Liquid Chromatographic Measurement of Solute Solvent/Solvent Partition Coefficients: Application to Solute/Container Interactions

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Abstract

Octanol–water partition coefficients ($P_{o\text{-}w}$) for model compounds used to study solute–polymer interactions are determined by reversed-phase high-performance liquid chromatography (HPLC). Marker compounds of known $P_{o\text{-}w}$ are separated using a C_8 stationary phase and mobile phases containing varying amounts of methanol, and the capacity factor vs. that methanol content data is used to obtain a capacity factor at 100% water content (log k_w). Regression of log k_w vs. log $P_{o\text{-}w}$ for the markers produces a relationship with an excellent linear correlation (r^2 = 0.994). The same procedure is used to obtain log k_w (and thus log $P_{o\text{-}w}$) for the polymer–solute interaction model compounds. Hexane–water partition coefficients ($P_{h\text{-}w}$) for the model compounds are determined by the shake flask method. Polymer–solute interaction constants (E_b) are determined for the interaction between the model solutes and a diethylhexyl phthalate (DEHP) plasticized polyvinyl chloride (PVC) polymer used as a parenteral product container. An interaction model was generated relating the interaction constant to the linear combination of $P_{o\text{-}w}$ and $P_{h\text{-}w}$. For the polymer studied the model takes the form: log E_b = 0.34 log $P_{o\text{-}w}$ + 0.29 log $P_{h\text{-}w}$ − 2.39 (r^2 = 0.934). The model is used to predict the fractional binding of several common drugs by the container material. The predicted behavior compares favorably with binding data reported in the literature.

Introduction

A material's usefulness as a container for aqueous products can be affected by either the ability of a container component to migrate out of the container and into the contained solution (leaching) or by the ability of the material to sorb solutes from the contained solution. Container compatibility assessments attempt to determine whether the magnitude of the interaction between the container and the contained solution is within acceptable limits. For leaching, the maximum permissible amount of migration is determined by the migrant's intrinsic toxicity or, less commonly, by optimum use constraints (e.g., solution clarity, color, and so forth). For the sorption of a solute from solution, the maximum permissible interaction is determined by product efficacy considerations (i.e., how much of the solute can we lose from solution and still maintain product efficacy).

Thermodynamically, the magnitude of the container/solution interaction is affected by the total amount of the migrant present, the solubility of the migrant in the phases of interest, and the equilibrium partitioning of the migrant between the container and the solution. Considering partitioning, several researchers (1–5) have demonstrated that polymer–solute partition coefficients ($P_{p\text{-}s}$) correlate well with a solute's solvent–solvent partition coefficient ($P_{s\text{-}s}$) via a Collander-type expression:

$$\log P_{p\text{-}s} = a \log P_{s\text{-}s} + b \qquad \text{Eq 1}$$

Determination of $P_{p\text{-}s}$ for several model compounds of known $P_{s\text{-}s}$ allows for the generation of an interaction model (by allowing for the calculation of the a and b constants in Equation 1) that can be used to predict the interaction properties of other compounds provided their $P_{s\text{-}s}$ is either known or determined. Reversed-phase high-performance liquid chromatography (HPLC) has been widely used in recent years as an accurate and effective means of estimating $P_{s\text{-}s}$ values [particularly octanol–water ($P_{o\text{-}w}$) partition coefficients] (6–15). Thus, the initial phase of this research involved the determination of $P_{o\text{-}w}$ and $P_{h\text{-}w}$ (hexane–water) for test model compounds chosen to develop a container interaction model. The determined partition coefficients, correlated with experimentally measured container interaction constants, generates the interaction model that can then be used to predict the interaction properties of additional solutes. Predicted fractional binding properties of the material studied [a plasticized polyvinyl chloride (PVC) polymer used as a common intravenous (IV) solution container] are then compared with interaction behavior reported in the literature.

Experimental

Chromatographic procedure for $P_{o\text{-}w}$ *determination.* The chromatograph consisted of a Kratos Spectroflow 400 pump, a Micromeritics 728 autosampler (coupled with an SSI Model 235 injection valve) and both a Kratos Spectroflow 757 UV detector and Knauer Model 108 refractive index detector. The chromatographic system was linked to both a strip chart recorder and laboratory data station for data collection.

