with 0.1% formic acid and 0.05% ammonium formate). The gradient ran from 5−95% B in 2.5 min and then 95% B in 2.5−3.0 min with a flow rate of 1.2 mL/min.

Chemicals. All solvents were of HPLC Chromasolv grade, from Sigma Aldrich (St. Louis, MO). Water was purified by a Millipore MilliQ Gradient (Billerica, MA). Most of the chemicals and buffers were purchased from Sigma Aldrich, and all were 97% in purity or higher and used as purchased.

Supporting Information Available: Analytical data used in the determination of log D values for both the shake flask and microfluidic methods. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Pauwels, R. Aspects of successful drug discovery and development. *Antiviral Res.* **2006**, *71*, 77–89.
(2) Kerns, E.; Di, L. *Drug-Like Properties: Concepts, Structure Design and Methods*.Elsevier: Amsterdam, 2008.

(3) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Delivery Rev.* **2001**, 3–26.

(4) Lombardo, F.; Shalaeva, M. Y.; Tupper, K. A.; Gao, F. $ElogD_{oct}$: a tool for lipophilicity determination in drug discovery. 2. Basic and neutral compounds. *J. Med. Chem.* **2001**, *44*, 2490–2497.

(5) Leo, A.; Hansch, C.; Elkins, D. Partition Coefficients and their Uses. *Chem. Rev.* **1971**, *71*, 525–616.

(6) Sangster, J. *Octanol−Water Partition Coefficients: Fundamentals and Physical Chemistry*; Wiley: New York, 1997.

(7) Liu, X.; Tu, M.; Kelly, R. S.; Chen, C.; Smith, B. J. Development of a computational approach to predict blood−brain barrier permeablity. *Drug Metab. Dispos.* **2004**, *32*, 132–139.

(8) Colmenarejo, G. In silico prediction of drug-binding strengths to human serum albumin. *Med. Res. Rev.* **2003**, *23*, 275–301.

(9) Kang, L.; Chung, B. G.; Langer, R.; Khademhosseini, A. Microfluidics for drug discovery and development: from target selection to product lifecycle management. *Drug Discovery Today* **2008**, *13*, 1–13.

(10) Kralj, J. G.; Sahoo, H. R.; Jensen, K. F. Integrated continuous microfluidic liquid−liquid extraction. *Lab Chip* **2007**, *7*, 256–263.

(11) Carlsson, K.; Karlberg, B. Determintation of octanol−water partition coefficients using micro-volume liquid−liquid extraction system. *Anal. Chim. Acta* **2000**, *423*, 137–144.

(12) Scherrer, R. A.; Howard, S. M. Use of distribution coefficients in quantitative structure−activity relations. *J. Med. Chem.* **1977**, *20*, 53–58.

JM8005228