Chromatography Solutions

Knowledge note #0014

Analyte properties: pK_a, logP and logD

INTRODUCTION

When developing a new chromatographic method, or understanding/troubleshooting an existing method, knowledge of analyte properties can prove invaluable. Analyte pK_a, logP and logD measurements are useful parameters that provide important information regarding the hydrophobicity and ionisation state of analyte molecules. These parameters can help determine the most suitable mode of chromatography, aid in diluent and mobile phase selection and help diagnose various issues such as poor peak shape.

PHYSICAL PROPERTIES OF ANALYTES

Where possible, it is advantageous to understand the physical properties of analytes of interest prior to developing a new chromatographic method. Knowledge of the pK_a of ionisable analytes helps to guide mobile phase pH and buffer selection, whilst logP and logD values can help predict retention and determine whether reversed-phase (RP) is suitable for a separation or if an alternative technique such as HILIC, could be considered.

ANALYTE pK_a

For ionisable analytes, knowledge of the pK_a is invaluable for liquid chromatography. At a mobile phase pH equal to an analyte's pK_a, the analyte is 50% ionised and 50% non-ionised in aqueous solution. An acidic compound is 50% negatively charged at its pK_a, whilst a basic compound is 50% positively charged at its pK_a (Figure 1).

A general rule in liquid chromatography (excluding ion exchange chromatography) is that it is always best, where possible, to work at a mobile phase pH >2 pH units away from an analyte's pK_a. This is to ensure that the target analyte is always present in a single ionisation state. If the analysis were to be carried out at a pH equal to the pK_a of the analyte, small changes in mobile phase pH would result in changes to the degree of analyte ionisation. This results in a change to the analyte's hydrophobicity and therefore a change in retention characteristics. This situation represents a non-robust method.

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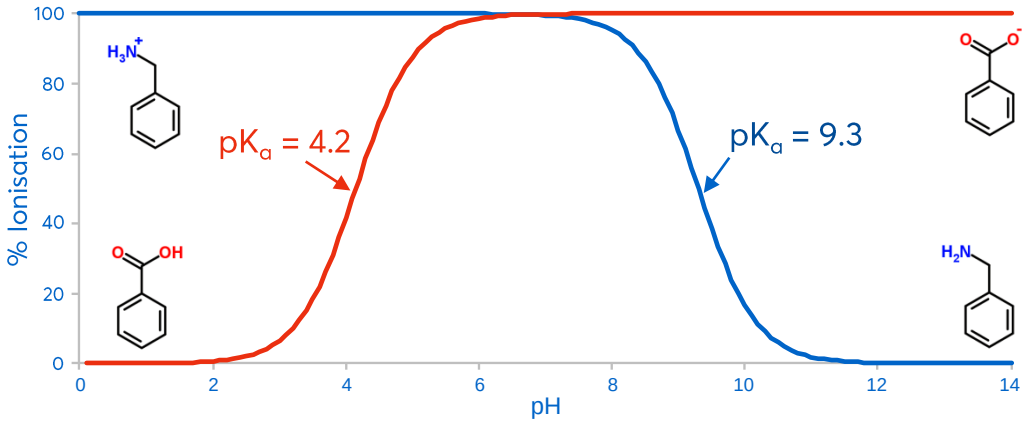

Figure 1: Percentage ionisation of acidic (red) and basic (blue) analytes at various pH values.

Operating away from the analyte pK_a is often not possible for complex samples which may contain a range of ionisable analytes. In this situation, it is important to use an appropriately buffered mobile phase to minimise the potential for small variations in pH.

PREDICTING RETENTION AND SEPARATION MODE

Analyte logP and logD values can be very useful indicators of whether the analyte is likely to be retained by RPLC or whether an alternative chromatographic mode, such as HILIC, may be more appropriate. It should be remembered that logP and logD values are useful when primarily hydrophobic interactions govern analyte retention and do not take into account secondary interactions, such as ion exchange, that may occur.

logP

logP values indicate the relative hydrophobicity of a non-ionised analyte and can therefore be used as a guide to predict likely analyte retention behaviour. If we consider a non-ionised analyte in a system consisting of two immiscible liquid phases in contact with each other, one aqueous and the other organic (octanol), we can define a partition coefficient (*P*), which is the ratio of the concentration of the non-ionised analyte in aqueous phase and organic phase. logP can therefore be defined by equation 1. As such, it represents the relative affinity of a compound for the organic and aqueous phases.

$$logP_{oct/H_2O} = log\left(\frac{[analyte]_{octanol}}{[analyte]_{H_2O}}\right) \quad (1)$$

An analyte's logP can be experimentally determined or predicted by using molecular modelling tools. A large, positive value for logP indicates that the analyte is hydrophobic in nature (i.e. has a greater affinity for the organic phase) and would be well retained in reversed-phase (e.g. naphthalene in Figure 2). Low or negative logP values indicate the analyte is hydrophilic and would therefore elute early in reversed-phase. In this case, other modes of separation, such as HILIC, may be more suitable. Analytes with a logP of approximately 0, such as caffeine can often be retained by RPLC or HILIC.

