CHAPTER V

CONCLUSION AND FUTURE WORK

In this work, microemulsion electrokinetic chromatography (MEEKC) was developed for separation and analysis of avermectins, such as abamectin, ivermectin and doramectin, in commercial formulations. The following MEEKC conditions for separation of analytes were optimized, such as types and concentrations of organic co-solvent, SDS concentration, types of co-surfactants, temperature and applied voltage. A change in resolution of avermectins in MEEKC with suppressed electroosmosis was explained using the derived equation reported in recent work of our research group [Nhujak *et al.* 2006], where resolution is related to selectivity, retention factor and efficiency.

In comparison with acetonitrile, methanol, ethanol and 2-propanol used as organic co-solvent, results showed that ethanol provided better resolution of avermectins, and avermectins separated from other compounds in the commercial formulations. With increasing the co-solvent concentration, the resolution of avermectins increased up to a maximum and then decreased at the concentration higher than 30 % v/v co-solvent, while longer analysis time was found due to the smaller retention factor and an increase in the viscosity of the buffer. An increase in the SDS concentration resulted in a slight change in selectivity and resolution, and gave the faster analysis time due to the greater retention factor. An increase in temperature and/or applied voltage provided faster migration time, but worse resolution, high background noise and imprecision in migration time were obtained due to the effect of Joule heating. Application of pressure during separation with applied voltage resulted in faster analysis time, but worse resolution. Therefore, the suitable MEEKC conditions were obtained to be the microemulsion buffer containing 50 mM phosphate buffer at pH 2.5, 1.1 % v/v *n*-octane as oil droplets, 180 mM SDS as surfactant, 890 mM 1-butanol as co-surfactant and 30 % v/v ethanol as organic co-solvent; applied voltage of -15 kV; and separation temperature of 25 °C. Achieved baseline resolution was obtained with $R_s \sim 4.9$ for ivermectin and doramectin and ~ 4.1 for doramectin and abamectin, and with analysis time within 25 min.

For quantitative analysis of individual avermectin, ethyl 4-hydroxybenzoate was used as internal standard, and the calibration plot was established using the ratio of corrected peak area of individual avermectin to that of internal standard ($A_{corr,ratio}$) as a function of the concentration of individual avermectin. The linear relationship gave a high value of correlation coefficient, with $r^2 > 0.998$. High accuracy of the MEEKC method were obtained for the determined amounts of avermectins spiked in the microemulsion and the solution of commercial formulations, with recoveries in a range of 98.7 to 103.4 % and with RSD < 2.0 %. In addition, high precision in migration times of analytes and $A_{corr,ratio}$ was also found with RSD $\leq$ 2.0 % for intraday and $\leq$ 2.7 % for interday.

The contents of avermectins in commercial formulations were determined by HPLC and MEEKC. Using paired *t*-test analysis at 95 % confidence interval of the mean, non-significant difference was obtained for the contents of avermectins determined by HPLC and MEEKC. Good agreement was found between the determined and labeled amount of all commercial formulations, except the sample of ivermectin formulation. Therefore, MEEKC can be used as an alternative method for quantitative determination of individual avermectins in commercial formulations with fast analysis time and lower amount of organic solvent consumption, in comparison with HPLC analysis.

In the future work, the developed MEEKC method could be used for analysis of avermectins in fermentation broth, where screening and monitoring products are needed. Furthermore, MEEKC may be used for quantitation of avermectins residue in real samples; however, off-line and/or on-column preconcentration should be used to enhance sensitivity of trace levels.