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Analytica Chimica Acta 545 (2005) 74–78
ANALYTICA CHIMICA ACTA
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2-[4-(Dimethylamino)phenyl]-3-hydroxy-4*H*-chromene-4-one: A H-bond-sensitive fluorescent probe for investigating binary mixtures of organic solvents

V.G. Pivovarenko [a,*], A. Wróblewska [b], J. Błażejowski [b]

[a] *Faculty of Chemistry, Kyiv Taras Shevchenko National University, Volodymyrska 64, 01033 Kyiv, Ukraine*
[b] *Faculty of Chemistry, University of Gdańsk, J. Sobieskiego 18, 80-952 Gdańsk, Poland*

Received 17 January 2005; received in revised form 4 April 2005; accepted 14 April 2005
Available online 31 May 2005

Abstract

Molecules of electronically excited 2-[4-(dimethylamino)phenyl]-3-hydroxy-4*H*-chromene-4-one (a fluorescent probe) co-exist in the normal (**N***) and tautomeric (**T***) forms and emit radiation in different spectral regions. The positions (expressed in wave numbers, ν) and intensities (I) of the emission bands are strongly affected by the ability of the medium's molecules to participate in hydrogen bonding with the probe. In such cases, I_{N^*}, I_{N^*}/I_{T^*}, ν_{N^*}, $\nu_{N^*} + \nu_{T^*}$ or $\nu_{N^*} - \nu_{T^*}$, depend on the concentration (over a certain range) or its base-10 logarithm (log) of the component interacting with the probe. These relationships form the basis for a quantitative assay of such compounds in binary mixtures. On the other hand, the $\log(I_{N^*}/I_{T^*})$ versus $\nu_{N^*} + \nu_{T^*}$ or $\nu_{N^*} - \nu_{T^*}$ relationships demonstrate unique features that can be used to distinguish components (alcohols) interacting with the probe and to quantify their contents. The prospects for the analytical application of these findings are outlined briefly.

Keywords: 2-[4-(Dimethylamino)phenyl]-3-hydroxy-4*H*-chromene-4-one; Hydrogen bonds; Fluorescent probe; Assay of components in binary liquids

1. Introduction

Molecular probes are compounds responding to stimuli or perturbations imposed on a system. These in turn, cause the physicochemical parameters characterising the probes to change in value, thus, providing important information about the system [1]. The spectral features of such probes, which usually operate as single parametric information transmitters, are widely applied in such fields as chemistry, biochemistry, cell biology, medicine and ecology [1,2]. Recently, however, there has been a demand for multi-parametric probes in which more than one system–characteristic parameter generates a response. Aminonaphthalene sulphonate [3–5] probes, whose fluorescence anisotropy, fluorescence band position and fluorescence quantum yield all depend on the medium's polarity and viscosity, were the first such probes used to investigate proteins, DNA, cell membranes and whole cells [6–8]. Another class of multi-parametric probes with numerous applications is derived from 2-dimethylamino-6-acylnaphthalenes [9]. In principle, most fluorescent dyes exhibiting distinct solvatochromism can be used as multi-parametric probes for investigating the properties of liquids [1,10].

Flavonols (derivatives of 3-hydroxy-2-phenyl-4*H*-chromene-4-one (**1**)) [11] appear to exhibit features of multi-parametric fluorescence probes; this is the group of compounds on which we have focused our interest. As a result of excited state intramolecular proton transfer (ESIPT), these compounds co-exist in the **N*** and **T*** tautomeric forms in the electronically excited state and exhibit dual fluorescence [12]. The excitation band positions, positions and intensities of the emission bands of the tautomers, as well as the fluorescence anisotropy of **1** have been

* Corresponding author. Tel.: +380 44 239 33 12; fax: +380 44 220 83 91.
E-mail address: pvg@univ.kiev.ua (V.G. Pivovarenko).

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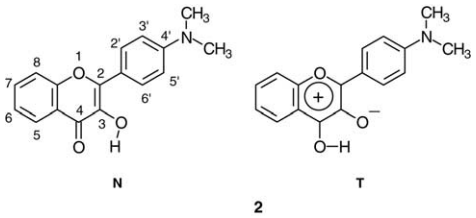
doi:10.1016/j.aca.2005.04.042



Scheme 1. Canonical structures of 2-[4-dimethylamino)phenyl]-3-hydoxy-4*H*-chromene-4-one (**2**) in the **N** and **T** forms. The non-hydrogen atoms are labelled.

determined by steady-state fluorimetry. This has provided complete information on micelle [13–16] and liposome [17–26] structure, and permitted the identification of cations of different radii [27–29].