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Retention time data for the marker and model solutes were collected using a Supelcosil LC-8-DB stationary phase packed into several column configurations and with several particle sizes. Specific column size–particle size couples used included 250 × 4.6 mm, 5 μm; 150 × 4.6 mm, 5 μm; and 33 × 4.6 mm, 3 μm. Marker and model solutes used are listed in Tables I and II. Mobile phases used in the solvent scouting experiments consisted of mixtures of HPLC-grade methanol and water. As appropriate for individual solutes, mobile phases were prepared to contain either 35 mM triethylamine (basic solutes), 4 mM trifluoracetic acid (acidic compounds), or were unmodified (neutrals). The mobile phase flow rate was 1.0 mL/min, sample injection size was 10 μL, and the column void volume was determined with pyridine-*N*-oxide.

Columns are equilibrated with each mobile phase for at least 30 min prior to the injection of any marker or model compound. Solutes were injected in triplicate and in a random order to obtain their retention characteristics.

Shake flask method for $P_{h\text{-}w}$ *determinations.* $P_{h\text{-}w}$ values were obtained by a conventional shake flask approach. Aliquots of an appropriate aqueous solution (spiked individually with a known concentration of model or marker solute) were equilibrated with a known volume of hexane and the equilibrium solute distribution between the phases was determined by measuring the equilibrium solute concentration in each phase by UV spectrometry.

Evaluation of the polymer–solute interaction. The polymer used was a diethylhexyl phthalate plasticized PVC [specifically the material used in VIAFLEX® containers (Baxter Healthcare Corporation) that are currently used for parenteral products (IV solutions)]. Model solutes used to generate the interaction model are listed in Table II. Approximately 1 g of polymer (cut into 1 cm^2 pieces) was placed in a glass reaction vessel containing 100 mL of a donor solution consisting of one or more of the model compounds at initial concentrations ranging from 10 to 100 ppm. The reaction vessels were closed and the samples stored at 45°C for a period of 10 days with constant gentle agitation. Controls containing the donor solution but no polymer were also stored under the same conditions. Upon equilibration, aliquots of the donor solution were analyzed to establish the magnitude of solute uptake. Duplicate vessels were prepared for each solute mixture.

The loaded polymers were rinsed with water and placed into a second glass vessel containing 25 mL of an extracting solution. The vessels were stored with constant agitation at 25°C, and upon equilibration the concentration of model solute in the extracting solution was determined. After analysis, the polymer was again rinsed with water and extracted with a second fresh aliquot of extracting solution. After equilibration in the second system, the solute concentration in the second solution was also determined.

The solute concentration in these donor and extraction solutions was determined by HPLC. Separation was accomplished with a binary methanol–water mobile phase (phase ratios used varied from 44:56 to 57:43) delivered at 1 mL/min and a Supelcosil LC-8-DB column (50 × 4.6 mm, 5-μm particles). Sample injection size varied from 20 to 50 μL and analytes were detected via UV at 220 nm. In all cases, solutes present in a particular interaction mixture were base-line resolved from one another. Solute concentration was determined from three-point calibration curves using peak-area response.

Both the extracting and donor solutions were 4 mM trifluoroacetic acid in water. Reported interaction constants represent the mean of constants determined individually in the loading and extraction phases of the experiment.

Reagents. Marker and model solutes were analytical-grade with a 98% or better purity. Reagents used to prepare mobile phase and other analytical solutions were reagent- or HPLC-grade as appropriate. Water was obtained from a Barnstead NANOpure II water polisher.

