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Calculate reagent log P values to determine solubility characteristics

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Introduction

Carefully planning chemical modification and labeling procedures includes determining the appropriate solvents for the specific reagents and reactants that will be used. Peptides and proteins are complex molecules whose solubility in particular aqueous or organic solvents must be determined empirically, although some estimation can be made by considering their isoelectric point (pI), composition with respect to hydrophilic vs. hydrophobic residues, and glycosylation. By contrast, the solubility characteristics of small compounds, such as crosslinkers, biotin- and fluorophore-labeling reagents, and chemical blocking reagents, can be determined easily by calculating their log P values.

log P

The log P value for a compound is the logarithm (base 10) of the partition coefficient (P), which is defined as the ratio of the compound's organic (oil)-to-aqueous phase concentrations:

Partition Coefficient (P) = [Organic] / [Aqueous], where [ ] is the compound's concentration in that solvent phase

log P = Log 10 (P)

The log P of a compound is constant for a given specific pair of aqueous and organic solvents, and its value can be determined empirically by one of several phase-partitioning methods. However, the calculated log P value for a compound in water vs. a simple organic compound like octanol or hexane can provide a guideline for predicting its solubility characteristics in other aqueous and organic solvents.

For example, a peptide with a measured log P equal to 1 indicates that its concentration is at a 10:1 ratio in organic to aqueous phase. This peptide is hydrophobic and requires dissolution in an organic solvent. In contrast, a peptide with a log P equal to -1 indicates that the concentration is at a 1:10 ratio in organic to aqueous phase. This peptide is hydrophilic and can be dissolved directly in an aqueous buffer. Finally, a peptide with a log P equal to 0 partitions at a 1:1 ratio in organic to aqueous phase; hence, the peptide is likely soluble in both organic and aqueous solvents, the choice of which depends on the specific application.

Calculated log P (clog P)

As stated above, the log P of simple compounds and reagents can be calculated rather than empirically determined, and this can assist in planning which solvents are likely to be effective for a particular experiment. The calculated log P (clog P) can be determined using an application available at the following website: http://www.daylight.com/daycgi/clogp. This website requires chemical structures expressed in the SMILES format. If using ChemDraw, draw the structure, select it with the lasso tool and select Copy As SMILES from the edit menu. Paste this structure into the box on the Daylight website and choose submit. The program reports the log P values for fragments of the final structure with the resulting clog P at the bottom.

The crosslinker and biotinylation reagent Selection Guides on our website report clog P values. These values generally correspond with categorization of the compounds by water-solubility and membrane-permeability. For example, the crosslinker DSS (Product No. 21655) has a clog P equal to -0.046, while the sulfonated version of the same compound (BS^3, Product No. 21580) has a clog P equal to -4.34. DSS is poorly soluble in water, while BS^3 is readily soluble in water or aqueous buffers. DSS can diffuse across cell membranes because it has sufficient organic (lipid) solubility, while BS^3 cannot diffuse across cell membranes.

Be aware that log P values do not directly correlate with solubility limits; they merely indicate whether compounds are more or less soluble in water vs. oil. Two compounds may both have log P values equal to -1, indicating that they are more soluble in aqueous than organic solvents; but they may nonetheless have very different solubility limits in aqueous buffers.

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Solvent clog P Values

For maximum solubility choose a solvent whose log P value (Table 1) is similar to the log P of the compound. Many solvents that are commonly classified as 'organic' are miscible (mix with water), rather than immiscible (do not mix with water). Generally, miscible solvents have negative log P values (Table 1). Therefore, the log P does not provide much information to predict whether a compound will dissolve more easily in water than in, for example, DMSO; both solvents have nearly identical log P values.

Table 1. The clogP of some common solvents.

Solvent	clogP	Pierce Product Number(s)
Heptane	4.397	
Octanol	2.939	
Dichloromethane	1.249	
Diethylether	0.870	
Ethyl acetate	0.711	
Pyridine	0.645	27530
Tetrahydrofuran	0.526	27860
Trifluoroacetic acid	0.365	28901-28904
2-Propanol	0.074	
Acetone	-0.208	
Ethanol	-0.235	
Acetonitrile	-0.394	20062, 51101
Methanol	-0.764	
Dimethylformamide (DMF)	-1.038	20672
Hydrochloric acid	-1.316	24308
Dimethylsulfoxide (DMSO)	-1.378	20684
Water	-1.380	51140

Making Use of Solvent Partitioning

Knowing how reactants and products will partition between an aqueous buffer and an immiscible solvent enables one to construct a protocol to purify reaction products from undesired byproducts. For example, the hydrolyzed byproducts of a reaction with excess BS^3 are sulfo-NHS and octanedioic acid. Although the sulfo-NHS is water-soluble (clog P = -1.830), the octanedioic acid is primarily organic-soluble (clog P = 1.034). Therefore, one could extract the octanedioic acid from an aqueous reaction mixture by phase separation into an immiscible organic solvent such as hexane.

Current versions of product instructions are available at *www.thermo.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.