論　文　の　要　旨

題　目　　Development of high performance analytical method for DNA fragments by capillary electrophoresis with electrokinetic supercharging preconcentration
（動電過給前濃縮キャピラリー電気泳動法による DNA 断片の高性能分析法の開発）

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With recognized advantages of instrumental simplicity, rapid analysis, minor sample and solvent consumption in comparison with HPLC, CE has became a popular separation technique in several application areas. However, the problem of relatively low concentration sensitivity Hindered the spread of CE. To address this drawback of CE, a variety of on-line preconcentration techniques have been proposed. Electrokinetic supercharging (EKS)-CZE is one of the efficient preconcentration technique, which implies an extended electrokinetic injection to introduce a great amount of analyte and followed by a tITP stacking step to refocus the injected analytes into a narrow zone. During the analysis of highly diluted sample, the EKS procedure was simplified by automatically generating the system-induced terminator without adding an external terminating ions. In combination with modification of electrode setting, a high sensitivity comparable to ICP-MS for rare earth ions was achieved. Furthermore, capillary gel electrophoresis (CGE) has been used for DNA anslysis based on the principle that soluble polymers were used as sieving matrix for size separation. Except for using highly sensitive fluorescence assay, it is difficult to improve the sensitivity of DNA analysis by conventional UV detection. Aiming to achieve high accuracy and sensitivity analytical method, the study was succeeded in developing a suitable EKS system for DNA analysis by CGE.

In Chapter 1, general introduction of CE including the developing history, wide applications from small ions to biomolecular, basic principles and instrumentation.

In Chapter 2, after discussing preconcentration by EKS, as one of the factors affecting the reproducibility of the quantitative analysis, we focus on the variation of the distance between electrode and capillary end, which discussed for (e.g. immobilization of electrode spacing) improved methods.

In Chapter 3, EKS system was applied to the analysis of rare earth ions. According to the 2D computer simulation, we can understand automatically generation of the terminal zone (system-induced terminator).

In Chapter 4, in order to apply EKS-CGE for analysis of DNA fragments, a low viscosity BGE was necessary since the mobility of leading ion is quite important. EKS performance was promoted due to the generated leading ion of co-ion (ions with the same charge sign as the sample ion) took over the role of leading ion for ITP preconcentration. To further improve the sensitivity, the electrode configuration and position was modified again. The obtained LOD of the weakest peak of 72 bp fragment was around 7.7 ng/L, apparently improved more than 10 000-fold in comparison with conventional CGE with UV detection.

In Chapter 5, as a result of applying a high voltage (10 kV/50 cm) during injection, very dilute DNA fragments (below 0.2 mg/L) could be damaged in aqueous solution during EKI. Computer simulation suggested that the high electric field could be generated partly during EKI process. It is practical that injection voltage should be kept as low as possible especially for the dilute DNA sample.

In Chapter 6, we focus on the problem of quantitative reproducibility in CGE analysis of DNA fragments, factors like electrode configuration and BGE carry-over were investigated. Finally, a nice reproducibility were obtained by optimization. RSD of peak area reduced from more than 20 % (without washing) to 7-8 % obtained by a optimized injection method.

In Chapter 7, conclusions of this dissertation were described finally.