Comparison of Three HPLC-based K_{OW} Estimation Methods for Polybrominated Diphenyl Ethers

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Introduction

Polybrominated diphenyl ethers (PBDEs) are highly hydrophobic compounds, which partition strongly into organic matter and bioaccumulate in successive levels of the food chain[1,2,3]. Although their environmental fate and toxicity depends on the bromine substitution pattern, only limited congener-specific physical-chemical property data are available. Considering that PBDEs are potentially toxic and act as endocrine disruptors[4], it is important to understand their partitioning characteristics into biological tissue. The octanol-water coefficient, K_{OW}, is one of the most commonly used properties to describe a chemical's distribution in a pharmaceutical and environmental context. The octanol-water system correlates well with the physiochemical properties of biomembranes[5] and is thus the most widely used solvent system for expressing partitioning into living matter.

Many experimental methods for determining K_{OW} rely on direct concentration measurements after equilibration in an octanol-water system. These methods are often lengthy, very difficult and inherently subject to inaccuracies, especially when dealing with highly hydrophobic compounds[6]. There is a long tradition of using reverse-phase high performance liquid chromatography (RP-HPLC) to estimate K_{OW} values. Different HPLC methods have been proposed[7,8,9]. Good reproducibility of retention time measurements yields highly precise results, but the accuracy depends on the calibration procedures, and the availability of reference and calibration compounds with well-known K_{OW} values. The suitability of HPLC-based methods for determining K_{OW}s for highly hydrophobic compounds, such as PBDEs, has received relatively little attention. HPLC methods may have difficulty dealing with these compounds, because there are few well-established, high K_{OW} values, which could serve as reference and for calibration.

The purpose of this study was to compare three HPLC-based methods for estimating the K_{OW} values of highly hydrophobic compounds. First, an isocratic experiment is shown to inaccurately estimate K_{OW} values, and to be limited by exceedingly long run times. To address these shortcomings, a gradient elution method[8] is applied to the PBDE congeners, shortening run times and increasing accuracy. Finally, a estimation method[9] to yield temperature-dependent K_{OW} values is used, and the three methods are compared.

Method

For chromatographic columns, the capacity factor k' of a compound partitioning in the column is given by

$$k' = (t_r - t_0)/t_0 \quad (1)$$

where t_r and t_0 are the retention times of the compound and of a substance that is not

retained by the stationary phase of the column. The estimation of K_{OW} values from k' is based on the assumption that the stationary phase of a RP-HPLC column acts as a surrogate for the octanol in an octanol-water partition system. Relationships between the K_{OW} and k' values of compounds have been observed in the form

$$K_{OW} = Ak'^{B} \quad (2)$$

or, in logarithmic form

$$\log K_{OW} = B \log k' + \text{constant} \quad (3)$$

An 1100 Series Agilent HPLC, with a binary pump, vacuum degasser, autosampler, and a controlled thermostat was used in all experiments. All analytes were dissolved in methanol (MeOH). Formamide was used to measure the void volume of the column. Analytes were detected with an 1100 Series Agilent Multiwavelength Detector, set at 254 nm and 210 nm with a bandwidth of 16 nm. Chlorobenzenes (CBz) and polychlorinated biphenyl (PCBs) were used as standard calibration compounds.

Method 1: Isocratic measurements were made at 25 °C on an Intersil ODS-2 column, 150 x 4.6 mm (5 um pore diameter) with a flow of 1.0 mL/min. Samples of 15 to 40 µL were injected over a range of 65 to 85 % MeOH. Capacity factors (*k'*) of the calibration compounds at 0 % MeOH were calculated from regressions of isocratic *k'* measurements against % MeOH. These *k'* values (at 0 % MeOH) were then regressed linearly against the literature log K_{OW} values. PBDE K_{OW} values were estimated by fitting the experimental PBDE k' values to this regression.

