

Influence of the water content of ethanol used to dissolve the spectral fluorescence standards of BAM-F001x and BAM-F005x kits

The spectral fluorescence standards included in the BAM-F001x and BAM-F005x kits are charge transfer (CT) operated dyes. The spectral position, shape of their absorption as well as their emission spectra may be affected by the solvent polarity. The certified normalized corrected emission spectra of these kits were determined in spectrometric grade absolute ethanol. As the water content of the chosen ethanol is an important factor that influences the polarity, only high purity ethanol is recommended for use with the kits. Using an ethanol solvent with a higher water content can introduce spectral shifts, which can amount up to 5 nm for the longer wavelength dyes: BAM-F003x, BAM-F004x, and BAM-F005x. Therefore, the ethanol used for dissolving the solid dyes must be carefully chosen and properly stored.

Suitable ethanol must be of high purity: ethanol absolute, spectrophotometric grade, $\geq 98\%$ (GC). We recommend the use of ethanol from Merck KGaA, Darmstadt, Germany, product number **1.00980 ($\leq 0.05\%$ water)** or similar ethanol, that is of an equivalent quality to the absolute ethanol used for the certification of the corrected longer available through Sigma Aldrich (product number 34923).

The impact of the water content of ethanol is illustrated in Figures 1&2 below for BAM-F005x. F005 is the most polarity-sensitive dye and thus illustrates best the results of a study on the influence of the water content of ethanol on the absorption spectra and corrected emission spectra performed for the kit. For these studies, different ethanol / water mixtures have been used to dissolve the BAM-F005x solid dye, with a water content ranging from 0 to 15%. The absorption spectra and corrected emission spectra then obtained from the resulting solutions are shown in Figure 1. The spectral position of the long wavelength absorption band and the emission maximum obtained are shown in Figure 2.

