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A practical deuterium-free NMR method for the rapid determination of 1-octanol/water partition coefficients of pharmaceutical agents

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ABSTRACT

A simple and rapid NMR method is described to determine the log *P* of pharmaceutical agents. This method is highly versatile and efficient, because it does not require the use of deuterated solvents or the addition of any internal/external standards to the sample. We demonstrate that log *P* can be accurately measured using NMR for pharmaceutical agents with known log *P* values. Our proposed method is made possible by the combination of state-of-the-art NMR techniques including the solvent concentration reference and robust solvent suppressions.

The octanol/water partition coefficient (log *P*) is the log of the ratio of the concentration of a compound at equilibrium partitioning between 1-octanol and water. In drug discovery, log *P* is an important physiochemical property of a drug candidate, as it is a critical parameter for QSAR and ADME.[1] The importance of log *P* has become more evident since Lipinksi's 'rule of five' was proposed for drug discovery.[2] Various methods have been developed for the routine estimation of log *P*[3–6] as well as measurement of solubility for potential drug candidates.[7–14] Although the theoretical calculation of log *P* is superior in efficiency, especially for large collections of compounds, the direct measurement can provide a higher level of accuracy and ultimately validate the calculations. New experimental techniques are continually being developed to increase the throughput of measurement of solubility (and log *P*) for libraries of molecules. For example in 2009, Tischler and co-workers described a novel NMR method to measure aqueous solubility using small quantities of deuterated solvents and an internal standard (3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid, sodium salt).[7] Also in 2009, Posner and co-workers detailed a microfluidic method to measure octanol–water partitioning in picoliter drops.[8] Both of these methods have key advantages over the traditional shake-flask method with HPLC–UV analysis.[15] In the case of the NMR method, it is uniquely suited for solubility and log *P* determinations, because NMR instruments are readily accessible in many research settings and also serve a dual role of structural determination and quantitative analysis. However, the requirement of deuterated solvents and internal standards for the measurement of aqueous solubility is not amenable to analyzing large numbers of compounds and the presence of additives can obscure a translation to pure solvents. In this Letter, we will describe an NMR method of direct detection to determine analyte concentrations in water and 1-octanol (after equilibrium partitioning) without deuterated solvents or the introduction of internal standards. This result was achieved with a combination of state-of-the-art NMR techniques using a novel solvent concentration reference method that we recently developed[16] and a robust solvent suppression sequence based on WET.[17] As such, this method provides an easy, efficient, and accurate way to determine log *P* using only NMR.

The feasibility and accuracy of NMR concentration determination from low micromolar concentrations to more than 100 M was demonstrated by us using only the solvent water as the concentration reference.[16] With minimal modifications, this method can be readily translated to other solvents, such as 1-octanol. We have selected pharmaceuticals with known log *P* values not only to demonstrate the implementation of our NMR technique but also to illustrate key issues when measuring log *P*. Our examples include acidic, basic, and neutral drugs to represent the typical scenarios in drug discovery.[7] The pharmaceutical agents were dissolved to near saturation in water and 1-octanol with the usual shake-flask equilibration, and log *P* was calculated from the concentrations determined in the two phases by NMR, as almost every pharmaceutical agent contains at least one proton signal for observation. All of the NMR spectra were acquired directly from the

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aqueous and octanol phases after separation. No additional special sample preparation (i.e., no additives as standards or deuterated solvents) or manipulations are needed. The importance and convenience in avoiding deuterated solvents has been highlighted by Hoye et al. in their elegant 'no-D NMR method' to determine the concentrations of organolithium reagents.[18,19] In our method, the analyte was observed in 1D proton spectra either after WET-based solvent (water or octanol) suppression[17] if their concentrations were low (<10 mM) or with the solvent if the concentrations were sufficiently high. If needed, the strong solvent signals were observed separately for accurate concentration referencing.[16] The solvent reference is superior in its universal presence, its predictability, and its accuracy for known concentrations. For example, the total proton concentrations of neat water and 1-octanol are 110.7 M and 114 M, respectively. Even in the presence of a modest amount of organic solutes (5% or less), the total proton concentrations are not expected to vary more than 5% from the neat solvent concentrations. Hence, water and 1-octanol (and others, see below) can be used as native internal concentration references in their own solutions. Under ideal conditions, the NMR peak integration A is proportional to the associated proton concentration C and the sine of the pulse excitation angle θ (no larger than 90°; subscripts s and a represent the solvent and analyte, see Eq. (1)). More frequent than not, the lipophilic pharmaceutical agent can be observed without any solvent suppression in 1-octanol. Hence, this equation readily leads to the determination of the solute, based on comparisons of peak integrations (Eq. (1)). On the other hand, the solute concentration in water tends to be much lower. As such, the solvent water can be observed with a very small excitation angle and solute observed in a separate but almost identical acquisition with the only changes as solvent suppression and larger excitation angle.

