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Simultaneous Determination of Fluorescein, Rhodamine 6G and Rhodamine B in Turbid Solution by Polarization Variable-Angle Synchronous Fluorescence Spectrometry

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Abstract: Polarization variable-angle synchronous fluorescence spectrometry was proposed to determine samples in turbid solution. A mixture of fluorescein, rhodamine 6G and rhodamine B was used to evaluate the technique. The background caused by scattering light was decreased remarkably. The limits of detection were 0.6 ng/ml for fluorescein, 2.3 ng/ml for rhodamine 6G and 4.1 ng/ml for rhodamine B, respectively.

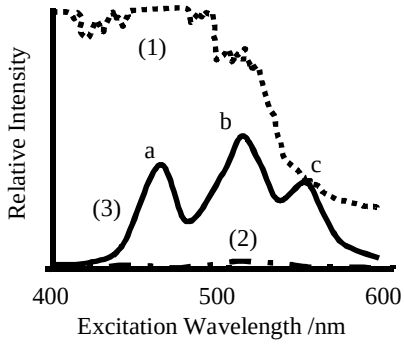
Keywords: Variable-angle synchronous fluorescence, polarization, fluorescein, rhodamine 6G, rhodamine B.

Fluorescence spectrometry, in practice, is hard to be applied to multi-component analysis due to the spectral overlap of complex mixtures. Moreover, environmental samples and biological samples are often turbid, and the direct determinations of such samples would suffer from the significant interference of scattering light. Synchronous fluorescence spectrometry is becoming popular concerning its simplicity, selectivity and sensitivity in dealing with complex samples. One drawback to conventional constant-wavelength synchronous fluorescence spectrometry (CWSFS) is that it is limited by a 45° section cut through the excitation-emission matrix. Variable-angle synchronous fluorescence spectrometry (VASFS) provides a more sensitive and selective fluorimetric method with a considerable flexibility in wavelength scanning[1~2]. The polarization technique can be used to suppress the interference of scattering light[3]. This technique has been applied to CWSFS, but not yet to VASFS. In this paper, polarization variable-angle synchronous fluorescence spectrometry (PVASFS) was proposed to determine three fluorescent dyes simultaneously in turbid solution. This work was performed on a laboratory-constructed versatile spectrofluorimeter[2].

The excitation and emission spectra of fluorescein, rhodamine 6G and rhodamine B (in 0.01mol/L NaOH) were overlapped severely. The simultaneous determination of these fluorescent dyes was ever performed by CWSFS[4] with $\Delta\lambda$ of 3 nm. Significant background appeared with CWSFS when the sample was turbid (talc powder), as shown in **Figure 1**(1), so that the fluorescence signals from the individual components were overwhelmed under such a big background. VASFS provides a technique to scan wavelengths more flexibly to reduce scattering interference and suit to different

fluorophores. A suitable scanning path with excitation and emission monochromators at the speeds of 120 and 102 nm/min respectively and the initial scan wavelengths of 400 and 436 nm, respectively, was chosen. Polarization technique was coupled with VASFS to suppress further the scattering light. Comparing **Figure 1**(2) with **Figure 1**(1), the background signal of the turbid blank solution was greatly suppressed by PVASFS. Fluorescein, rhodamine 6G and rhodamine B in a turbid solution were well resolved in the PVASF spectra as shown in **Figure 1**(3). The PVASF peaks at a, b and c can be used to determine fluorescein, rhodamine 6G and rhodamine B, respectively. The correlation coefficients of the calibration graph for them were all above 0.997. The limits of detection were 0.6 ng/ml for fluorescein, 2.3 ng/ml for rhodamine 6G and 4.1 ng/ml for rhodamine B, respectively. PVASFS should be a useful tool to determine individual components in complex matrix due to the flexibility in wavelength selection and the suppression of scattering light.

Figure 1. CWSF blank spectrum (1) and PVASF blank spectrum (2) of a turbid blank solution, and PVASF spectrum (3) of a turbid mixture of fluorescein, rhodamine 6G and rhodamine B



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