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Light-scattering detection with a fluorimetric detector in high-performance liquid chromatography

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Abstract

Non-fluorescence compounds were detected by a fluorescence detector based on scattering light. The fluorescence detector was used without any modification, and the scattering light was observed at the wavelength twice as long as the excitation wavelength. Actually the wavelength of the observed scattering light was the same as that of the excitation light. The maximum signal was achieved at around 280 nm. The signal was increased with increasing molecular weight or size of analytes. Colloidal silica with nanometer sizes, ethylene glycol oligomers, saccharides and cyclodextrins could be visualized by the present detection method. The detection limit at S/N = 3 for colloidal silica with 78 nm was 39 pg for 20-μL injection.

Keywords: HPLC; Light-scattering detection; Fluorescence detector; Colloidal silica; Saccharides

1. Introduction

Fluorimetric detectors usually provide higher sensitivity and they have been frequently used in HPLC. Fluorescence detectors are selective and non-fluorescent compounds have to be therefore derivatized prior to detection. Since derivatization processes are sometimes time-consuming and less reproducible, they should preferably be avoided.

Refractive index detectors and mass spectrometers are universal detectors employed in HPLC. However, the former detectors are not sensitive, whereas the latter detectors are expensive. Detectors acquiring scattering light are also expected to provide universal and sensitive detection in HPLC. So far, low-angle laser light-scattering detectors [1,2] and evaporating light scattering detectors [3–5] have been developed in HPLC. It is recognized that evaporative light scattering detection is compatible with solvent gradient elution and achieves the detection limits of 50–100 ng for common compounds [3]. Evaporative light scattering detectors have been widely applied because they are not limited to compounds that contain UV-absorbing chromophores, and they are not very sensitive to mobile phase variations [3]. It should be noted that solvent contaminations and column bleeding can cause severe baseline noise as for scattering light detection [4].

Dollinger et al. [6] used an HPLC fluorimeter as a 90° light-scattering detector for biopolymers. They demonstrated that a simple HPLC fluorimeter can be used as a 90° light-scattering detector for biopolymer molecular weight determinations. They modified the fluorimetric detector placing a second-order filter for the measurement of scattering and detected 7.7 μg of soybean trypsin inhibitor (20,000 dalton) and 1.1 μg of IgG (150,000 dalton).

We have tried to use a fluorimetric detector as a simple 90° light-scattering detector without any modification based on the same detection principle as demonstrated in ref. [6]. The present paper describes preliminary results on light scattering detection of non-fluorescent colloidal silica as well as lower-molecular-weight compounds using a fluorimetric detector.

2. Experimental

2.1. Apparatus

The eluent was supplied by a model PU-880 or a PU2080i plus Intelligent HPLC pump (Jasco, Tokyo, Japan) at a flow-rate of 1.0 mL min^{-1}. Separation columns commercially available

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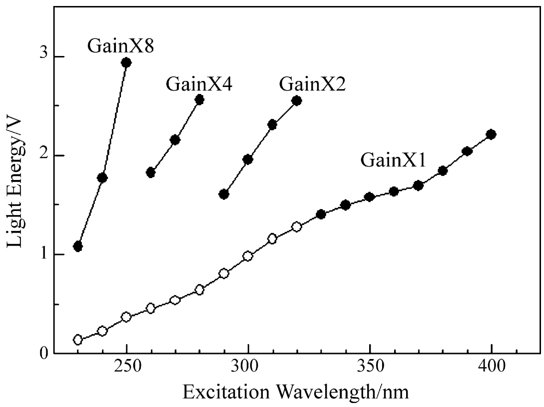

Fig. 1. Light energy of the xenon lamp employed in this work. Open symbols are original light energy.

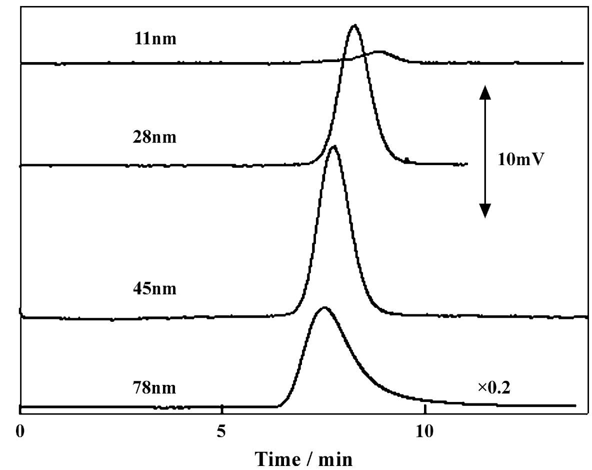

Fig. 2. Light scattering detection of silica colloids in size-exclusion mode. Column: TSKgel 6000 PW, 300 mm × 7.5 mm I.D; eluent: 10 mM $Na_2HPO_4 + NaH_2PO_4$ (pH 9.1); flow-rate: 1.0 mL min^{-1}; concentration of analytes: 3.7 ppm for 11 nm, 6.6 ppm for 28 nm, 5.3 ppm for 45 nm and 5.2 ppm for 78 nm; injection volume: 20 μL; detector: FP-920 fluorescence detector; excitation wavelength: 280 nm; emission wavelength: set at 560 nm.