The use of flavonols to determine the properties and composition of liquid phases has been attempted by several research groups [30–34]. Klymchenko and Demchenko [34] found three physicochemical properties of liquids – the dielectric constant, the electronic polarisability functions and the presence or absence of hydrogen bonds in them – to be related to their composition. Apart from [32], none of these papers demonstrated that flavonols could act as probes to assess the content of polar (protic) components in liquid phases. In the present paper, we propose a method for the qualitative and quantitative assay of a component interacting with 2-[4-dimethylamino)phenyl]-3-hydroxy-4*H*-chromene-4-one (**2**) (Scheme 1) (fluorescent probe) in non-polar media. We have already reported on the propensity of **2** to participate in hydrogen bonds [35].

2. Experimental

Probe **2** was synthesised according to ref. [36]. Its purity was confirmed by TLC (50 mm × 150 mm Silufol UV-254 plates, 98:2 to 85:15% (v/v) chloroform–methanol eluent) and fluorescence spectra analysis (synchronous scans). The concentration of **2** was 5.4×10^{-6} M and the temperature was 20 °C in all experiments. Fluorescence spectra were recorded on a Perkin-Elmer LS-50 spectrofluorimeter (excitation wavelength = 400 nm in all cases). The solvents were of spectral grade (Merck) and were used as supplied. The fluorescence spectra were deconvoluted with the "Spectral Data Lab" computer program developed by Doroshenko [37], which uses the iterative non-linear least-squares method based on the Fletcher–Pauell algorithm. The shapes of the individual emission bands were approximated by a log–normal function, which unlike the more frequently applied Gauss and Lorenz functions, accounts for the asymmetry of the spectral bands [38]. In most cases, the band half-width and asymmetry were selected freely during deconvolution. However, where bands relevant to the $\mathbf{N}^*$ and $\mathbf{T}^*$ tautomers were not well separated, certain spectral parameters were assumed on the basis of data for well-separated bands. An example of a deconvoluted spectrum is illustrated in Fig. 1. The deconvoluted spectra and other graphs were plotted using the Origin 5.0 program [39].

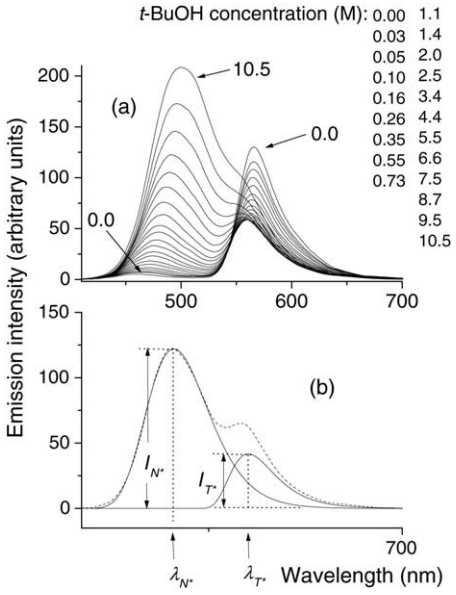


Fig. 1. Fluorescence spectra of **2** in benzene and benzene-*t*-butanol mixtures (*upper plot*), and an example of a deconvoluted spectrum for a *t*-BuOH concentration of 7.5 M (*lower plot*).

3. Results and discussion

3.1. Influence of liquid phase properties on the fluorescence characteristics of 2

Several earlier investigations showed that the more stable form of flavonols was **N** in the ground electronic state, but $\mathbf{T}^*$ in the excited electronic state (Scheme 1) [12,30,31]. This is the case when these compounds are dissolved in neutral solvents (not specifically interacting ones) (see Fig. 1). However, when a specifically interacting component is added, the long-wavelength emission due to $\mathbf{T}^*$ weakens and the short-wavelength emission caused by $\mathbf{N}^*$ strengthens (Fig. 1) [30]. This now makes it feasible to use the intensity (I) or intensity ratio, e.g. I_{N^*} or I_{N^*}/I_{T^*}, to measure the content of the component specifically interacting with flavonols. Another feature is that the $\mathbf{N}^*$ and $\mathbf{T}^*$ band maxima change position with increasing content of the specifically interacting component (Fig. 1) [35] and so can be regarded as a measure of this content.

