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A wide-range fluorescent pH-indicator based on 3-hydroxyflavone structure

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Abstract

With the aim to develop wide-range pH-indicators, we synthesized a new water-soluble compound, 3,4′-dihydroxy-3′,5′-bis-(dimethylaminomethyl)flavone (FAM345), which displays a spectral sensitivity to pH in the range from 2 to 12 in absorption spectra as well as in fluorescence excitation or emission spectra. In all three cases, the estimation of pH is possible, either from the position of the maxima or from the absorbance or fluorescence intensity at one or several separated wavelengths, or also from absorbance or fluorescence intensity ratios. This last feature allows determining pH value of the medium independently from the probe concentration, from its possible photobleaching, as well as from any variation of light source intensity (at least in fluorescence methods).

Keywords: Absorbance; Fluorescence; pH-Indicator; Flavonol; 3-Hydroxyflavone; Ratiometric probe

1. Introduction

Flavonols (3-hydroxyflavones, 3HF) are presenting a considerable interest not only in drug design [1], but also in the design of new fluorescence probes with broad applications in the study of molecular interactions in solutions and biological systems. The attractive features of the flavonols are related to their high sensitivity to physico-chemical parameters of the environment. Flavonols usually exhibit two bands in their fluorescence spectrum, due to an excited state intramolecular proton transfer (ESIPT) reaction [2], leading to two excited forms, the normal N* and the tautomer T* ones, and thus resulting in two strongly separated bands in the fluorescence spectrum. Their positions and relative intensities depend on several parameters of the medium. Due to this unique phenomenon many flavonol derivatives were shown to be very effective probes in the analysis of the structure of micelles [3–6] and phospholipid vesicles [6–13], as well as in the fluorescence recognition of cations of different radii [14–16].

The presence of a single hydroxyl group in the position 3 of the molecule renders the flavonols able to sense pH in the range from 8 to 12 [17]. Such a sensor molecule can be further derivatized by other chemical groups also submitted to some acid–base equilibrium, but at quite distant pHs. Provided all the corresponding species are fluorescent, this sensor should work properly in a relatively large pH range.

Several examples of dyes with two pH transitions are described in the literature. There are many different molecules like phenols or nitrogen-containing substances such as aminonaphtols [18], 2-(pyridyl)benzimidazoles [19,20], polyamines [21], hydroxyphenylbenzoxazoles [22] or acridones [23]. However, for most of them, their practical use is quite uneasy because of low solubility, low fluorescence quantum yield, low fluorescence sensitivity to pH or of acid–base transitions too far away on the pH-scale.

With the aim to develop wide-range pH-indicators, we propose in the present work a new fluorescence pH sensor based on flavonol structure that works properly in a

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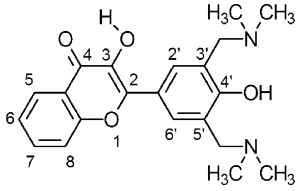

FAM345

Scheme 1.

pH range from 2 to 12. This probe, 3,4′-dihydroxy-3′,5′-bis-(dimethylaminomethyl)flavone (FAM345), contains two hydroxyl groups, both of them being conjugated to a carbonyl group pertaining directly to the chromophore system of the molecule (Scheme 1). Due to this feature, it is possible to drive the position of excitation and/or emission spectrum simply by the deprotonation of one of the hydroxyl groups. Due to their phenolic nature, the acidity constants pK_a of the hydroxyls are in a pH range from 8 to 10. Since the molecule also contains two dimethylaminomethyl groups localized in close proximity to the positive head of the molecular dipole, we can expect that not only deprotonation of hydroxyl groups, but also protonation of dimethylaminomethyl groups should have an influence on intensities and positions of both excitation and emission bands of FAM345. Additional spectral effects could be caused by intramolecular acid–base interactions between hydroxyl and amino groups of FAM345. For example, such an interaction can cause the deprotonation of 4′-OH group at a lower pH [24], rendering possible pH measurements in a pH range near neutrality.