Method 2: The gradient method[8] ran at 25°C on a Supelco ODP-50 cartridge column, 20 x 4.0 mm. A flow of 1.8 mL/min was used, and the MeOH fraction increased linearly from 10 to 100 % over 40 minutes. K_{OW} values were estimated by regressing the calibration compounds' *k'* values against literature log K_{OW} values.

Method 3: Retention time measurements were performed at various temperatures between 5 to 45 °C on an ODS-2 column, 100 x 4.6 mm, (5 um pore diameter) with a 50 mm ODS-2 guard column in place. The elutions were performed isocratically at 80 % MeOH. *k'* values for five chlorobenzenes (1,2-CBz, 1,3,5-CBz, 1,2,3,5-CBz, penta-CBz, hexa-CBz) were used to determine temperature-dependent *B* values[9], using eq^{n} 3 and temperature-dependent K_{OW} values reported by Bahadur[10]. This method[9] requires an internal standard for which K_{OW} is well known as a function of temperature, preferably measured by slow-stirr or shake-flask methods. 1,3,5-CBz served this purpose, and K_{OW} values reported by Bahadur[10] were employed. $\ln\left(k'/k'_{ref}\right)$ values at 5 to 45 °C were plotted against log(K_{OW}/*B*) at the same temperatures for each PBDE congener, and K_{OW} values estimated as described by Lei et al.[9].

Results and Discussion

Using method 1, CBzs and PCBs yielded widely different k' vs. K_{OW} relationships (Fig. 1). K_{OW} values for 3-BDE and 2,4-BDE, estimated using the two different regressions, differ by an order of magnitude (Table 1). Because of long run times, it is usually not feasible to perform isocratic elutions with 100 % water. The k' values displayed in Fig. 1 are therefore not measured directly, but are linear extrapolations of k'values measured at

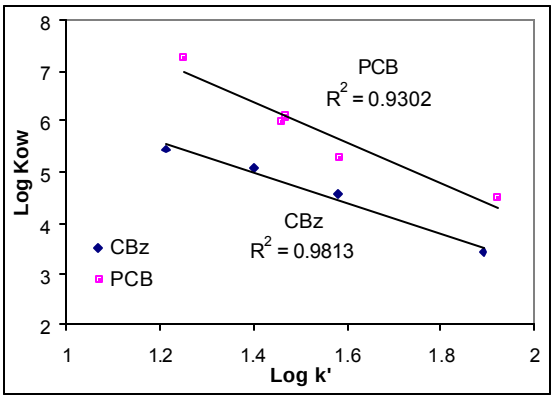

Figure 1: Linear regression of isocratic log *k'* against literature K_{OW} values

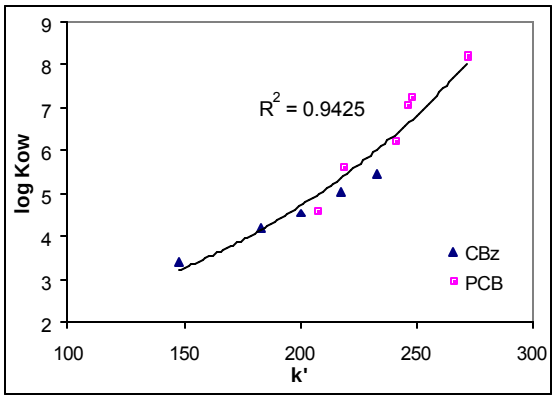

Figure 2: Exponential regression of gradient *k'* values against literature K_{OW} values

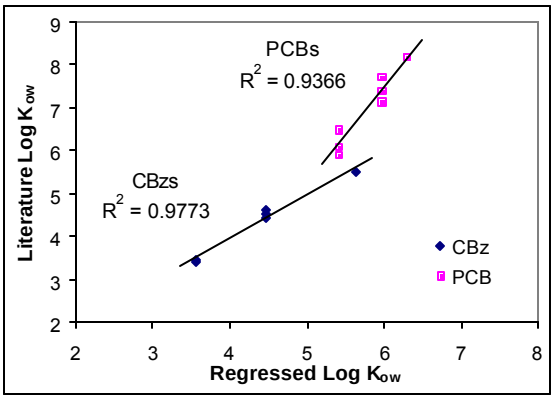

Figure 3: Final calibration of temperature-dependent values, where CBzs and PCBs are plotted separately.