In our investigations, the specifically interacting compounds were alcohols (protic of medium polarity) (methanol (MeOH), *n*-butanol (*n*-BuOH) and *t*-butanol (*t*-BuOH)) dissolved in benzene or ethyl acetate (EtOAc), water (protic, highly polar) dissolved in 1,4-dioxane (dioxane) and dimethylsulphoxide (DMSO) (aprotic, polar) dissolved in toluene. Fig. 1 illustrates their influence on the fluorescence characteristics of **2** [35]. It shows that the rising concentra-

tion of a specifically interacting component always causes a bathochromic shift in the fluorescence $\mathbf{N}^*$ band (positive solvatochromism [30,35]) (Fig. 1). This band gradually intensifies with increasing content of the specifically interacting component, but in the case of MeOH and water, it reaches a maximum and then decreases [35]. With $\mathbf{T}^*$, the band situation is more complex. A distinct bathochromic shift is always observed in the case of DMSO [35], but with protic components, the shift is hypsochromic [35] (Fig. 1). The intensity of the $\mathbf{T}^*$ band decreases gradually with rising content of the specifically interacting component in the medium. The effect is most distinct for water and alcohols (Fig. 1).

The above phenomena are discussed in detail in our recent publication [35]. Protic alcohols or the aprotic but polar DMSO appear to form H-bond complexes involving the carbonyl oxygen atom and the hydroxyl group of **2** in positions 3 or 4. These complexes are 1:1 when moderate concentrations of these components are involved, but two molecules of alcohol or DMSO at higher concentrations are complexed by **2**.

3.2. Use of **2** as a fluorescent probe to determine the protic (polar) component content in non-polar media

The use of **2** as a fluorescent probe to determine the content of a component interacting with it in a medium requires that the monitored feature(s) of **2** be linearly dependent on this component's concentration (or some function of it). Analysis of the various possible relationships has shown that over a certain range of concentrations, I_{N^*} or I_{N^*}/I_{T^*} are linearly dependent on the concentration of a polar (protic) component in a non-polar medium. Furthermore, over a certain range of concentrations, ν_{N^*} or $\nu_{N^*} - \nu_{T^*}$ (where ν is the wave number ($1/\lambda$, Fig. 1) indicating a band's position) depend linearly on the base-10 logarithm of the concentration of the polar (protic) component in solution. Table 1 lists the linear least-squares regression coefficients (slopes and intercepts), along with those of other relationships also found to be linear. These latter relationships indicate how the method can be applied directly in analytical procedures.

The data in Table 1 demonstrate clearly that the properties of **2** allow this compound to be classified as a typical multi-parametric probe. This is because several of its fluorescent characteristics, I_{N^*}, I_{N^*}/I_{T^*}, ν_{N^*}, $\nu_{N^*} + \nu_{T^*}$ or $\nu_{N^*} - \nu_{T^*}$, depend linearly on the concentration or its base-10 logarithm over a broad range of concentration—from 0.1 to 11 M. Hence, when **2** is used to monitor one or more of the fluorescent characteristics of a polar (protic) component in non-polar media, these relationships yield information on its content.

3.3. **2** as a fluorescent probe enabling the simultaneous identification of an alcohol and the determination of its content in non-polar media

In the light of what was said in Section 3.2, we can determine the concentration of a polar component in a liquid mixture once all the components have been identified. Often, however, we need to identify components before their quantitative assay. To discover whether this is possible with **2** as a probe, we plotted the relationships of $\log(I_{N^*}/I_{T^*})$ first versus $\nu_{N^*} - \nu_{T^*}$ (Fig. 2; Table 2) and then versus $\nu_{N^*} + \nu_{T^*}$ (Fig. 3, Table 2). The former relationship does not differentiate between these systems to a sufficient extent, similarly as between polar and non-polar solvents [34]. The distinction between the systems under study and neat solvents [34] is much improved if $\log(I_{N^*}/I_{T^*})$ is plotted against $\nu_{N^*} + \nu_{T^*}$ (Fig. 3). The data in Fig. 3 show that the relationships of $\log(I_{N^*}/I_{T^*})$ versus $\nu_{N^*} + \nu_{T^*}$ become linear for isomolar solutions of selected alcohols in pertain solvents. Such relationships are shown in Fig. 4, together with the linear relationships, within the same coordinates, approximating the points corresponding to different concentrations of an alcohol in a given solvent (the latter relations are also given in Fig. 3). Fig. 4 is, thus, a diagram enabling the qualitative and the

Table 1
Linear dependences of various spectral characteristics on the concentration (c) of the polar component, or on log c