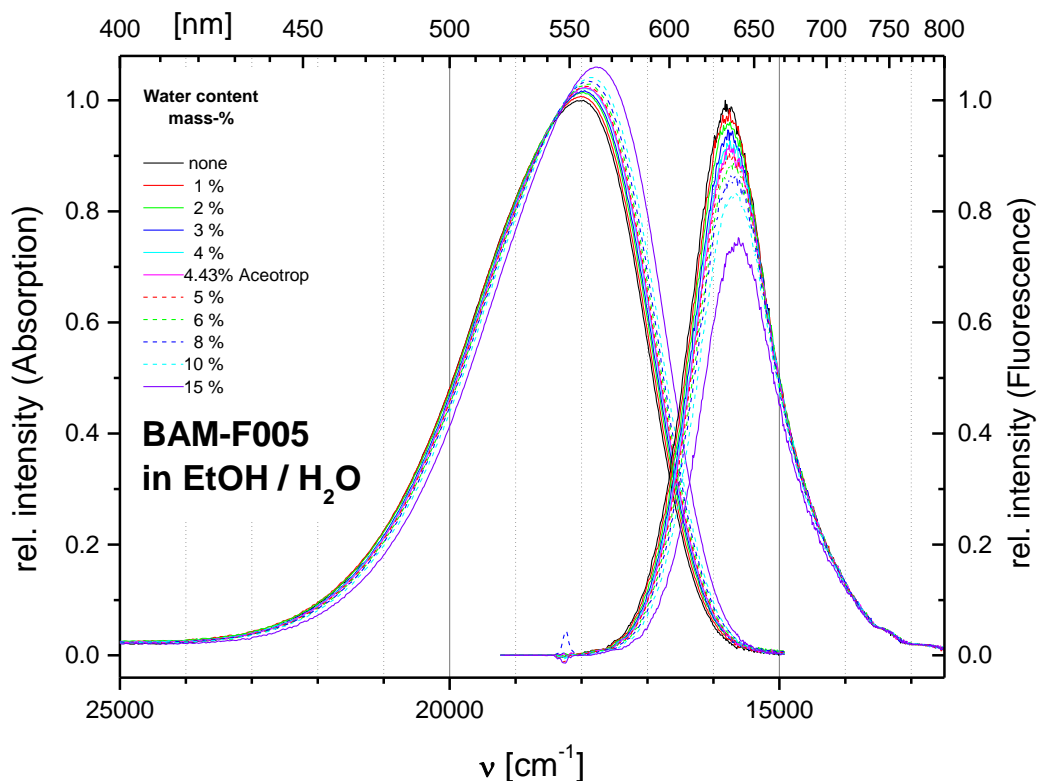


Figure 1. Absorption spectra (left) and corrected emission spectra (right) of BAM-F005x measured in ethanol (EtOH) - water (H₂O) mixtures of varying water content. All emission measurements were performed with identical instrument settings. The emission spectra were corrected for matrix-induced changes in the absorbance at the excitation wavelength.

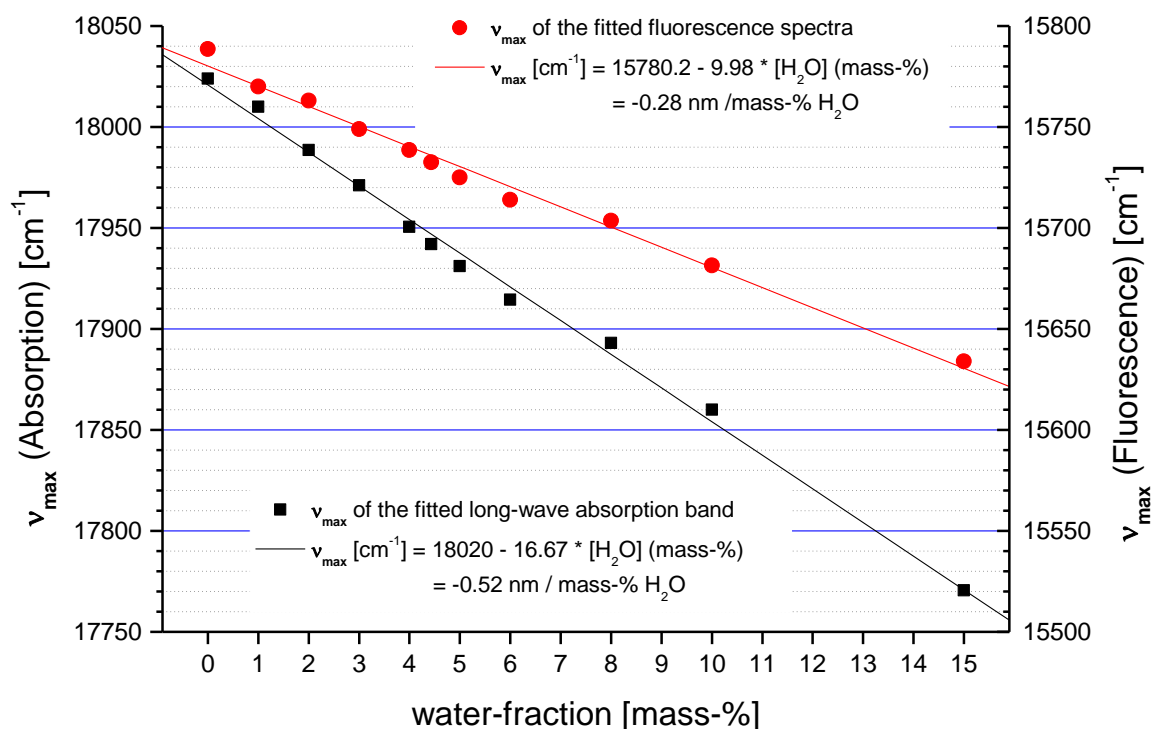


Figure 2. Influence of water content on the spectral position of the long wavelength absorption band and the emission maximum of BAM-F005x.