Measuring Log P of Samples that are Hard to Dissolve in water and Octanol

To measure log P by the pH-metric method the sample must be in solution throughout the assay - no solid sample should be present. Three techniques are generally used to get the sample into solution.

Pion's SiriusT3™

The assay can be started at a pH where the ionized form will dissolve in the aqueous phase, but this may fail if the salt form is poorly water-soluble. Another technique is to start at a pH where the neutral form will dissolve in the octanol, but this may fail if the octanol volume is small. Yet another technique is to start a triple titration with undissolved sample and anticipate that by the end of the first titration it will have dissolved in one phase or the other*. This technique can be effective for some systems, although the result from the first titration will be unreliable. This Application Note presents an alternative approach – to dissolve the sample in a small volume of cosolvent before the aqueous phase or octanol are added.

Background

Whilst it is possible to dissolve poorly soluble samples in a cosolvent before the start of a log P assay, it is important to understand how the presence of the cosolvent affects the partitioning of the sample into the lipid phase. To ensure that cosolvent experimental conditions do not cause unacceptable shifts in log P values, we undertook a systematic investigation and published results in a poster, which is available as a download from the Pion website.[1] The poster concludes that within limits, the cosolvent approach is valid and can be used for sample preparation prior to pH-metric measurement of log P.

Experimental

Five samples with log P values above 4 that are poorly water-soluble in neutral form (amitriptyline, diclofenac, imipramine, propranolol and warfarin) were investigated on the SiriusT3 instrument. In the first part of the study, pKa values were measured in aqueous solution (0.15 M KCl, 25 °C). Afterwards, log P octanol-water values were measured under similar conditions, typically using sample weights of 0.5 – 0.7 mg. All titrations started at a pH where the samples were ionized. In some experiments, an aliquot of between 5 and 100 µL of pure cosolvent

At the start of the second titration no additional octanol should be added, however additional octanol can be added at the start of the third titration.

(methanol, dioxane, acetonitrile, DMSO) was pipetted directly onto the weighed sample in a dry vial, and the vial agitated until the sample dissolved in the cosolvent. All log P measurements were made by triple titration in 1.5 mL of aqueous volume with octanol volumes of 20, 70, and 1070 µL during successive experiments. Results of 39 log P assays designed for statistical significance using a D-optimal model were analyzed by Design-Expert® software and expressed graphically. Figure 1 shows the average reduction in measured log P as a function of the volume of cosolvent.

Discussion and Recommendations

Figure 2 shows a pH-metric titration for log P. The curves represent the shift in pKa of warfarin in the presence of increasing volumes of octanol. The log P result is derived from these curves. Titrations of the type shown in Figure 1 were used to generate log P data for the five compounds studied, and Table 1 shows the maximum volume of cosolvent that could be present in a log P titration in 1.5 mL aqueous volume that would cause a result to be reduced by no more than 0.2 log P units. The data show that up to 100 µL of methanol could be used to dissolve a sample for analysis in 1.5 mL aqueous volume with no more than 0.2 shift in log P. However, methanol is volatile, and would be a poor choice for setting up overnight runs of log P measurements on the SiriusT3 with autoloader. DMSO is not volatile and is a better choice for setting up overnight runs. When this cosolvent method is required in the Pion applications service, our normal practice is to pipette 50 µL of DMSO on to a weighed sample to dissolve it before the start of a log P titration.

Measuring Log P of Oily or Caked Samples

It is often hard to weigh small amounts of oily samples for log P measurement. Weighing problems also occur for small amounts of caked material stuck to the walls of sample vials. Samples like these which are difficult to recover from the vial can be dissolved *in situ* in the cosolvent to prepare a stock solution. An aliquot of this can then be used for titrimetric analysis.

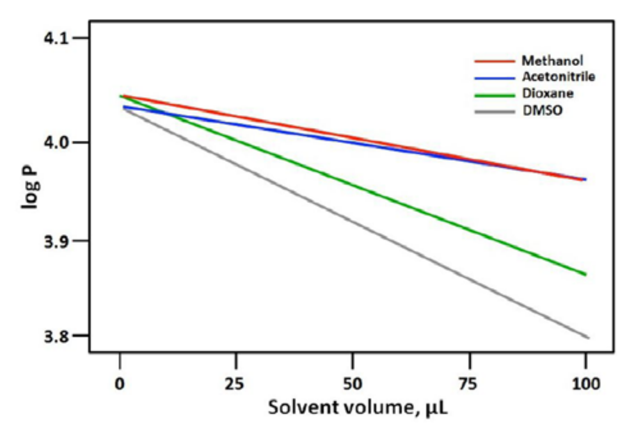

Figure 1. Average reduction in measured log P

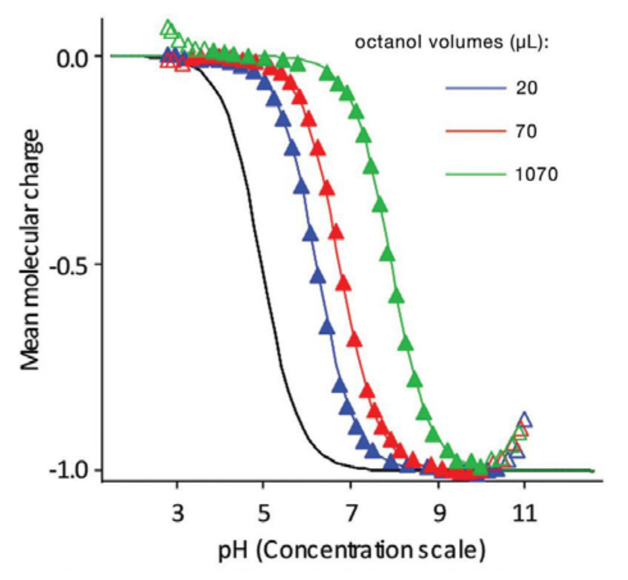

Figure 2. pH-metric triple titration for log P of warfarin (0.7 mg in 1.5 mL aqueous volume) in the presence of 40 µL of dioxane.

Reference

1. Use of cosolvent to aid solubilization of poorly soluble molecules during log P measurement; a study on the impact of cosolvent on measured log P values. Matthews, D. Yin, J. Towes, L. Stockton, B. Box, K. Comer, J. Mole, J. 2012. Poster, AAPS Annual Meeting, Chicago, USA.

Table 1. Maximum cosolvent volumes to cause a log P shift of < 0.2

Maximum cosolvent vol(µL) causing < 0.2 reduction in log P					
Cosolvent	**Amitriptyline**	**Diclofenac**	**Imipramine**	**Propranolol**	**Warfarin**
Methanol	92	99	100	99	100
Dioxane	70	100	97	96	63
Acetonitrile	97	99	100	100	100
DMSO	64	75	97	47	50