

Generally, 10-mL stopped centrifuge tubes or 200-mL centrifuge bottles are used. They are inverted gently for 2–3 min and then centrifuged at 1000–2000 *g* for 20 min before the phases are analyzed. Analysis of both phases is highly recommended, to minimize errors incurred by adsorption to glass walls at low solute concentration. For highly hydrophobic compounds, the slow stirring procedure of de Bruijn and Hermens is recommended (114). The filler probe extractor system of Tomlinson et al. is a modified, automated, shake-flask method, which is efficient, fast, reliable, and flexible (115).

Partition coefficients from different solvent systems can also be compared and converted to the octanol/water scale, as was suggested by Collander (116). He stressed the importance of the following linear relationship:  $\log P_2 = a \log P_1 + b$ . This type of relationship works well when the two solvents are both alkanols. However, when two solvent systems have varying hydrogen bond donor and acceptor capabilities, the relationship tends to fray. A classical example involves the relationship between  $\log P$  values in chloroform and octanol (117, 118).

$$\begin{aligned} \log P_{\text{CHCl}_3} &= 1.012 \log P_{\text{oct}} - 0.513 \quad (1.42) \\ n &= 72, \quad r^2 = 0.811, \quad s = 0.733 \end{aligned}$$

Only 66% of the variance in the data is explained by this equation. However, a separation of the various solutes into OH bond donors, acceptors, and neutrals helped account for 94% of the variance in the data. These restrictions led Seiler to extend the Collander equation by incorporating a corrective term for H-bonding in the cyclohexane system (119). Fujita generalized this approach and formulated Equation 1.43 as shown below (120).

$$\log P_2 = a \log P_1 + \sum b_i \cdot \text{HB}_i + C \quad (1.43)$$

$P_1$  is the reference solvent and  $\text{HB}_i$  is an H-bonding parameter. Leahy et al. suggested that a more sophisticated approach incorporating four model systems would be needed to adequately address issues of solute partitioning in membranes (121). Thus, four distinct solvent types were chosen—apolar, amphiprotic, proton

donor, and proton acceptor—and they were represented by alkanes, octanol, chloroform, and propyleneglycol dipelargonate (PGDP), respectively. The demands of measuring four partition coefficients for each solute has slowed progress in this particular area.

**3.2.1 Determination of Hydrophobicity by Chromatography.** Chromatography provides an alternate tool for the estimation of hydrophobicity parameters.  $R_m$  values derived from thin-layer chromatography provide a simple, rapid, and easy way to ascertain approximate values of hydrophobicity (122, 123).

$$R_m = \log(1/R_f - 1) \quad (1.44)$$

Other recent developments in chromatography techniques have led to the development of powerful tools to rapidly and accurately measure octanol/water partition coefficients. Countercurrent chromatography is one of these methods. The stationary and mobile phases include two nonmiscible solvents (water and octanol) and the total volume of the liquid stationary phase is used for solute partitioning (124, 125).  $\log P_{\text{app}}$  values of several diuretics including ionizable drugs have been measured at different pH values using countercurrent chromatography; the  $\log P$  values ranged from  $-1.3$  to  $2.7$  and were consistent with literature values (126).

Recently, a rapid method for the determination of partition coefficients using gradient reversed phase/high pressure liquid chromatography (RP-HPLC) was developed. This method is touted as a high-throughput hydrophobicity screen for combinatorial libraries (127, 128). A chromatography hydrophobicity index (CHI) was established for a diverse set of compounds. Acetonitrile was used as the modifier and 50 mm ammonium acetate as the mobile phase (127). A linear relationship was established between  $\text{Clog } P$  and CHIN for neutral molecules.

$$\text{Clog } P = 0.057 \text{ CHIN} - 1.107 \quad (1.45)$$

$$n = 52, \quad r^2 = 0.724, \quad s = 0.82, \quad F = 131$$

A more recent study using RP-HPLC for the determination of  $\log P$  (octanol) values for