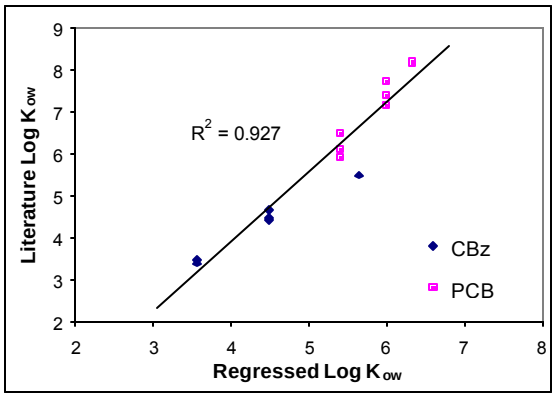

Figure 4: Final calibration of temperature-dependent K_{OW} values

Tab. 1 log K_{OW} values for selected polybrominated diphenyl ethers, determined by RP-HPLC, and reported in the literature[6,12].

	Method 1				Final	Slow-
	PCB regr.	CBz regr.	Method 2	Method 3	Adjusted Values[12]	Stirr[6]
3-BDE	4.74	3.74	4.94	4.37	5.08	4.94
2,4'-BDE	4.65	3.68	6.03	4.62	5.48	
2,4-BDE				4.98		
2,4',6-BDE				5.51		
2,4,6-BDE				5.50	5.8	5.94
3,4,4'-BDE				5.24		
2,3',4,4'-BDE				6.46		
3,3'4,4'-BDE			6.77	6.46	6.39	6.81
2,2',4,4',5,5'-BDE			7.66	6.85	7.08	7.90

high MeOH fractions (> 65 % MeOH). Because this relationship is often not linear[11], extrapolations over such a large compositional range are highly uncertain and may yield unreliable results. This is the most likely explanation for the observed differences between the two groups of reference compound.

The results of the gradient elutions (Method 2) appear to best fit an exponential regression (Fig. 2). Some difference between the elution behaviour of the PCBs and CBzs is still apparent, however the exponential fit tends to better account for this variability, which is likely due to the slightly different structures of the compounds. The K_{OW} values estimated by the gradient method agree well with the results of recent slow-stirr experiments[6] (Table 1). However, they are at least 0.5 log units higher than the Final Adjusted Values (FAVs) for K_{OW} values derived by Wania and Dugani[12].

In the temperature-dependent measurements of method 3, the CBzs and PCBs again show different elution behaviour (Fig. 3). However, the combined regression (Fig. 4) has a reasonably high R^2 of 0.927, allowing for the estimation of K_{OW}-values Only the higher values given in Table 1 agree well with the FAVs[12], and all are lower than the slow-stirr values[6]. This consistent underestimation of K_{OW} is presumably caused by the calibration of the method. The CBzs are the only compounds for which temperature-dependent K_{OW} values are available[10], but they have considerably lower K_{OW} values than the PBDEs. Thus, the method currently relies on a calibration with compounds with a K_{OW} mostly out of the range of highly hydrophobic compounds.

Conclusion

Though structurally very similar, CBzs and PCBs consistently showed different RP-HPLC elution behaviour, as revealed by differences in the regressions between k' and K_{OW}. This makes the use of such regressions for estimating the K_{OW} of other substances problematic, especially if they fall into a different K_{OW} range then the calibration compounds. Simple isocratic measurements are strongly affected by this problem, as are the temperature-dependent measurements. Due to its simplicity, ability to better account for differences between groups of calibration compounds, and agreement with directly determined K_{OW} values, the gradient method currently appears to be the most efficient method to rapidly estimate K_{OW} for highly hydrophobic compounds, such as the PBDEs.

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