Results and Discussion

Determination of the partition coefficients. The HPLC method used in this study involves the generation of log k' vs. the percent methanol in the mobile phase plots that are extrapolated to the y-axis (100% water mobile phase) to produce an estimated log k_w value. Partition coefficients for the model compounds are correlated with the extrapolated log k_w values to produce a calibration plot. The partition coefficient of

Table I. Solutes Used as HPLC Log $P_{o\text{-}w}$ Markers

Compound	Abbreviation	Methanol range in mobile phase (%)	Fit of solvent scout plot (r^2)	log k_w	log $P_{o\text{-}w}$*
Aniline	AN	25–50	0.995	0.85	0.89
Acetanilide	AC	25–50	0.997	1.15	1.17
4-Methylbenzyl-alcohol	MBA	30–50	0.998	1.70	1.60
p-Chloroaniline	PCA	30–50	0.998	1.90	1.83
Dimethyl phthalate	DMP	30–50	0.998	2.03	2.22
4-Methylbenzoic acid	MBOH	30–55	0.995	2.22	2.27
Chlorobenzene	CB	40–60	0.998	2.52	2.84
Diethyl phthalate	DEP	35–50	0.998	3.20	3.22
1,4-Dichloro-benzene	DCB	50–70	0.999	3.21	3.37
Biphenyl	BP	45–70	0.998	4.06	4.11
Pheonothiazine	PT	50–65	0.999	3.88	4.15
Anthracene	AN	55–80	0.991	4.26	4.45
Pentachlorophenol	PTP	60–80	0.997	5.01	5.12
Hexachlorobenzene	HB	70–90	0.996	5.65	5.70

* From Reference 16. $P_{o\text{-}w}$ = octanol-water partition coefficient.

Table II. Model Solutes Used and their Determined Properties*

Solute	Abbreviation	log $P_{o\text{-}w}$	log $P_{h\text{-}w}$	log E_b
Dimethyl phthalate	DMP	2.00†	0.70	−1.36
Diethyl phthalate	DEP	3.22†	1.8	−0.53
Dipropyl phthalate	DPP	4.20	2.7	−0.25
4-Methylbenzoic acid	MBH	2.35	−0.20	−1.87
4-Ethylbenzoic acid	EBH	3.10	0.50	−1.33
4-Butylbenzoic acid	BBH	3.70	1.5	−0.93
Ethyl paraben‡	ETPB	2.57	−1.1	−1.76
Butyl paraben	BUPB	3.57	0.40	−0.95
n-Ethylbenzyl amine	NEHA	2.17	−1.7	−2.15
Carbazole	CAR	3.59	2.2	−0.40
Ethyl benzene	EB	3.31	3.0	−0.57
Propyl benzene	PB	3.96	3.0	−0.40
Ethyl-4-aminobenzoate	EBA	2.24	−0.07	−1.82
Butyl-4-aminobenzoate	BBA	3.37	1.2	−0.85

* $P_{o\text{-}w}$ = octanol-water partition coefficient and $P_{h\text{-}w}$ = hexane-water partition coefficient.
† From reference 16.
‡ 4-hydroxybenzoic acid, ethyl ester.

unknown or model solutes is determined via the calibration plot from their experimentally determined log k_w value.

Results of the solvent scouting experiment performed to generate the calibration plot from the marker compounds are summarized in Table I. In general, the resulting log k' vs. methanol content plots are quite linear ($r^2 > 0.99$). Extrapolated values of log k_w are related to reported $P_{o\text{-}w}$ values (16) to produce the calibration plot shown in Figure 1. Least squares linear regression of the calibration data produces a best fit equation:

$$\log P_{o\text{-}w} = 1.029 \log k_w + 0.010 \qquad \text{Eq 2}$$

which is characterized by a high degree of correlation between the variables ($r^2 = 0.994$). The correlation is particularly impressive when one considers that the $P_{o\text{-}w}$ range studied spans nearly 5 orders of magnitude. Attempting to expand the calibration range to either lower or higher log $P_{o\text{-}w}$ values is complicated by the poor chromatographic performance of compounds with these properties (that is, retention is either very weak or exceedingly strong). The slope of the calibration plot is a reflection of the relative ability of the HPLC system to respond to changing solute lipophillicity versus the same ability of octanol. The near unit slope obtained confirms that the HPLC system employed is virtually an exact octanol model. The intercept of the calibration plot is a measure of the relative intrinsic lipophillicities of the HPLC stationary phase versus that of octanol. Again, the near zero intercept confirms that the HPLC system is an adequate and direct model for the octanol–water system.