were employed. Analytes were injected with a model 5095 injector equipped with a 20 μL sample loop (Rheodyne; Cotati, CA, USA) or a model 7000 six-way switching valve (Rheodyne). The sample loop for the latter injector was prepared in the laboratory. An FP 920 or an FP-1520 fluorescence detector with 16 μL flow cell was obtained from Jasco, and its light source (xenon lump) energy dispersion as a function of the wavelength is illustrated in Fig. 1. At lower wavelengths, e.g., shorter than 320 nm, the gain is automatically adjusted to compensate the lower light energy, as shown in the figure. All of the data were collected by a Chromatopac C-R7Ae plus data processor (Shimadzu, Kyoto, Japan).

2.2. Reagents and materials

HPLC-grade acetonitrile and distilled water were obtained from Nacalai Tesque (Kyoto, Japan). Silica colloids with different size were obtained from Catalysts & Chemicals Industries Co. (Kawasaki, Japan), β-cyclodextrin, ethylene glycol and triethylene glycol were purchased from Tokyo Chemical Industry (Tokyo, Japan). Other reagents were of guaranteed reagent grade and were obtained from Nacalai Tesque. All reagents were used without any further treatment.

The separation columns employed in this work were TSKgel G3000PW (10 μm particle diameter, 300 mm × 7.5 mm I.D.; TOSOH, Tokyo, Japan) and G6000PW (17 μm particle diameter, 300 mm × 7.5 mm I.D.; TOSOH) for size exclusion chromatography, L-column ODS (5 μm particle diameter, 150 mm × 4.6 mm I.D.; Chemicals Evaluation and Research Institute, Tokyo, Japan) and Develosil C30-UG-5 (5 μm particle diameter, 150 mm × 4.6 mm I.D.; Nomura Chemical, Seto, Japan) for reversed-phase mode separation, and Develosil NH_2-5 (5 μm, 250 mm × 4.6 mm I.D.; Nomura Chemical) for normal-phase mode separation.

3. Results and discussion

3.1. Detection of colloidal silica

When the excitation wavelength of the fluorescence detector is set at some value (λ), light with the wavelength λ, which scatters from the flow cell, can reach to the grating for the emission. If the emission wavelength is set at twice the excitation wavelength (2λ), the scattered light with the wavelength λ reaches to the photomultiplier and the signal can be observed. For example, when the excitation and emission wavelengths of the fluorescence detector were set at 280 and 560 nm, respectively, the scattered light with the wavelength of 280 nm can be monitored.

Fig. 2 demonstrates the detection of colloidal silica with different sizes between 11 and 78 nm. The signal for the 78 nm colloid is so large that the signal is attenuated in the figure. In Fig. 2a, 20 μL volume of the sample solution containing 3.7 ppm of 11-nm silica colloid, 6.6 ppm of 28-nm silica colloid, 5.3 ppm of 45-nm silica colloid or 5.2 ppm of 78-nm silica colloid is separately injected onto the size-exclusion column. In other words, 0.07–0.1 μg of each analyte is injected and detected by observing scattered light. It can be also seen that the retention times of the analytes are slightly different, viz., the larger the colloid size, the shorter the retention time. This retention behavior supports that the analytes are eluted from the separation column in the size-exclusion chromatography mode.

In order to illustrate the effect of analyte size on the signal intensity, the size-exclusion mode column with smaller exclusion limits was employed. The silica colloids with 5–78 nm were actually eluted from the TSKgel 3000PW column at the same time and the signal intensity could be compared. Fig. 3 illustrates the effect of the peak height on the size of the colloidal silica, where a 20 μL volume of the sample solution containing 3.3–6.6 ppm of each silica colloid is separately injected and each signal is adjusted as 5 ppm. It is apparent that the signal intensity increases with increasing the size of the silica colloid. The present detection system is quite sensitive for large species. For example, the detection limit at S/N = 3 for the colloidal silica with 78 nm is 39 pg under the conditions as in Fig. 3. It should be noted that the peak height was linear to the colloid concentration and that the linear range was dependent on the colloid size