

# Chapter 11

## Concluding Remarks and Future Perspectives

Capillary electrophoresis for the separation of small pharmaceutically or biomedically interesting compounds can be subdivided into two major modes. The first and most simple mode, in which its selectivity is based on the electrolyte system (pH and the ionic strength) is called capillary zone electrophoresis (CZE). When the selectivity in such a system is not sufficient, often expressed as the selectivity factor  $\alpha$ , the use of additives to the background electrolyte may offer extra separation power. The latter approach is commonly addressed as capillary electrokinetic chromatography (CEKC, or EKC), due to the introduction of a (*pseudo*-) stationary phase. Hence, it is analogous with conventional chromatographic methods.

Based on the nature of this (*pseudo*-) stationary phase, EKC can be further subdivided in micellar electrokinetic chromatography (MEKC) and capillary electrochromatography (CEC). This thesis describes the use of (chiral) selectors that are (partially) based on molecular recognition for the chiral or achiral separation of pharmaceutically interesting compounds with closely resembling structures. These selectors may provide a unique kind of selectivity, as compared to organic modifiers or micelles, and may lead to an increase in analysis time for only one or a limited number of analytes.

Chiral selectors added to the background electrolyte are considered to be an EKC mode, while the change in migration rate of the enantiomers is a direct consequence of the interaction of the analyte with a *pseudo*-stationary phase.

Cyclodextrins are by far the most widely used class of chiral selectors in separation science. We have shown that incorporation of these CDs in a removable and replaceable gel can significantly improve the selectivity. The latter was illustrated by the enantioseparation of several  $\beta$ -mimetics. Furthermore, the use of these chiral selectors for the achiral separation of structurally related phenothiazines was shown. The latter proves that additives influencing the selectivity based on chiral and/or molecular recognition are not only applicable to provide chiral separations, but that they can also be used to fine-tune the selectivity in non-chiral separations. One of the aims of this thesis was to investigate the different selectivities that the various types of cyclodextrins can offer. It was shown that some cyclodextrin derivatives are able to separate the enantiomers of a selected

pharmaceutical, whereas they are unable to accomplish a baseline separation of a structural analog. A relatively new class is that of the charged cyclodextrins. Especially the chiral separation of charged analytes by using an oppositely charged cyclodextrin was a challenging and theoretically interesting approach. In our study on the chiral separation of cationic ofloxacin with an anionic cyclodextrin derivative, it was shown that chiral baseline separations were possible within 4 minutes. Also the use of dual cyclodextrin systems appeared to result in useful selectivity changes, although some of the results could not be explained. A challenge for future research will be the coupling of specific functional groups to the naturally occurring cyclodextrins, which may provide even higher selectivity towards certain analytes. One of the major limitations in this approach is the reproducibility of the synthesis and the characterization of the obtained cyclodextrin-derivatives. Furthermore, to determine the principle behind the recognition process of the cyclodextrin derivatives, future research should focus on the nature of inclusion complexation, e.g. by NMR and by molecular modeling.

The use of molecularly imprinted polymers (MIPs) is an even more tailor-made approach in investigating chiral and other separations. In this thesis it was shown that spherical molecularly imprinted polymer particles added to the run buffer in capillary electrokinetic chromatography are a challenging and promising application. Precipitation polymerization seems to be an effective way to produce spherical particles less than 1  $\mu\text{m}$ , without the necessity of a time-consuming grinding process. We have shown in some preliminary experiments that chiral separation using these MIPs can be achieved. A problem that arises is the fact that only partially filling techniques can be used to prevent the microspheres from passing the detector window: that would subsequently lead to major fluctuations in the baseline signal due to light scattering. Therefore, electroosmotic flow has to be prevented by the choice of a low pH value. Another problem is peak broadening caused by slow mass transfer of the analyte from stationary to mobile phase due to a strong interaction with the MIPs, which can lead to a poor resolution. The production of even smaller particles, nanospheres, might help to further overcome this problem and may lead to *pseudo-*

stationary phase materials that can provide unique and programmable selectivity in capillary electrochromatography.