At first glance, the ability of the alkyl-bonded phase to mimic the solute interaction properties of a solvent having a modest hydrogen bond activity (e.g., octanol) is somewhat unexpected. However, it has been demonstrated that methanol is absorbed as a monolayer onto the alkyl ligand (17), thus affording the stationary phase some hydrogen bond ability. Additionally, the presence of unreacted silanol groups in the bonded phase allows for dipole induced interactions, hydrogen bonding, and electrostatic interactions between the stationary phase and aqueous solutes.

Methanol (as opposed to other common mobile phase organic modifiers) is the solvent of choice for generating octanol-water models (8). Methanol, like water, has a strong hydrogen bonding character, so a wide range of methanol volume fractions will not significantly change or disrupt the ordering of the water

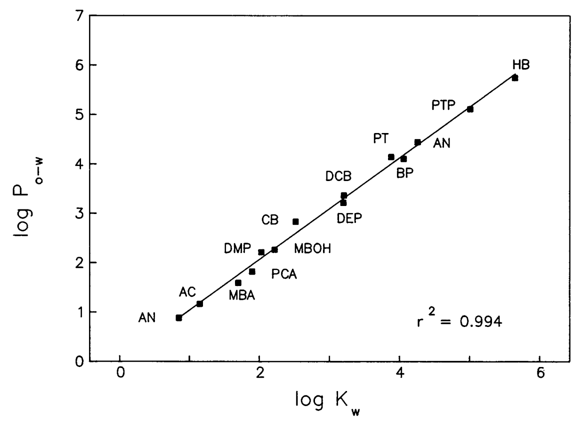

Figure 1. Calibration plot relating the marker solute's octanol-water partition coefficient ($P_{o\text{-}w}$) and its theoretical capacity factor for a 100% aqueous mobile phase (log k_w). Abbreviations used are described in Table I.

molecules. By contrast, acetonitrile and tetrahydrofuran (THF) are weak hydrogen bond acceptors. Acetonitrile also has a significant dipole moment, allowing it to participate in selective dipole–dipole interactions with solutes. Additionally, both acetonitrile and THF exhibit multilayer sorption onto the stationary phase, indicating that these two solvents have a stronger interaction with the stationary phase than does methanol.

Polymer/solute partitioning. The equilibrium interaction constant (E_b) reflects the partition-mediated equilibrium distribution of a solute between the polymer and aqueous phase and is defined as follows:

$$E_b = (m_p/W_p)/(m_s/V_s) \qquad \text{Eq 3}$$

where m equals the mass of solute in a phase at equilibrium, W equals the weight of polymer (g), V equals the volume of solution (L), s refers to the solution phase, and P refers to the polymer phase. E_b is analogous to $P_{p\text{-}w}$ (in Equation 1), differing as a gravimetric (as opposed to volumetric) expression of the solute concentration in the polymer. Experimentally determined values of log E_b, log $P_{o\text{-}w}$, and log $P_{h\text{-}w}$ for the model compounds studied are summarized in Table II.

The existing E_b and P database allows for the development of a PVC–solution interaction model based on the Collander-type formulation shown in Equation 1. Of course, the utility of the model depends on the ability of the model solvents chosen to mimic the physicochemical nature of the polymer being studied. In fact, octanol and hexane alone are relatively poor models for the plasticized PVC studied herein. The correlation between E_b and either log $P_{o\text{-}w}$ or log $P_{h\text{-}w}$ is poor ($r^2 = 0.77$ and 0.88, respectively). The single solvent models are particularly poor for those solutes which are hydrogen bond donors and thus can interact with the polymer by more than one mechanism.

In principle, the relationship between partition coefficients and interaction constants can be improved for hydrogen bond donors by adding a term to the equation which corrects for hydrogen bonding ability. Specifically, the corrected model equation would take the form:

$$\log E_b = a \log P_{o\text{-}w} + b \log K_{HB} + c \qquad \text{Eq 4}$$

where K_{HB} is the hydrogen bond formation constant. Such an approach has been successfully employed in correlating partitioning behavior between multiple solvent systems (18) and between polymer and solvent systems (19). In practice, however, measurement of K_{HB} is difficult and an alternate approach is proposed. An interaction model based on the linear combination of two partition coefficients related to two solvents of differing hydrogen bond character (e.g., octanol and hexane) intrinsically includes a correction for the solute's hydrogen bonding character. Essentially, such a model takes the form:

$$\log E_b = a \log P_{o\text{-}w} + b \log P_{h\text{-}w} + c \qquad \text{Eq 5}$$

