Spectrophotometric determination of pK_a values for fluorescein using activity coefficient corrections

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Abstract

The absorbance of the organic water tracer compound fluorescein is known to be pH dependent but differences between the reported pK_a values make it difficult to predict these absorbance changes. A new pK_a determination method, which incorporated activity corrections, was used to calculate the pK_a values of fluorescein. Several published pK_a values were re-evaluated and were in agreement once activity corrections were applied.

Introduction

Fluorescent and coloured organic tracers are used in a wide variety of water investigations such as evaluating the mixing regime of treatment systems (Levenspiel, 1972), characterising the performance of stabilisation ponds (Shilton et al., 2000), or calculating the parameters used in activated sludge modelling (Makinia and Wells, 2000). A number of tracers are pH-sensitive and this can affect the spectrophotometric and transport behaviour of the tracer (Behrens, 1986). Should precise tracer data interpretation be required it is vital to have accurate pK_a values to account for this pH response. This paper describes a technique that was developed to measure the three pK_a values and four absorptivity factors of fluorescein.

The most rudimentary ionic model that accounts for the pH response of fluorescein (Klonis and Sawyer, 1996) uses the six different ionic species proposed by Zanker and Peter (1958). These include a cation, monoanion and dianion ionic species and a lactone, zwitterion and quinonoid neutral species. While each ionic species has its own characteristic absorbance spectrum, the strongest absorptivity is associated with the dianion and there are substantial differences in the species absorptivities at the dianion analytical wavelength of 490 nm. Compared to the dianion absorptivity at 490 nm, the monoanion has only 19%, the neutral species only 3%, and the cation species only 0.04% of the absorptivity (Diehl, 1989, and Klonis and Sawyer, 1996) and it is these large relative absorbance differences that make the consequences of inaccurate pK_a values so important.

Unfortunately, it is difficult to predict exactly when these ionic changes occur because there is little agreement between the published fluorescein pK_a values. A wide variety of pK_a values have been reported and these range from a three pK_a ionic model using values of 2.25, 4.23 and 6.31 (Klonis and Sawyer, 1996) to a single pK_a value of 5.1 (Kasnavia et al., 1999). While the differences between some of the reported pK_a values appear to be small, they have a large impact on the apparent fluorescein concentration. For instance, if the Klonis and Sawyer (1996) pK_a values and absorptivities are used as reference values but fluorescein actually has the pK_a values of 2.2, 4.4 and 6.7 (Lindqvist, 1960) then at a pH of 6.1 only 70% of the expected fluorescein would be detected.

Apart from the pK_a value differences, questions have also been raised about the fluorescein absorptivity values by Boets et al. (1992) who compared their absorptivity value of 8.7×10^4 with other published values of 7.4×10^4 (Larsen and Johansson, 1989), 8.4×10^4 (Hammond, 1979), 8.9×10^4 (Delori et al., 1978, and Melhado et al., 1982) and 1.6×10^5 $M^{-1}cm^{-1}$ (Grotte et al., 1985). These inconsistent values may be the result of using an absorbance test to quantify a highly fluorescent compound (Braude et al., 1950), and as the magnitude of this error depends on the type of analytical instrument (Gibson and Keegan, 1938) it will be important to determine values specific for the spectrophotometer used in each study.

Additionally, some fluorescent compounds are influenced by the nature of the cations present (Smart and Laidlaw, 1977) so it will be important to eliminate any such effects from a pK_a determination method.

A number of different methods have been reported (Albert and Serjeant, 1984) for pK_a determinations, which involve monitoring the response of the test compound throughout a titration. A simple and rapid pK_a determination method (Clark and Cunliffe, 1973) may be adequate for some applications, however this technique does not incorporate activity corrections and the prescribed test buffers have concentrations of greater than 0.08M, which will yield ionic strengths greater than the 0.01M limit above which activity corrections are recommended (Albert and Serjeant, 1984). More recent pK_a determination methods use mathematical techniques to simultaneously solve for the pK_a values, however these methods either do not correct for activity effects (Klonis and Sawyer, 1996) or do not account for the activity complications caused by the test buffer (Sjöback et al., 1995).

The ideal pK_a determination method would have the accuracy of the Albert and Serjeant (1984) techniques, the simplicity of the Clark and Cunliffe (1973) method, and the minimal equipment requirements of the mathematical approaches (Klonis and Sawyer, 1996, and Sjöback et al., 1995), but must also include activity and temperature corrections. Thus, the proposed method gathers data in a manner similar to that of Clark and Cunliffe (1973) and eliminates the equipment required for precise temperature control. In addition, the method processes the data using a simultaneous

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