4.5.6 Capillary Zone Electrophoresis

Capillary electrophoresis (CE) has emerged as a method of choice for determining compound pK_a values, as it possesses many favorable qualities, as outlined in the following:

- Potential impurities and degradants can be separated from the target compound.
- Knowledge of sample concentration is not required for analysis.
- Sparingly soluble compounds with a suitable UV chromophore can be analyzed.
- No changes in spectral properties are required for the detection of a pK_a value.
- Minimal sample amounts are required for analysis (<1 mg).

Single CE–UV systems possess a throughput of approximately one compound per hour when analyzing 12 pH points per compound. Using the 96-capillary cePRO 9600™ system (9), it is possible to analyze 12 compounds per hour over 24 pH points. This breakthrough results in a significant increase in sample throughput, in combination with an extended pH range and improved data quality.

High-performance liquid chromatography techniques have also been used in the determination of log *P* values. A potential problem with the use of HPLC retention data is that it is not a direct method and thus requires calibration. Furthermore, there may be problems with performing experiments at pH greater than 8.

4.5.7 Plate Method for Solubility Testing

The MultiScreen Solubility filter plate method is a screening method that provides a fast, convenient, automation-compatible, and high-throughput means to estimate the aqueous solubility of hundreds of compounds per day. It correlates well with standard shake-flask methodology and can be implemented readily for this method in the typical drug discovery laboratory. Using a single-point calibration, the screening ratio is derived simply and quickly, and compound solubility is approximated easily. Multiple samples, each requiring approximately 200 nmol (~100 μg) per result, can be run in parallel. This method allows for the analysis of approximately 45 compounds (duplicate determinations) per plate, with the capability of completing four or more plates in a standard 8-hour day. The assay is inherently compatible with the method by which most compound libraries are produced (e.g., as stock solutions in dimethylsulfoxide [DMSO]) and is integrated easily into the existing chemical profiling and early absorption, distribution, metabolism, and excretion (ADME) workflows. The method's resultant filtrate quickly provides a particulate-free and known soluble compound that can be used with confidence for other downstream ADME analysis.

Using this method, entire libraries can be screened for solubility. This narrows the number of candidates to only those that can meet solubility levels consistent with predicted oral bioavailability levels (especially when combined with permeability results) necessary to proceed down the pipeline.