Multiple regression analysis of the existing E_b and P database for the model compounds provides the following best fit equation for the PVC interaction model:

$$\log E_b = 0.34 \log P_{o\text{-}w} + 0.29 \log P_{h\text{-}w} - 2.39 \qquad \text{Eq 6}$$

As is shown in Figure 2, the model is quite effective in terms of mimicking the PVC–solution solute distribution for model compounds of varying ionic character and hydrogen bonding ability ($r^2 = 0.934$). Apparently the PVC exhibits a hydrogen bond character roughly intermediate between that of the two model solvents chosen.

Prediction of drug sorption by VIAFLEX containers. For a solute of known P_{o-w} and P_{h-w} and a specific VIAFLEX container configuration (solution volume and polymer mass), one can calculate E_b from Equation 6 and estimate the fractional uptake of the solute by the container (F_b) from the expression:

$$F_b = (W_p E_b)/(V_s + W_p E_b) \qquad \text{Eq 7}$$

Such a calculation was performed for several drugs whose partition coefficients were available and whose interaction with VIAFLEX containers had been studied. The results of the calculation (based on an 11-g container holding 100 mL of solution), along with the reported interaction magnitude, is shown in Table III. For highly sorbed solutes, predictions and experimental observations show good agreement. However, considerable disagreement exists for solutes exhibiting a lesser interaction with the polymer. Specifically, for hydrocortisone acetate and pentobarbital sodium, the model predicts a fractional binding of nearly 50% while no significant container sorption is reported. While one certainly expects some discrepancy between the model and observed behavior because of the intrinsic variability in the data used, the difference between the predicted and observed behavior for these solutes is much larger than the intrinsic variation in the experimental data. One anticipates that the lower binding reported from experimental observations results from ionization of the solutes under the conditions used. Because ionized solutes do not participate in polymer binding, one would expect the model (which calculates an E_b assuming complete solute participation in binding) to overestimate the magnitude of interaction observed for ionized solutes. Of course, the model-derived E_b can be corrected for the effect of a solute's degree of dissociation via expressions involving the pH and dissociation constant such as those documented in Reference 20.

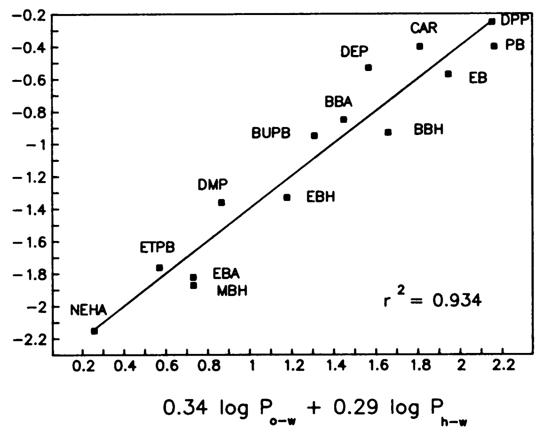

Figure 2. Plot of the equilibrium interaction constant (E_b) vs. the linear combination of the model solute's octanol-water (P_{o-w}) and hexane-water (P_{h-w}) partition coefficients. The best fit line represents Equation 6.

Table III. Predicted vs. Reported Fractional Binding*

	Fractional binding (% of initial in solution)		
Drug	**Predicted**	**Reported**	**Reference**
Medazepam	98.5	85	2
Diazapam	86.3	90	2
Warfarin	80.4	90,85	2,20
Nitroglycerin	73.4	83	2
Thiopental	82.8	73	2
Oxazepam	70.1	46	2
Nitrazepam	67.6	47	2
Hydrocortisone acetate	47.3	0	2
Pentobarbitol sodium	47.7	0	2
Thiopentone	76.9	70	20
Chlorpromazine	100	100	20
Diltiazem	86.2	75	20

* Note, the above calculations are valid for an 11-g container holding 100 mL of solution.

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