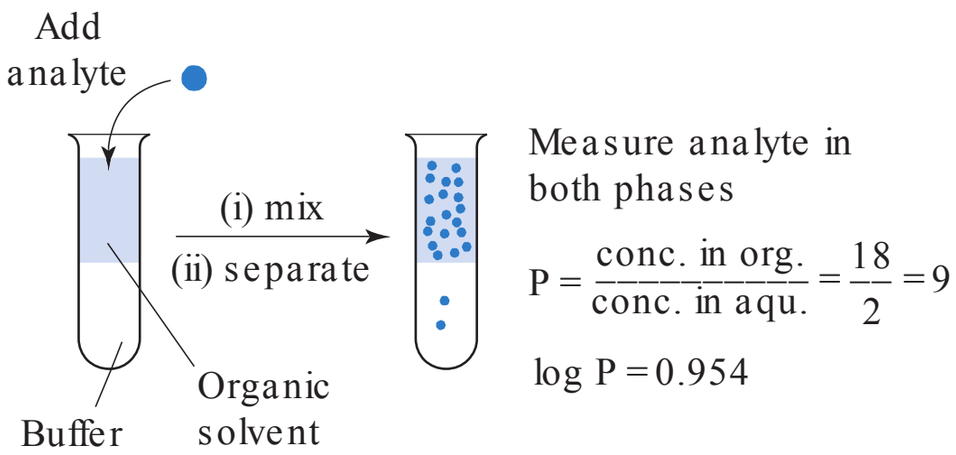


**Fig. 21.2** • Diagram of the shake-flask method for determining partition coefficient.

elsewhere in Chapters 2, 20 and 23. As discussed in Chapter 20, *n*-octanol is most commonly chosen as the solvent for the oil phase as it has similar properties to biological membranes although other oil phases have been used (as considered in Chapter 23). One of the most common ways of measuring partition coefficients is to use the shake flask method (Fig. 21.2). It relies on the equilibrium distribution of a drug between oil and an aqueous phase. Prior to the experiment the aqueous phase should be saturated with the oil phase and vice versa. The experiment should be carried out at constant temperature. The drug should be added to the aqueous phase and the oil phase which, in the case of *n*-octanol, as it is less dense than water, will sit on top of the water. The system is mixed and then left to reach equilibrium (usually at least 24 hours). The two phases are separated and the concentration of drug is measured in each phase and a partition coefficient calculated. This technique is discussed further in the context of preformulation in Chapter 23.

If the aqueous phase is at a particular pH, the distribution coefficient at that pH is measured (log*D*); this then accounts for the ionization of the molecule at that pH. In the case of a weakly acidic or a weakly basic drug, the log*D* measured at an intestinal pH (e.g. 6.8) is liable to give a better prediction of the drug's ability to cross the lipid gastrointestinal membrane than its partition coefficient, log*P*, which does not take the degree of ionization into account.

As discussed in Chapter 20, within a homologous series, increasing lipophilicity (log*P* or log*D*) tends to result in greater absorption. A molecule is unlikely to cross a membrane (i.e. be absorbed via the transcellular passive route) if it has a log*P* less than 0.

Instead of determining log*P* experimentally, computational methods can be used to estimate it. There are a number of software packages available to do this. There is a reasonably good correlation between calculated and measured values. Log*P* can be estimated by breaking down the molecule into fragments and calculating the contribution of each fragment to overall lipophilicity (often called the *c*log*P*). Another way of estimating log*P* is the Moriguchi method, which uses 13 parameters for hydrophobic and hydrophilic atoms, proximity effects, unsaturated bonds, intramolecular bonds, ring structures, amphoteric properties and several specific functionalities to obtain a value for the partition coefficient. This is often called the *m*log*P*. The advantages of these methods are in drug discovery, where an estimate of the lipophilicity of many molecules can be obtained before they are actually synthesized.

Another, more sophisticated physicochemical means of estimating how well a drug will partition into a lipophilic phase is by investigating how well the molecule can be retained on a high-performance liquid chromatography (HPLC) column. HPLC columns can be simply coated with *n*-octanol to mimic *n*-octanol-aqueous partition or, more elaborately, designed to mimic biological membranes. For example the immobilized artificial membrane (IAM) technique provides a measure of how well a solute (i.e. the drug) in the aqueous phase will partition into biological membranes (i.e. be retained on the column). Good correlations between these methods and biological in vitro methods of estimating transcellular passive drug absorption have been obtained.

## Cell culture techniques

Cell culture techniques for measuring the intestinal absorption of molecules have been increasingly used over recent decades and are now a well-accepted model for absorption. The cell line that is most widely used is Caco-2.

Caco-2 cells are a human colon carcinoma cell line that was first-proposed and characterized as a model for oral drug absorption by Hidalgo. In culture, Caco-2 cells spontaneously differentiate to form a monolayer of polarized enterocytes. These enterocytes resemble those in the small intestine, in that they possess microvilli and many of the transporter systems present in the small intestine, for example those for sugars, amino acids, peptides and the P-glycoprotein efflux transporter. Adjacent Caco-2 cells adhere through tight junctions. However, the tightness of these junctions is more like those of the colon than those of the leakier small intestine.