CHAPTER 8

SUMMARY AND SOME SIMPLE RULES

We began Chapter 2 with a simple Fick's law of diffusion model for absorption, with the key components: permeability, solubility, and charge state (the pH effect). The BCS scheme is more or less constructed along these lines. Closely related to permeability are partitioning in the well-trodden octanol–water and in the lesser-traveled liposome–water systems. We carefully examined the recent literature, with a focus on describing experimental methods which can yield high-quality results, including fast methods. Sometimes forgotten classic works were also revisited. The ''it is not just a number'' idea was drilled thoroughly with the tetrad–equilibria speciation diagrams for octanol, liposomes, and solubility. The log–log plots having $(0,\pm 1)$ slopes were evoked in several places, to relate the true pK_a to the apparent pK_a and learn something about the ''apparency.'' Out of these efforts emerged the practical concepts underlying pK_a^{oct}, pK_a^{mem}, pK_a^{gibbs}, and pK_a^{flux}.

The charge-state section highlighted the value of Bjerrum plots, with applications to 6- and a 30-pK_a molecules. Water-miscible cosolvents were used to identify acids and bases by the slope in the apparent pK_a/wt% cosolvent plots. It was suggested that extrapolation of the apparent constants to 100% methanol could indicate the pK_a values of amphiphilic molecules embedded in phospholipid bilayers, a way to estimate pK_a^{mem} using the dielectric effect.

Using such dielectric-based predictions, when the methanol-apparent solubility, $\log S_0^{\epsilon}$ versus wt% methanol is extrapolated to 0% cosolvent, the aqueous solubility, $\log S_0$, can be estimated; when $\log S_0^{\epsilon}$ is extrapolated to 100% cosolvent, the membrane solubility, $\log S_0^{mem}$, can be estimated. The approximate membrane partition

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coefficient can be calculated from the difference between the two solubilities: $\log P^N_{\text{mem}} = \log S_0^{\text{mem}} - \log S_0$, a concept we called the ''solubility-partition unification.'' Very little of this kind of prediction has been reported elsewhere.

Ion pair partitioning in octanol–water was carefully reviewed. The 'parabola vs. step' shape log *D* plots of peptides should no longer be subjects of controversy. But ''Ion-pairing, fact or fiction?'' needs to be further explored. The significance of the partitioning behavior of quaternary ammonium drugs in octanol–water is not entirely resolved. For example, when the permeability of warfarin across *phospholipid*-impregnated filters at high pH is measured, no evidence of warfarin in the acceptor compartment is seen. Given the complex structure of octanol and observations of the sodium dependence of the permeability of warfarin at high pH through *octanol*-impregnated filters suggests that such permeability is more a characteristic of octanol than ''real'' biological membranes. A postulate that orally administered amphiphilic molecules, even charged ones, can enter the bloodstream by going across the epithelial cell barrier ''under the skin of the tight junction,'' as depicted in scheme 3a → 3b → 3c in Fig. 2.7 is worthy of exploration. Not enough is really known of how such amphiphilic molecules can cross the tight junction.

The study of octanol–water ion pair partitioning has suggested the ''*diff* 3–4'' rule. With it, ion pair partition coefficients can be predicted from knowledge of just the neutral-species log *P*. With the liposome–water system, the rule slips to ''$diff_{\text{mem}}$ 1–2.'' Knowing these rules-of-thumb can prevent ill-guided use of equations to convert single-point log *D* values to log *P* values. An analogous ''*sdiff* 3–4'' rule for solubility was proposed. This may help to predict effects of salts in the background of a physiological concentration of NaCl or KCl.

The study of drug partitioning into liposomes has revealed some puzzling observations, in terms of the δ parameter. Why does acyclovir have such a high liposome–water log *P* and such a low octanol-water log *P*? Are such anomalies observed in IAM chromatography? The review of the literature hints that the high liposome–water log *P* values indicate a surface phenomenon (H-bonding, enthalpy-driven) that attenuates or even prevents membrane transport. Sometimes, high membrane log *P* or long retention times in IAM chromatography just means that the molecule is stuck on the membrane surface, and does not permeate. This idea needs to be further explored.

The concept of the Δ shift in HTS solubility measurements is quite exciting. It means that DMSO can be used in solubility measurements and the measured values later corrected to DMSO-free conditions. So we can have speed and accuracy at the same time! The pharmaceutical industry needs speed and accuracy, and will need these more in the future. In silico methods are no better than the data used to train them.

Solubility and dissolution are processes that take place in the gastric and the luminal fluids, not on the surface of epithelial cells. Measurement of solubility ideally needs to take place at pH 1.7 (stomach) and pH 5–8 (small intestinal tract). Ideally, the screen media should resemble intestinal fluids and contain bile acid-lecithin mixed micelles. Fast and reliable techniques for assessing solubility in

such environments are available. Industrywide consensus on solubility measurement protocols is needed, so that clinically relevant measurements are produced.

Permeability is a property closely tied to the environment of the epithelial cell surface. There is little point in measuring permeability at pH 1.7, if the microclimate barrier has pH $\geq$5 and $\leq$8, averaging $\sim$6. An in vitro permeability screen based on donor pH 5.0–7.4 and acceptor pH 7.4 seems about right. It will be useful to correct the data for the unstirred water layer effect, using computational methods.

Weak acids and bases can be better assessed if the shapes of the flux–pH profiles were considered, as far as predicting the outcome of a particular choice of assay pH.

The lengthy permeability chapter (Chapter 7) recounts the study of many different artificial membrane formulations, comparing transport results of each to human jejunal permeabilities. A very promising in vitro screening system was described: the double-sink sum-P_e PAMPA GIT model. It is most applicable to molecules that are classified as ''soluble'' in the BCS scheme.

When molecules have the ''insoluble'' BCS classification, the expected absorption profile is exemplified in Fig. 2.2. The upper horizontal line (solid) in Fig. 2.2, representing log P_0, can be determined by the methods described in Chapter 7. The ''slope 0,$\pm$1'' segments curve (dashed), representing log C_0, the concentration of the uncharged form of an ionizable molecule, can be determined by the methods described in the Chapter 6. The summation of log P_0 and log C_0 curves produces the log flux–pH profile. Such plots indicate under what pH conditions the absorption should be at its highest potential.

The Dressman–Amidon–Fleisher absorption potential concept [45], originally based on octanol–water partition coefficients, can be made more predictive, by using PAMPA permeabilities, instead of partition coefficients, for all the reasons discussed in Chapter 7. Such a scheme can be used to minimize false positive predictions of HIA.

Semiquantitative schemes, like the *maximum absorbable dose* (MAD) system described by Curatolo [53], can be made more predictive by applying solubilities measured by clinically-relevant protocols and PAMPA permeabilities.

The BCS scheme can be made more useful by incorporating a further improved basis of physicochemical profiling. For example, the role of pH in permeability measurements could be better defined. The use of simulated intestinal fluids for solubility measurements could be better promoted. The effects of fed/fasted states on absorption could be better address, in methods that have optimum clinical relevance.

In this book, a conceptually rigorous effort was made to describe the state-of-the-art physical methods that underlie the processes related to absorption. The aim was to give conceptual tools to the analytical chemists in pharmaceutical companies who do such measurements, so that they could in turn convey to the medicinal chemists, who make the molecules, how structural modifications can affect those physical properties that make candidate molecules ''drugable.''

As Taylor suggested in the introductory chapter, ''There are great advances and great opportunities in all this, . . .''