

博士論文（要約）

**A long bare open tubular capillary  
chromatography. Methodological  
developments, separation mechanism and  
elution behavior characterization**

（長い中空キャピラリークロマトグラフィーに  
関する方法論開発及び特性評価）

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High-performance liquid chromatography (HPLC) is a widely used separation technique. A continuous quest for new principles, instrumentation, and technologies has been undertaken to satisfy these demands. The chromatographic separation is achieved in a column using solid and mobile phases. Despite their growing number, separation methods face certain problems such as analyte adsorption or interaction with the solid phase, size range limitation, and protein denaturation in the mobile phase.

In this study, a liquid chromatography technique was developed on a long bare open tubular capillary using water/phosphate buffered saline as mobile phases. Interactions between biomolecules and solid phases were reduced using the open tube capillary column and large analytes readily penetrated the column. Separations were difficult to achieve under these conditions because of the weak driving force; however, the column was lengthened to solve this problem. A long column usually provides high efficiency and sharp peaks. The peak width is evaluated by the number of theoretical plates. Therefore, lengthening the capillary column is expected to facilitate analyte peak separation, even with very small retention time differences.

A new HPLC method using marked LC equipment. A very long open tubular capillary was used as the column while water and phosphate buffered saline (PBS) acted as the pressure driven mobile phase. The methodological developments, separation mechanism and elution behavior characterization of such a system, a long bare open tubular capillary chromatography, were described here.

The successful analysis by OT capLC essentially dependent on equipment techniques described, such as small volume injection, small volume solution sending and void volume minimization through every part of the instrumentation system to prevent the sample spreading. Under such analysis conditions, the long capillary column without column-to-column connection was used to obtain high separation efficiency and sharp peaks with  $89 \times 10^5$  theoretical plates for thiourea. A new electrostatic diffusion layer repulsion model will be used to further elucidate this phenomenon. Liquid molecules in a confined nanospace behave differently from the bulk because of the high surface-to-volume ratio<sup>1</sup>. This effect was most pronounced at the electric double layer (EDL). The elution behavior of negatively charged analytes was explained qualitatively and quantitatively using a theoretical HDC model basing on Poiseuille (parabolic velocity distribution profile). This HDC model was proved by examining by inner wall coating, inner diameter, salt concentration, temperature, mobile phase linear velocity effect and capillary length. This HDC

model proved useful for measurements of anion analyte distributions in the EDL. The special phenomenon has been evaluated by the parameters of relative retention time, exclusion distance in EDL, and discussed by the concept of theoretical exclusion model, normal distribution, DLVO theory. (1) The thin layer exhibits a dynamic shear flow instead of a steady-state regime. The attractive van der Waals and the repulsive electrostatic potentials form the total interaction energy in the EDL. Van der Waals forces are present in combination with electrostatic forces. So the analyte should keep a relative stable state in the EDL. The EDL reached its maximum thickness on a bare OT capillary column using water as a mobile phase.(2) The Reynolds number is small than 1, consistent with a laminar flow. The laminar flow displays a Poiseuille (parabolic) velocity distribution profile in the radial direction. The eluent develops a velocity gradient normal to the flow direction exhibiting a maximum near the center of the interstitial space and vanishing to zero at the inner wall surface. The local velocity for a stream line at radial position  $r$  is typically parabolic and the maximum flow rate at the center of the capillary is twice as high as the average flow rate. The experimental data of capillary length effects indicate that a sample distribution is varying in a long open tubular capillary column. A sample distribution has been theoretically explained by ion concentration, the number of unit charge on an ion, the flow rate and temperature of mobile phase and capillary radius and capillary length. In probability theory, the analyte distribution in an open tubular capillary column should be a normal distribution. Analyte normal distribution exhibits a dynamic distribution, and analyte formed different position in the capillary cross-section. All the affecters should be based on a common continuous probability distribution that is dynamic state but not static state.

Analyte formed different position in the capillary cross-section. In the exclusion region near the capillary inner wall, the concentration of analyte is nearly zero; the concentration become higher and higher with the position near to the capillary center and it has a maximum at the center. The concentration become higher and higher near to the center of capillary and far from the injection of the capillary column.

The elution behavior of negatively charged analytes was explained using a new theoretical HDC model superstructured with not only a poiseuille velocity distribution profile but also an analyte normal distribution profile. The new model proved useful for measurements of anion analyte distributions in the capillary.

The long capillary was further applied to the separation of high-molecular-weight proteins

using phosphate buffered saline. Retention time and peak shape of proteins were depended on by their pIs. Retention time increased and peak became wider with increasing the pI. The OT capillary column displaying a low-specific surface area and high theoretical plate number produced improved peak shape. Results showed that the protein separation was mainly driven by electrostatic interactions, which dominate in ion-exchange chromatography. Such a weak ion exchange effect are advantageous for a number of reasons, including a reduced tendency to sample denaturation, their inability to bind weakly charged impurities and the enhanced elution resolution. The elution takes place under mild conditions (without column packing and with PBS buffer), so that the protein can maintain its native conformation during the chromatographic process. The impact of mixed concanavalin A, catalase, and chymotrypsin composition on the characteristics of protein detection chromatograms was discussed. The peak shape changes due to the concentration of rhodamine B or rhodamine 110. This analysis system is expected to be useful for monitoring and distinguishing proteins that exhibit weak properties changes.

Therefore, the separations by weak hydrodynamic chromatography on thin electrical double layer using long open bare tubular capillary and pressure water/ PBS were achieved. Because the capillaries are uncoated, the column lifetime is virtually infinite. In addition to extremely low operational costs, this approach benefits from a unique daily consumable, which is the eluent, and generates environmentally friendly waste; however, the retention times are long. Improvements may be achieved by optimizing the instrumentation, such as small volume during solution sending, autoinjection, detection, and system void volume minimization. Scaling down the analytical conditions is expected to shorten the retention times.

Future studies involve the feasibility of chromatography applications. Some questions such as the potential effect of the open capillary coil radii also need to be addressed. Data with enhanced reliability may be obtained to describe protein characteristics and biological function. OT cap LC still has some potential attraction. This newly developed analytical method may significantly impact protein analysis, especially for protein misfolding related to neurodegenerative diseases and protein drugs.