4.1 The Purpose of the Method

The approach to method development will be partly determined by the purpose of the method and the constraints upon it. An important factor is whether the method is required for long term use or for short term problem solving. Methods which are intended for long term use must be designed with robustness and validation in mind. Another important consideration is the sample matrix. Samples of a pure drug substance in water for example are easier to analyse than those in complex biological matrices. Samples in matrices such as blood may require extraction and purification before analysis is possible. High ionic strength matrices in particular can be especially problematic.

A key factor for consideration is the approximate enantiomeric purity of the samples to be analysed. Samples in which both enantiomers are present in approximately equal amounts are significantly less demanding upon the method than those samples which have a high degree of enantiomeric purity. As most new pharmaceutical agents with an asymmetric centre are developed as a single enantiomer, the minor enantiomer may be regarded in the same light as any of the other related substances. Because of the high purity requirements for pharmaceutical agents the analytical methods must be able to quantify the minor enantiomer at the level of fractions of one percent.

The difficulties posed by samples with very different ratios of the two enantiomers are illustrated in the computer simulations shown in Figures 4.1 and 4.2. Figures 4.1 and 4.2 are idealised representations of CE peaks generated by using the assumption of normal probability distributions for each of the enantiomer peaks. The Figures cover variations in the level of the second enantiomer peak across two orders of magnitude, and at two different levels of selectivity. In each case the simulations show cases where the two enantiomers are present in the ratios 50:50, 95:5, and 99.5:0.5. Figure 4.2 differs from Figure 4.1 in that the degree of separation between the enantiomers is twice as large. Whilst the degree of separation in Figure 4.1 is entirely adequate to give good quantification for a ratio of enantiomers of approximately 50:50, the uncertainty for a ratio of 95:5 would be significant and that for a ratio of 99.5:0.5 very considerable. For good quantification of the samples with the low levels of the minor enantiomer the degree of separation must be much higher than that depicted in Figure 4.1, for example that shown in Figure 4.2.

The representations shown in Figures 4.1 and 4.2 are idealised using perfectly symmetrical peak shapes. Such ideal behaviour is rarely seen in practice and usually the problem of peak tailing due to overloading or other problems means that the degree of separation required for good quantification of the minor enantiomer is even higher.

Low levels of the minor enantiomer place additional demands upon the analytical method. Unless the analyte has a very strong chromophore it may be difficult to detect the minor enantiomer at a level of 0.5% or below. In order to achieve the required sensitivity for the minor enantio-
4.2 Factors Controlling Resolution

As has been discussed previously resolution between the enantiomer peaks depends upon two factors: the efficiency and the selectivity of the separation method. These two factors are incorporated in equation (4.1) which is used to describe resolution in CE [2].

\[ R_s = \left( \frac{V}{32D} \right)^{0.5} \frac{(I)^{0.5}}{L} \frac{\Delta \mu_{ep}}{(\mu_{ep} + \mu_{eo})^{0.5}} \]

(4.1)

where \( V \) is the applied voltage, \( D \) the average diffusion coefficient for the two enantiomers, \( L \) is the total capillary length, \( I \) the length of the capillary from the inlet to the detector, \( \Delta \mu_{ep} \) the electrophoretic mobility difference between the two enantiomers, \( \mu_{ep} \) the average electrophoretic mobility of the two enantiomers, and \( \mu_{eo} \) the electroosmotic mobility.

The first two terms of equation (4.1) are related to the efficiency of the separation system i.e. the extent to which the enantiomer peaks become dispersed during movement from the injector to the detector. The last term in equation (4.1) is related to the selectivity of the system i.e. the extent to which the peak maxima are separated.

4.2.1 Efficiency

The increase in resolution with the square root of the applied voltage that is expected from equation (4.1) has been demonstrated experimentally by Hutterer and Jorgenson [3]. The resolution between pairs of analytes was measured at voltages of 28 and 120 kV and the expected increase in resolution by a factor of 2.1 was in good agreement with that found experimentally.

Giddings has considered the ideal case in CE in which peak dispersion is caused by diffusion along the length of the capillary alone [4]. Under these conditions, and in the absence of electroosmotic flow, the efficiency can be related to both the voltage and the charge on the analyte as shown in equation (4.2).

\[ N = \frac{FV_z}{2RT} \]

(4.2)

where \( N \) is the number of theoretical plates, \( F \) is the Faraday constant, \( V \) is the potential difference between the capillary inlet and the detector, \( z \) is the number of charges on the analyte, \( R \) is the gas constant, and \( T \) is the absolute temperature. At 298 K the value of \( F/2RT \) is about 20 and so equation (4.3) may be employed as an approximation to equation (4.2).

\[ N \approx 20 \frac{V_z}{\Delta \mu_{ep}} \]

(4.3)

Equation (4.2) has also been investigated by Kenndler and Schwer in experimental work on aromatic sulphonic and carboxylic acids [5]. Plate counts for mono- and disulphonated benzene and naphthalene derivatives were recorded using potential drops of 8.84 and 4.42 kV. At the lower voltage the plate counts for the disulphonated analytes were greater than those for the monosulphonated analytes by a factor of 1.9. Doubling the potential drop lead to an increase in the plate count by an average of 1.95 for the monosubstituted analytes and an average of 1.75 for the disubstituted analytes. The measured plate counts were between 67 and 82% of the values expected from equation (4.2), with the agreement being closer for the monosubstituted analytes and with the smaller potential drop.

The differences between the theoretical and measured efficiency values was ascribed to differences in the conductivities between the analyte and sample zones, and to Joule heating. By using buffers of different \( pH \)s Kenndler and Schwer were also able to alter the degree of dissociation of three substituted benzoic acids. It was found that the measured plate count was directly proportional to the degree of dissociation (i.e. the partial charge on the analyte).

The results from other experiments can also be interpreted with the aid of equation (4.2). Figure 4.3 shows the separation