$$\frac{A_a}{A_s} = \frac{c_a \sin(\theta_a)}{c_s \sin(\theta_s)} \tag{1}$$

For example, Figure 1 demonstrates how the log P of acetaminophen was determined with ease by NMR. Figure 1a shows the spectrum of acetaminophen in the aqueous layer after a 0.53° pulse excitation. The peak integration ratio between the four aromatic protons (Fig. 1a inset) and all protons in the solution (from 0 to 10.0 ppm) is found to be 0.357:100. According to Eq. (1), with the total proton concentration assumed as 110.7 M, the concentration of acetaminophen in the aqueous phase can be calculated as 98.7 mM. In Figure 1b, acetaminophen can be cleanly observed along with the solvent octanol after a small angle pulse excitation. Based on the peak integration ratio (0.669:100), and the assumption of total proton concentration of 114 M, the concentration of acetaminophen in the octanol layer can be calculated as 191 mM. Then log P of acetaminophen is determined as 0.29.

For more lipophilic compounds, the solubility in the aqueous phase is likely to be low. It is frequently preferred and more time-effective to observe the analyte signal after the solvent suppression and larger pulse angle excitation, while the much stronger solvent signal can be observed separately. Figure 2 demonstrates how the concentration of naproxen can be determined by such an approach. Naproxen is an acidic compound and typically, the log P of these types of compounds is measured at low pH (e.g., 0.1 M HCl). In Figure 2b, the solvent signal is observed with a (0.30°) pulse excitation and the integration of all protons in the solution can be normalized to 1005. Figure 2a shows the full spectrum of naproxen acquired under the same conditions except that excitation angle is 90° and the solvent is suppressed. The six aromatic protons (Fig. 2a inset) can be integrated as 5.74 (using the same normalizing factor as above). Hence the total concentration of naproxen (mainly in acidic form, due to the presence of 0.1 M HCl during partition) is 68 μM. On the other hand, its concentra-

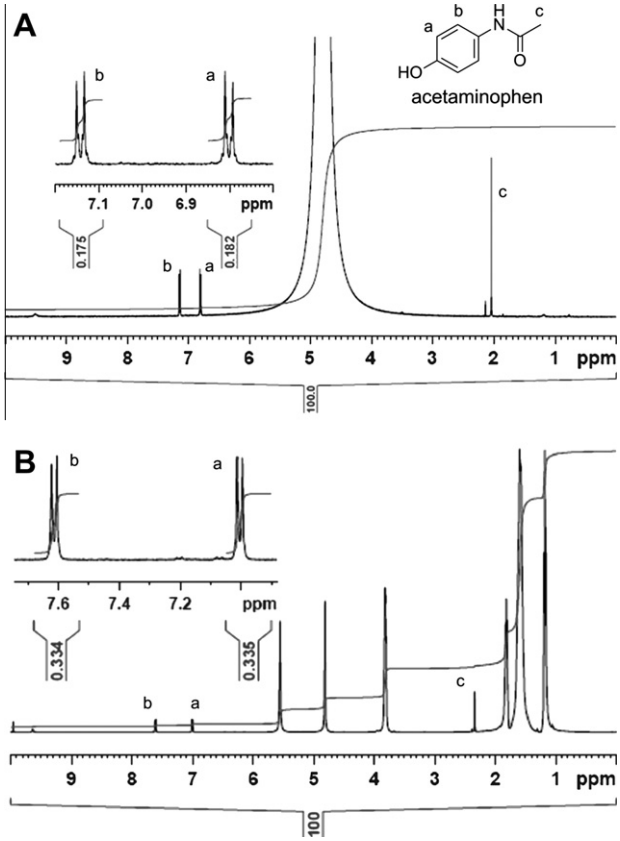

Figure 1. (A) Acetaminophen in the aqueous layer can be observed after a small angle pulse excitation along with solvent water. Its concentration is calculated as 98.7 mM, from the peak integration ratio between the aromatic resonance (inset) and all protons in the solution (from 0 to 10 ppm). (B) Acetaminophen in the octanol layer can be observed similarly along with solvent octanol. Its concentration is calculated as 191 mM, from the peak integration of the aromatic resonance (inset) and all protons in the solution (from 0 to 10 ppm).