System	Coefficients of the linear dependence		Linearity limit (c, M)
	a	b	
$I_{N^*} = ac + b$			
MeOH–benzene	49.6	10.5	0.1–4
n-BuOH–benzene	20.0	14.6	3–7
t-BuOH–benzene	14.1	15.7	0.5–9
MeOH–EtOAc	28.6	7.80	0.1–5
Water–dioxane	17.3	10.7	0.1–6
DMSO–toluene	3.15	23.0	1–11
$I_{N^*}/I_{T^*} = ac + b$			
MeOH–benzene	1.75	−2.12	2–11
n-BuOH–benzene	0.391	0.0575	1–10
t-BuOH–benzene	0.359	0.0842	0.1–8
MeOH–EtOAc	1.19	−0.192	0.7–5
Water–dioxane	0.556	0.0804	0.2–8
DMSO–toluene	0.208	0.580	2–11
$\nu_{N^*} = ac + b$			
t-BuOH–benzene	−104	21000	0.5–11
$\nu_N - \nu_{T^*} = ac + b$			
t-BuOH–benzene	−104	3250	0.7–11
$\nu_{N^*} + \nu_{T^*} = ac + b$			
t-BuOH–benzene	−102	38800	0.4–11
Water–dioxane	−165	38300	1.8–8.5
$\nu_{N^*} = a(\log c) + b$			
MeOH–benzene	−1180	20300	0.4–8
n-BuOH–benzene	−1410	20800	1–11
t-BuOH–benzene	−390	20900	0.2–3
MeOH–EtOAc	−950	20100	0.7– 10
Water–dioxane	−1190	20800	0.7–3
DMSO–toluene	−1350	20800	0.7–14
$\nu_{N^*} - \nu_{T^*} = a(\log c) + b$			
MeOH–benzene	−1110	2560	0.3–7
n-BuOH–benzene	−1120	2910	5–11
t-BuOH–benzene	−470	3180	0.1–3
MeOH–EtOAc	−908	2500	0.7–10
Water–dioxane	−1210	3210	0.7–10
DMSO–toluene	−1040	3300	0.7–10

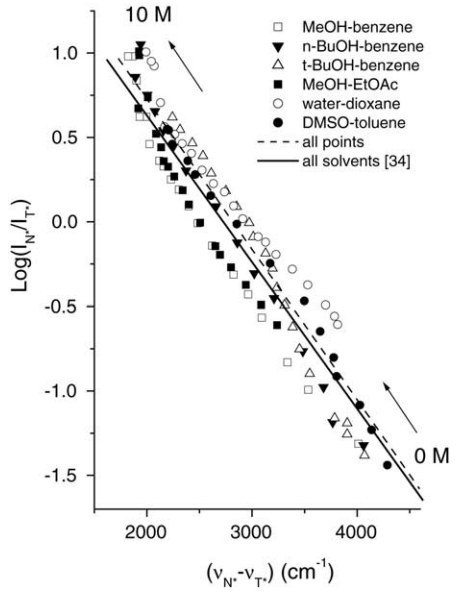


Fig. 2. Base-10 logarithm (log) of the emission intensity ratio (I_{N*}/I_{T*}) vs. the difference in positions (ν) of $\mathbf{N}^*$ and $\mathbf{T}^*$ maxima for 0–10 M concentrations of the polar component in the liquid phase. The dotted line is the linear least-squares regression dependence for all experimental points, and the solid line is the analogous dependence corresponding to neat protic and aprotic solvents (taken from [34]). Slopes and intercepts are given in Table 2.

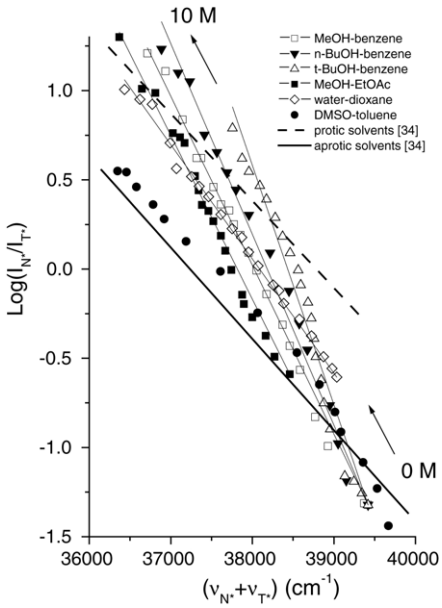


Fig. 3. Base-10 logarithm (log) of the emission intensity ratio (I_{N*}/I_{T*}) vs. the sum of wave numbers (ν) of $\mathbf{N}^*$ and $\mathbf{T}^*$ maxima for 0–10 M concentrations of the polar component in the liquid phase. Slopes and intercepts are given in Table 2.

quantitative assay of alcohols (protic components) in non-polar media. Therefore, if we know, for example, that one of the alcohols – MeOH, *n*-BuOH or *t*-BuOH – is dissolved in benzene, we can add **2** to the solution as a probe, measure I_{N*}, I_{T*}, ν_{N*} and ν_{T*}, and locate the point corresponding to the coordinates $\log(I_{N*}/I_{T*})$ and $\nu_{N*} + \nu_{T*}$. This point will identify the alcohol and provide information on its concentration.