2. Experimental

FAM345 was prepared from 3,4′-dihydroxyflavone and *N*,*N*,*N*′,*N*′-tetramethyldiaminomethane by a well known procedure [25]. A solution of 0.254 g (1 mmol) of 3,4′-dixydroxyflavone (INDOFINE, U.S.A.), and 0.404 g or 0.54 ml (4 mmol) of *N*,*N*,*N*′,*N*′-tetramethyldiaminomethane (from Aldrich) in 5 ml of dry dioxane was boiled 4–5 h, until a yellow–orange precipitate was formed. It was filtered off and washed with dioxane. The obtained reaction yield was 41% (0.151 g). The product was homogeneous according to ^{1}H NMR spectroscopy data and TLC (eluent–chloroform-methanol–triethylamine mixtures, 95:4:1 or 85:14:1, v/v). Melting point was 154–155 °C (uncorrected). It was determined on a PHMK apparatus (“VEB Analytik” Dresden). ^{1}H NMR spectrum was obtained in DMSO-d_6 with TMS as internal standard on Varian Mercury-400 spectrometer. Below chemical shifts (δ, ppm), form of a signal (s, singlet; d, doublet; t, triplet), coupling constants (J, Hz) and signal intensity are given: 8.11d (J = 8) 1H; 7.94s 2H; 7.71t (J = 8) 1H; 7.65d (J = 8) 1H; 7.38t (J = 8) 1H; 3.57s 4H; 2.30s 12H. Mass spectrum (obtained on Thermabeam Mass Detector, Waters Integrity System, U.S.A.) showed a molecular ion peak at 368 corresponding to the calculated molecular mass.

For absorption and fluorescence spectroscopy experiments, FAM345 was first dissolved at 5 μM concentration in phosphate–citrate–borate buffer, 15 mM (5 mM of each component, ionic strength μ = 0.05 M, pH 7.2), and the pH were then adjusted to the desired values by addition of microquantities either of 5 M NaOH or 5 M HCl, never exceeding 3% of the initial volume. The absorption spectra were recorded on a Cary 4 spectrophotometer, and the fluorescence spectra on a Jobin–Yvon Fluoromax spectrofluorimeter. pH were determined directly into the quartz cuvette (1 cm × 1 cm) on a 713 pH-meter from Metrohm. Temperature was kept at 20 °C for all the experiments.

The effective pK_a values (presented in Table 1) were calculated by the Fletcher–Pawl algorithm using a nonlinear least squares method, as developed in Doroshenko’s program [26,27], which minimizes the sum of squared deviations of the experimental and calculated absorbance (A) or fluorescence data in the approximation of one-step protonation process for each transition. The program utilizes the common formula [28]:

$$A = \frac{A_{HA}10^{-pH} + A_A 10^{-pK_a}}{10^{-pH} + 10^{-pK_a}},$$

where A_{HA} is the absorbance or fluorescence intensity of each protonated species (H_4R^{2+} and H_3R^+ for the first and second transitions, respectively, see Scheme 2), A_A is the absorbance or fluorescence intensity of each deprotonated species (H_3R^+ and R^{2-} for first and second transitions, respectively). In such an approximation, we obtained for the second transition (pK_2

Table 1
Effective pK_a values of FAM345 determined by the absorption, fluorescence excitation and emision parameters

Method	Constant	By maximum position	Registration wavelength			Averaged value
			λ = 350 nm	λ = 385 nm	λ = 430 nm	
Absorption	pK_1	4.43 ± 0.10	4.29 ± 0.08	4.17 ± 0.04	4.32 ± 0.08	4.30 ± 0.08
Excitation		4.38 ± 0.10	4.51 ± 0.15	4.29 ± 0.08	4.47 ± 0.16	4.41 ± 0.12
Emission		[b]	4.33 ± 0.08[a]	[b]	4.34 ± 0.09	4.34 ± 0.09
Absorption	pK_2	9.28 ± 0.06	8.97 ± 0.05	9.03 ± 0.04	8.93 ± 0.05	9.05 ± 0.05
Excitation		8.33 ± 0.06	9.00 ± 0.13	8.90 ± 0.08	8.88 ± 0.05	8.78 ± 0.08
Emission		8.84 ± 0.12	8.67 ± 0.17[a]	[b]	8.55 ± 0.10	8.69 ± 0.13

[a] Calculated from the intensity ratio I_{440}/I_{530}.
[b] There is no possibility to determine.