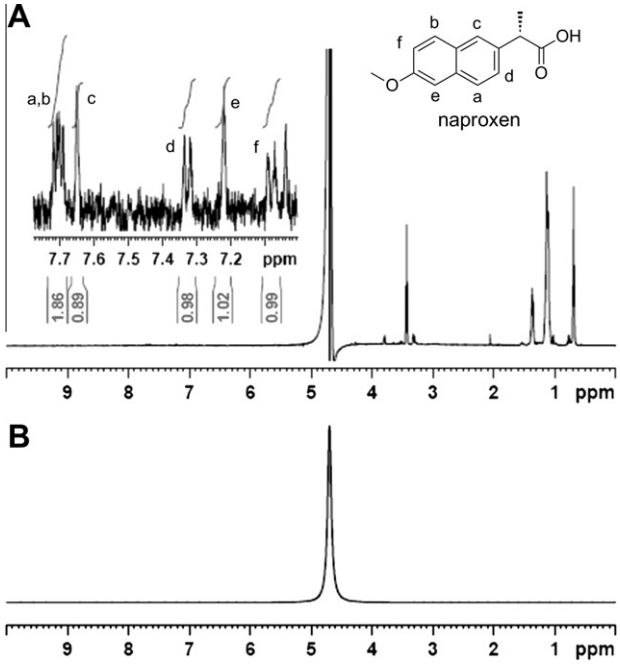

Figure 2. (A) Naproxen in the aqueous layer (i.e., 0.1 M HCl) can be observed after WET solvent suppression and 90° pulse excitation (64 scans; inset is zoomed in by 32 times). (B) The solvent (water) signal is observed with a 0.30° pulse excitation (eight scans) with the peak integration as 1005 (not shown).

tion of in the octanol layer can be found the same way as described in Figure 1 as 144 mM. Hence, the log *P* of naproxen is determined to be 3.32 for this sample.

It is also quite easy to evaluate the quality of NMR concentration determination. As shown in Eq. (1), the accuracy depends on peak integrations for solvent and analyte, the reference solvent concentration, and pulse angle calibrations. First, modern NMRs have linear transmitters enabling error in pulse angle determination as small as 1%.[20] Second, the reference solvent concentration rarely changes under our typical experimental conditions, especially when the solute concentration is very modest. It is as unbiased as any other internal reference, because all protons, regardless of chemical shift or line-shape or instrument set-up, have the same efficiency in eliciting observed NMR signal amplitude (peak integration). Third, the error in analyte signal integration can be readily evaluated through its signal-to-noise ratio analysis and multiple measurements. Within a single measurement, self-consistence test can be conducted for stoichiometry by integrating individual resonance of known compounds. In our experience, proton signals above 100 μM concentration can be reliably measured in several minutes. Furthermore, the direct observation of the clean analyte NMR signal minimizes concern for interference of impurities, decomposition, or solvent/analyte reactions (i.e., hydrolysis). For an example, in Figure 2a, it can be easily seen that the six aromatic protons are free from any other spectroscopic interference and their integrations are self-consistent in meeting the proton ratios predicted by the molecular formula.

The log *P* values for acetaminophen, aspirin, chloramphenicol, erythromycin, lidocaine, naproxen, nitrofurantoin, phenacetin, and procaine were determined by NMR and compared to their reported values (Table 1).[6,9,10,21–23] These drugs are representative of different therapeutic categories and span a reasonable range of molecular structures. Lidocaine, naproxen, and procaine are examples of basic or acidic drugs; the other drugs are neutral. Our log *P* values are the average of three measurements by NMR and compared favorably to the known literature log *P* values for each pharmaceutical. In the case of naproxen, to replicate the literature value of 3.34,[21] we conducted the log *P* measurement using 0.1 M HCl rather than water. Likewise for procaine, water was exchanged for 0.1 M NaOH. Indeed, the aqueous solubility (and measured log *P*) for compounds with strongly acidic or basic functional groups is highly dependent on pH.[13,14] Also, other factors can affect log *P* measurements, and many other additives are commonly included in the aqueous solution, such as salts, DMSO, solubilizers, and emulsifiers.[7,24,25] These situations routinely occur when comparing measured log *P* values to previously reported values and even the presence of 1-octanol dissolved in water can further obscure an accurate log *P* assessment.[25]