HOW DOES logD DIFFER FROM logP?

Whilst logP values can be useful, it must be remembered that they refer to the non-ionised form of an analyte. Often, analytes are ionisable and therefore logD values may be more appropriate. LogD is the logarithm of the distribution coefficient at a specific pH and is therefore more applicable for ionisable species, i.e. acidic and basic compounds. For ionisable compounds, logD is altered by pH as the relative abundance of ionised and non-ionised species changes as a function of pH. In chromatographic terms, logD values for ionisable analytes can be used in much the same way as logP values for non-ionised analytes, to aid in predicting likely retention behaviour and, alongside pK_a, to determine an appropriate mobile phase pH.

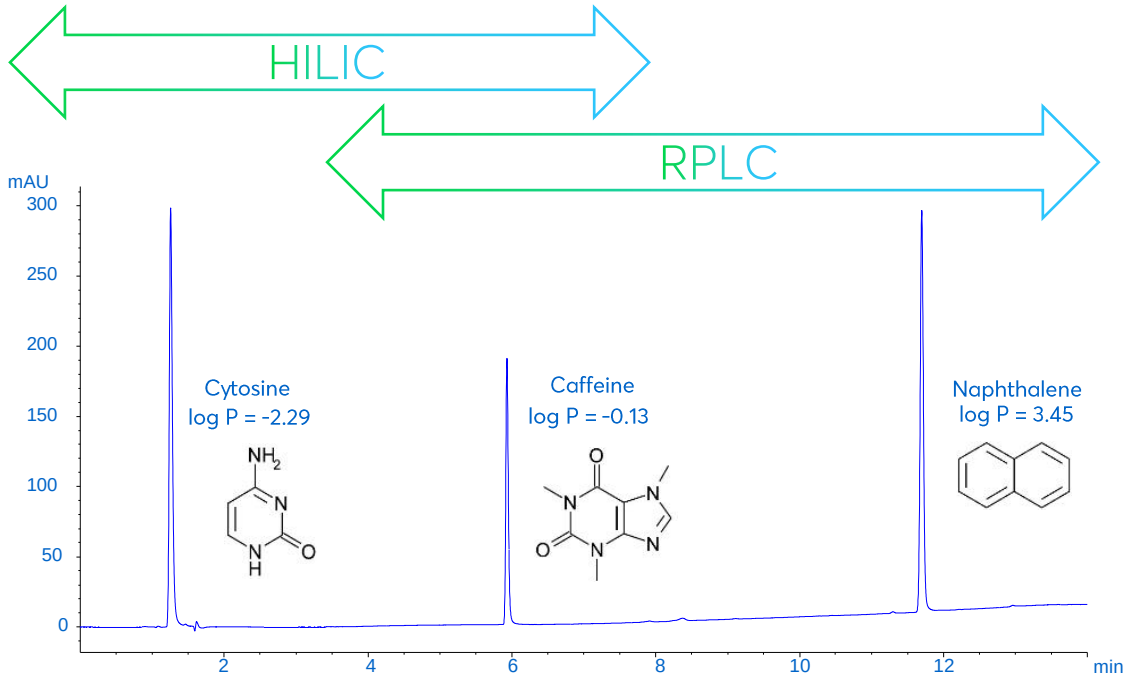

Figure 2: Elution of three analytes with varying logP values on a reversed-phase gradient and illustration of approximate reversed-phase and HILIC operating regions. Column: Avantor® ACE® Excel 2 C18, 100 x 3.0 mm, Mobile phase: A: 10 mM NH_4HCO_2 pH 3.0 in H_2O, B: 10 mM NH_4HCO_2 pH 3.0 in ACN/H_2O (90:10 v/v), Gradient: 5 to 100% B in 10 minutes, Flow Rate: 0.4 mL/min, Temperature: 30 °C, Detection: UV, 254 nm.

CONCLUSION

This knowledge note has discussed the usefulness of considering analyte physicochemical properties in order to develop robust separations. Analyte pK_a, in particular, is important for selecting appropriate mobile phase pH conditions. Developing methods with a mobile phase pH at least 2 pH units from the analyte pK_a is highly recommended where possible, to aid method robustness. Analyte logP and logD values are also worthy of consideration before commencing method development, to help understand and predict likely retention behaviour and select the most suitable mode of chromatography for the analysis.

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