Technical Note: Affects on Relative Fluorescence of Fluorescein

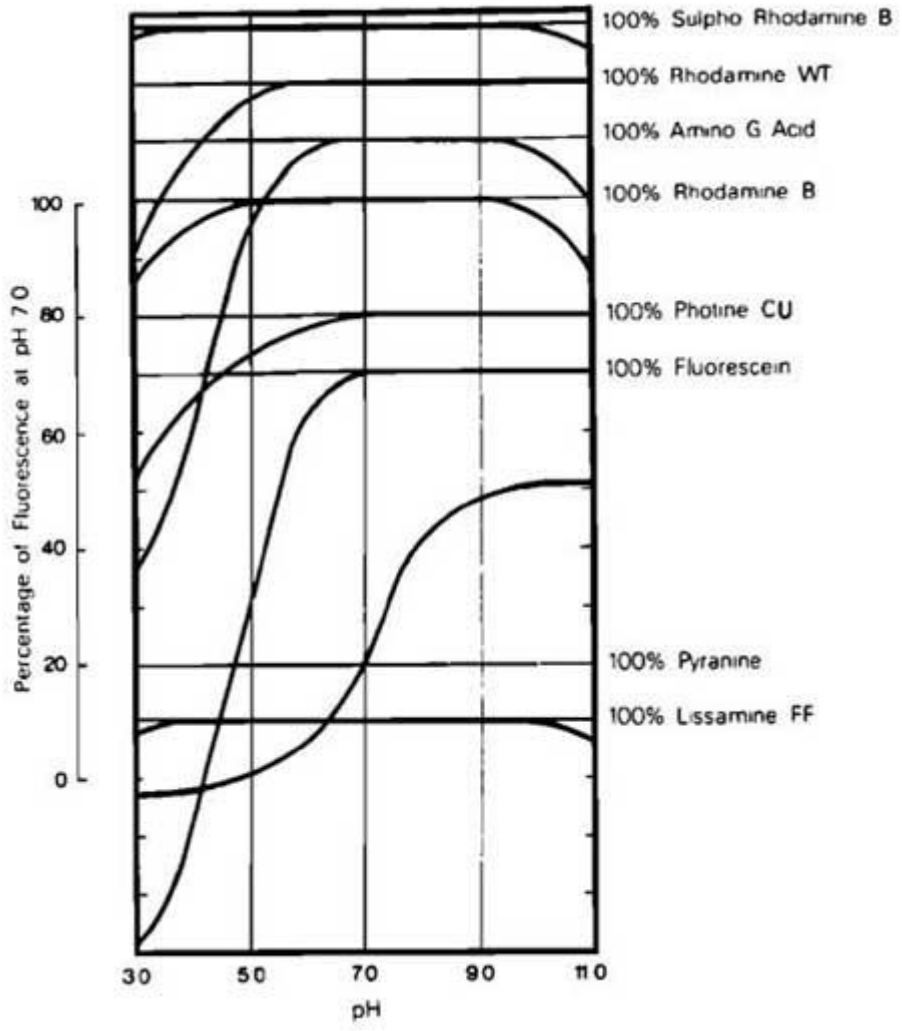
Fluorescein is a tracer dye used in flow studies, environmental, industrial, and biotech applications. There are many variations of fluorescein dye and each compound will produce slightly shifted excitation and emission maxima. However, these small shifts shouldn't greatly affect the signal detected by fluorometers that are configured with broad band excitation/emission filters, especially if the compound used in the study is the same compound used to calibrate the fluorometer.

Aside from the molecular structure of different fluorescein dyes, temperature is a parameter that affects the fluorescence response of fluorescein. Generally, as temperature increases, the fluorescence response of fluorescein decreases, and vice versa. Temperature correction coefficients are available for correcting this change in fluorescence:

[Fluorescein Temperature Coefficient = -0.0036 per degree Celsius]

There could be as much as 10% percent error if temperature is uncorrected and the temperature swings are 30 degrees or greater, such as boiler or cooling water applications.

The most significant effect on the fluorescence response of fluorescein is actually pH. The figure below is taken from a study done by Smart & Laidlaw (1977) and shows that a pH drop from 7.0 to 6.0 can account for as much as a 10% drop in the relative fluorescence of fluorescein. At pH values lower than 6.0 the relative fluorescence response of fluorescein drops sharply with almost a 40% drop in fluorescence at a pH of 5.0.



The recommended practice for conducting dye studies using fluorescein is to calibrate your sensors using water sourced from your study site or pH-adjusted deionized water in an effort to reduce pH effects which may be a significant source of error when measuring fluorescence response of fluorescein.

Turner Designs sells Fluorescein standards made at pH values between 6.5 – 6.8 (PN: 10-509).