3.4. Accuracy and precision of analytical assays

In order to evaluate the parameters characterising the quality of these assays, we prepared five solutions each of *t*-BuOH in benzene and of water in dioxane at concentrations 0.5, 4 and 8 M, containing **2** at a concentration of 5.4×10^{-6} M. The fluorescence characteristics were measured (one spectrum per solution) for all these solutions and the spectra deconvoluted as described in the experimental section. The extracted values of I_{N*}, I_{T*}, ν_{N*} and ν_{T*} were then used to determine the concentrations from the relationships given in Table 1. The mean values of these measurements were compared with known concentrations. Deviations affecting the

Table 2
Slopes (a) and intercepts (b) of linear least-squares regression dependences

Figure	Description (experimental points, system)	a	b
2	All points	−0.000889	2.51
	All solvents [34]	−0.000867	2.36
3	Protic solvents [34]	−0.000488	18.9
	Aprotic solvents [34]	−0.000512	19.1
3 and 4	MeOH–benzene	−0.000947	36.0
	n-BuOH–benzene	−0.00107	40.8
	t-BuOH–benzene	−0.00134	51.6
	MeOH–EtOAc	−0.000917	34.7
	Water–dioxane	−0.000640	24.4

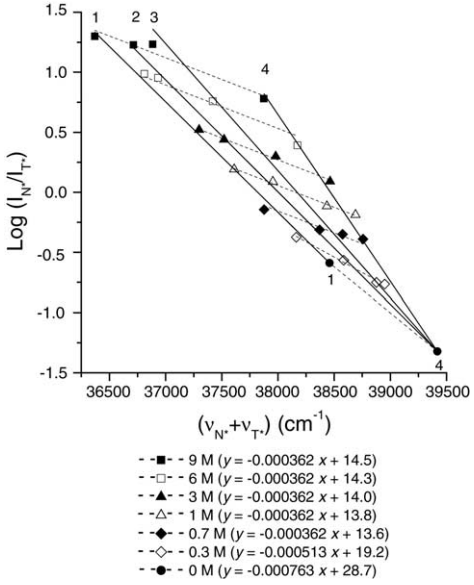


Fig. 4. Linear fits of base-10 logarithm (log) of the emission intensity ratio (I_{N*}/I_{T*}) (y) vs. the sum of wave numbers (ν) of $\mathbf{N}^*$ and $\mathbf{T}^*$ maxima (x) for various concentrations of alcohol in a given system (solid lines—slopes and intercepts are given in Table 2) or isomolar solutions of alcohols in two solvents (dashed lines). (1) MeOH–EtOAc system or pure EtOAc (lower value), (2) MeOH–benzene, (3) *n*-BuOH–benzene, (4) *t*-BuOH–benzene system or pure benzene (lower value).

accuracy of the assays did not usually exceed ±0.01 for the 0.5 M solution and ±0.2 for the 4 and 8 M solutions. The accuracy was better with relationships containing c instead of $\log c$. Reflecting the precision, the relative standard deviations were of the order of 3% for 0.5 M and 4% in the case of 4 and 8 M. The reasonable accuracy and precision of these test measurements provide good grounds for the routine use of the method in assays.

4. Conclusions

The results of these investigations indicate that, for a known composition of a liquid-phase containing a polar (protic) component in a non-polar solvent, the fluorescence characteristics of the probe (**2**) enable this component to be assayed quantitatively. Furthermore, the fluorescence characteristics of **2**, if plotted appropriately, allow alcohols to be differentiated, and their content to be assayed in binary mixtures with non-polar solvents.

The results of the investigations demonstrate that **2** is a convenient multi-parametric fluorescent probe with prospective analytical applications. The proposed approach opens up possibilities for the qualitative and quantitative assay of molecules containing hydroxyl groups, including naturally-occurring ones.

Acknowledgements

The authors are grateful to A.P. Demchenko and A.S. Klymchenko for the fruitful discussions, and to W. Wiczk and T. Ossowski for their kind assistance with the fluorimetric experiments. The research was partially financed by the Polish State Committee for Scientific Research (KBN) through the Polish–Ukrainian Executive Program of Research and Technical Co-operation (Grant No. PRO: III. 28/1998; Contract No. 157), BW/8000-5-0173-4 and DS/8000-4-0026-5 grants.

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