Table 1
Comparison of log *P* values determined by NMR to values reported in the literature

Compd	NMR log *P*[a]	Lit. log *P*
Acetaminophen	0.29 ± 0.001	0.33[e], 0.51[d]
Aspirin	1.15 ± 0.01	1.19[d]
Chloramphenicol	1.05 ± 0.01	1.05[e], 1.14[d]
Erythromycin	2.01 ± 0.03	2.54[h]
Lidocaine	2.24 ± 0.02	2.26[d], 2.33[f]
Naproxen	3.34 ± 0.02[b]	3.24[g], 3.34[d]
Nitrofurantoin	−0.36 ± 0.06	−0.68[i]
Phenacetin	1.45 ± 0.04	1.58[d]
Procaine	0.90 ± 0.02[c]	−0.89[e], 0.88[d]

[a] Values are presented as the average ± standard deviation of three samples in water and 1-octanol after measurement by NMR.
[b] Values were measured by NMR after partitioning 0.1 M HCl and 1-octanol.
[c] Values were measured by NMR after partitioning between 0.1 M NaOH and 1-octanol.
[d] See Ref. 6.
[e] See Ref. 9.
[f] See Ref. 10.
[g] See Ref. 14.
[h] See Ref. 22.
[i] log *P* value for pH 7.0, see Ref. 23.

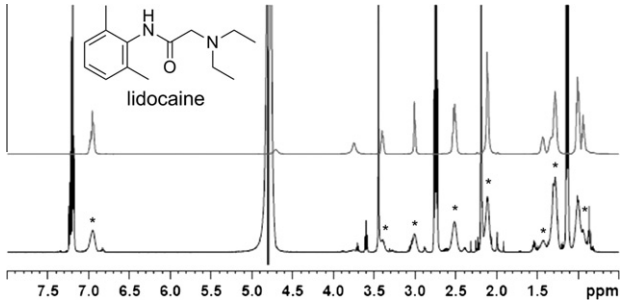

Figure 3. Lidocaine contained in octanol droplets (indicated by *) can be observed in the aqueous layer due to incomplete separation of phases. Top trace: lidocaine in octanol; bottom trace: lidocaine in aqueous layer with some octanol droplets due to incomplete phase separation.

Another benefit of determining log *P* by NMR is that the dissolved 1-octanol in water can be observed and its exact amount determined with ease. Also, the presence and concentration of analyte (i.e., pharmaceutical agent) dissolved in the small quantities of octanol droplets in the aqueous layer due to incomplete separation can be determined. An example is illustrated in Figure 3 where a small set of peaks correspond to lidocaine dissolved in octanol droplets that are, in turn, suspended in the aqueous layer. Small quantities of emulsified octanol in the aqueous phase can erroneously lead to higher concentrations of analyte in water.[25] This final point distinguishes NMR from other analytical tools used for log *P* measurement, because structural information (chemical shift, J-coupling, line-shape, etc...) for the analyte is gathered simultaneously.

In summary, this NMR method offers an alternative to other procedures for the measurement of log *P* in drug discovery. It is superior in its direct detection of analyte, simple data interpretation, and minimal sample manipulation. On the other hand, one main concern is the NMR's sensitivity, which may require hours acquisition time if sample concentration falls under 1 μM. Another concern is the lack of clear analyte signal due to interference by the solvent (water or 1-octanol). Nevertheless, both of those situations occur rarely in drug discovery. First, a drug candidate typically needs at least a modest level of aqueous solubility for good bioavailability. Second, most drug-like compounds have proton signals that can be readily identified and are distinct from the solvent; therefore, they can be quantified with high accuracy and confidence. In conclusion, we have demonstrated that NMR is a robust tool to determine the log *P* for pharmaceutical agents, without the use of deuterated solvents and internal standards, and our log *P* values are consistent with the literature reports. This method is also fast and highly versatile in that other solvent systems can be readily analyzed for the determination of analyte partitioning. Therefore, the determination of log *P* by NMR provides as a practical way to rapidly analyze lead compounds in the drug discovery process.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.08.145.

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