

Separation and Chiroptical Detection of Biomolecules Using an LC-SRCD System: A Case Study on Amino Acid Profiling

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Circular dichroism (CD) spectroscopy is a powerful tool for analyzing the stereochemical structures of chiral molecules. However, conventional CD instruments are typically limited to the ultraviolet (UV) region (>190 nm). Consequently, acquiring structural information for molecules that have characteristic absorption bands in the vacuum-ultraviolet (VUV) region, such as saccharides and amino acids, remains challenging [1, 2]. In the present study, a high-intensity synchrotron radiation source at the Hiroshima Synchrotron Radiation Center (HiSOR) is utilized to develop an integrated system combining High-Performance Liquid Chromatography (HPLC) with a VUV-CD spectrophotometer [3].

A specialized flow-cell system capable of meeting the requirements of VUV measurements while coupled to an HPLC unit has been installed at BL12. The system's performance was evaluated through real-time separation and analysis of amino acids (*this study*) and sugars (*currently under investigation*).

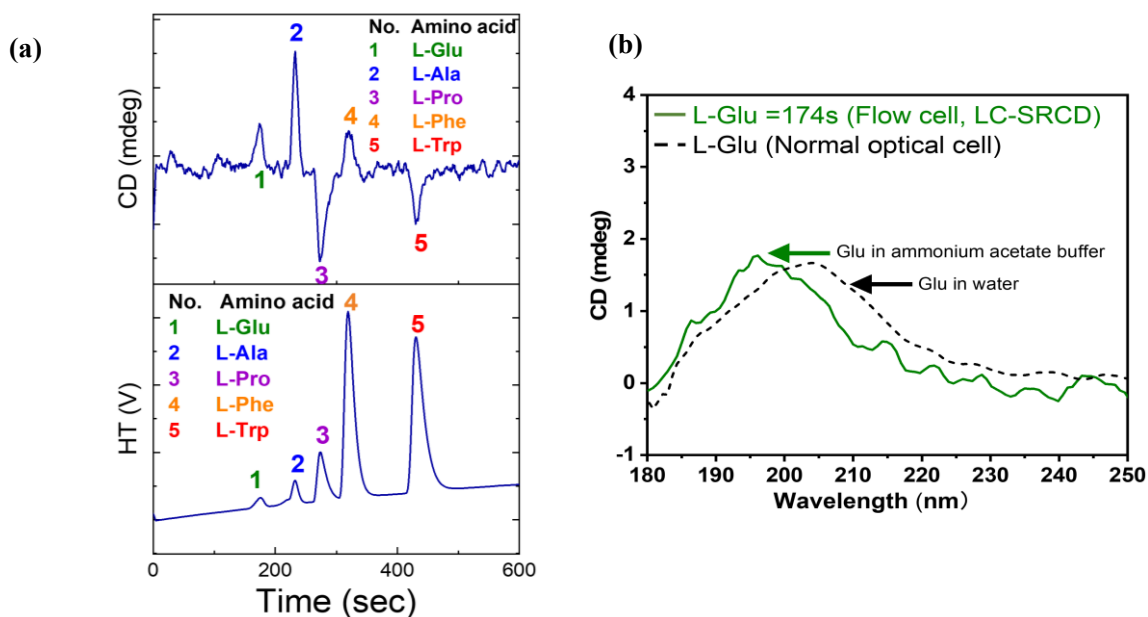


FIGURE 1. LC-SRCD separation and conformational study of an amino acid mixture: (a) online monitoring of the LC separation of five amino acids; and (c) CD spectrum of L-Glu separated by LC and simultaneously monitored by CD, compared with the conventional CD spectrum.

The results demonstrated that the integrated LC–SRCD system successfully separated five amino acids namely L-glutamic acid (L-Glu), L-alanine (L-Ala), L-proline (L-Pro), L-phenylalanine (L-Phe), and L-tryptophan (L-Trp) while simultaneously recording their CD spectra in the VUV region down to ~170 nm (**Figure 1a,b**). This development enables the direct identification and structural analysis of chiral compounds in complex mixtures, offering a novel analytical approach for biochemical and pharmaceutical research. Future work will focus on separating mixtures of sugars, upgrading the LC–SRCD system to be fully automated, and improving the signal-to-noise ratio.

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