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On-Line Multichannel Raman Spectroscopic Detection System For Capillary Zone Electrophoresis

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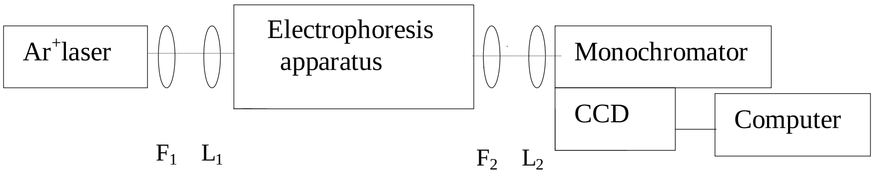
Abstract: An on-line multichannel Raman spectroscopic detection system for capillary electrophoresis was established by using an Ar^+ laser and a cryogenically cooled ICCD. Resonant excitation Raman spectra of methyl red and methyl orange were employed to test the system. The result shows that it could yield on-line electrophoretogram and time series of Raman spectra.

Keywords: Raman spectroscopy, capillary electrophoresis, online coupling, methyl orange, methyl red

Detection and peak identification are key factors in developing CE (capillary electrophoresis) methodology. They have become hotspots for many reasons. The most obvious one lies in the revealing of unknown species in the application of CE to biological samples. CE–MS and CE–NMR have since been developed. CE–Raman spectroscopy was also reported[1]. As compared to the former two methods, the Raman spectroscopy method is highly attractive because it has the potential to provide high-information-content spectra for solutes dissolved in the aqueous media most often employed in CE. We have thus started a project to build a laboratory coupling system since 1998 and have reached a stage for practical use. In this communication, we present some preliminary results.

Figure 1 Diagram of the Raman spectroscopy detection system for CZE
F_1,F_2—filter L_1,L_2—lens.



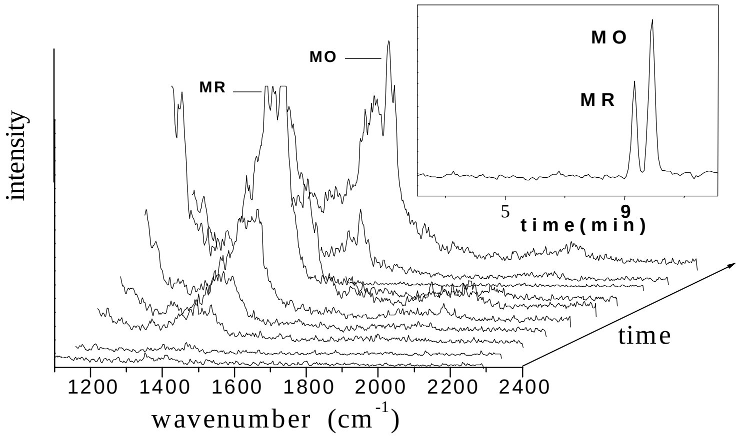
In **Figure 1**, is shown the on-line Raman spectroscopic detection system for CE. It consists mainly of an Ar^+ laser (Nanjing Sanle Corp.), a 0.275m focal length monochromator (Acton Research Corp. SpectraPro 275) with a 1200 groove/mm grating, and a cryogenically cooled ICCD detector (Princeton Instruments. inc. ICCD-576 G/1). The ICCD has 512x512 pixels and a ten micron spectrograph entrance slit which gives a resolution of about $4cm^{-1}$. The laser radiation at 488nm is focused into the detection window of the CE capillary and the Raman signal is filtered through filter F_2 and gathered by focusing on the array of optical fibers. The fibers are set at an angle of 15° away from the direction of the laser beam. The monochromator is operated at f/3.8.

The Raman spectra are monitored and treated by a computer with the software package of CSMA.

Electrophoresis is carried out in a fused silica capillary (Yongnian Optical Factory, Hebei, China) of 520μm i.d.x64cm (effective length). Samples are introduced into the positive end of the capillary by hydrodynamic injection for 10s at 3cm height. They are separated at 5kv (55-65μA) at room temperature. Methyl orange (MO) and methyl red (MR) are used as test samples. Their Raman spectra recorded have the maximal absorption of 464nm and 440nm, respectively[2]. The peaks around 1410cm^{-1} show the strongly enhanced N=N stretches of the basic forms of MO and MR, which agrees well with the literature[3].

Figure 2 demonstrates a time series of Raman spectra measured from 10^{-3}M MO and MR at the integration time of 5s. This is a compromise between the signal collection needs of Raman spectroscopy and the resolution requirement of CE, although 1-2s integration time has acceptable signal/noise ratios. By increasing the integration time to 10-20s, the detection limits can be lowered, but the spectrum appears to suffer from a peak broadening. To remove heat and manipulate easily, fairly low voltage is adopted in this work (5KV).

Figure 2 Time series of Raman spectra obtained from 10^{-3}M MO and MR at 5s integration for each spectrum. Raman-detected (1420cm^{-1}) electrophoretogram is shown in the up-right frame.



The results shown in **Figure 2** have proved the possibility of our system to be used for on–line Raman spectroscopy detection of solutes. The detection sensitivity has however to be further improved and an approach is being taken. The main idea is to adopt the principles of surface enhanced Raman spectroscopy. More discussion will be made elsewhere.

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