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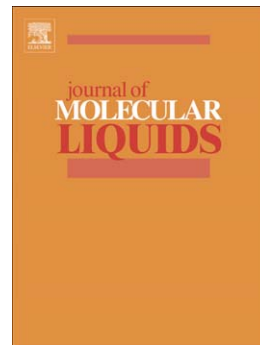
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Acid-base dissociation and tautomerism of two aminofluorescein dyes in solution

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ABSTRACT

The four-step dissociation of two fluorescein dyes bearing the amino group in 4'- and 5'-positions of the phthalic moiety was studied in water. The constants describing the equilibria $H_4R^{2+} \rightleftharpoons H_3R^+ \rightleftharpoons H_2R \rightleftharpoons HR^- \rightleftharpoons R^{2-}$ were determined using the spectrophotometric method, at 25 °C. In water, four steps of aminofluoresceins dissociation occur within pH range of 0–9. On going to 50 mass % aqueous ethanol, the increase in the pK_{a1} , pK_{a2} and decrease in the pK_{a0} , $pK_{a(-1)}$ values take place. The visible absorption spectra, referring to all the ionic (molecular) forms, were singled out. On the whole, the behavior of 4'-aminofluorescein is closer to that of the mother compound. The shift of the equilibria on introducing 50 mass % ethanol into the aqueous solution allowed better understanding the detailed scheme of prototropic equilibrium. The tautomeric equilibrium of the molecules shifts towards the colorless lactone. This effect is expressed stronger for the 5'-aminofluorescein. For this dye, even signs of such tautomers of the single-charged anion as anion-lactone and phenolate anion are observed in aqueous ethanol, which are atypical for fluorescein in water-based solvents.

KEY WORDS: aminofluorescein; dissociation constant; water–ethanol solvent system; glass electrode; visible spectroscopy; tautomerism

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