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USE OF ORGANIC SOLVENTS FOR THE ENHANCEMENT AND LIFETIME INCREASING OF LUMINESCENCE IN FLUORIMETRIC APPLICATION BY FLUORESCAMINE

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Abstract

In this study, the fluorogenic efficiency of fluorescamine for spectrofluorimetric analysis of primary amines was investigated. Different mixtures of dichloromethane and other organic solvents were used for evaluation of fluorescence stability and intensity of fluorescamine derivative (pyrrolinone). Solvents such as acetonitrile (ACN), tetrahydrofuran (THF) and dioxane increase stability and intensity of fluorescence, whereas methanol, acetic acid and dimethyl sulfoxide have a decreasing effect. Calibration graph equations in optimum solvent system were obtained for spectrofluorimetric determination of norepinephrine as a model compound in the range of 0.1-1.0 ppm in 10 minute intervals. Results obtained show a good correlation coefficient and excellent reproducibility (RSD% = 0.82, C = 0.5 ppm, n = 6). Using the first set of solvents, reproducibility of spectrofluorimetry is excellent (RSD% = 0.82).

Introduction

Fluorimetric analysis is one of the most suitable tools for the analysis of organic, biological and pharmaceutical compounds such as hormones, nucleic acids, lipids, amino acids, amines, peptides, proteins, carbohydrates, etc., [1]. The main advantages are its selectivity and sensitivity which are necessary for ultratrace analysis [1,2]. However, many of the mentioned substances do not fluoresce and in order to make sensitive and specific analysis, the use of fluorogenic reagents for the coupling or derivatization of such compounds is indispensable [3].

Keywords: Fluorescamine derivatives; Norepinephrine; Spectrofluorimetry

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4-Phenylspiro (furan-2 (3H), 1-phthalan)-3,3'-dione (FluramR or Fluorescamine) has been developed as an effective reagent for the fluorimetric determination of amino acids, peptides, proteins, catecholamines, aliphatic amines, aromatic amines and nitroaromatic compounds [4-13] and others. The unique features of this reagent are its high reactivity and specificity toward the derivatives of primary amines. The reaction occurs within a few seconds at room temperature at pH 7-10 to give a highly fluorescent pyrrolinone derivative compound [5, 14-16].

The fluorescence quantum yield of many compounds is very sensitive to the environment of the excited state [17,18]; for example, it has been shown that water molecules are capable of interacting with the excited state

of the fluorophores [19] to form an excited state complex (exciplex). This reaction is competitive with fluorescence and can therefore reduce the quantum yield of fluorescence. The presence of inorganic ions in aqueous media also decreases the intensity of fluorescence. Fluorescamine itself is rapidly hydrolyzed to form a non-fluorescent product under certain reaction conditions [4].

The factors mentioned previously affect the sensitivity and precision of the fluorimetric method. A logical approach to the prevention of exciplex formation and the elimination of inorganic ions is to carry out the assay with organic media [18,20,21]. In previous work, we showed that the pyrrolinonic compound(s) can be extracted at about pH 1 into dichloromethane, which results in an increase of luminescence intensity and emission lifetime [9].

In this work, we have extended earlier studies on the use of organic solvents such as acetonitrile (ACN), tetrahydrofuran (THF), dioxane and methanol and their effects on fluorescence intensity and lifetime.

Experimental Section

Chemicals and Reagents

Acetonitrile (ACN), tetrahydrofuran (THF), dioxane, acetic acid, methanol, dichloromethane and borax were from E. Merck (Germany). Fluorescamine was purchased from Fluka (Buchs, Switzerland) and norepinephrine (NE) from Aldrich-Chemie (U.K.).

Apparatus

A Shimadzu spectrofluorimeter model RF-540 U-4.0 with a xenon lamp and 1-cm quartz cell was used for fluorimetric measurements.

Solutions

Stock solutions of 10 mg/l norepinephrine (NE) in 0.01 M HCl, 0.05% (w/v) fluorescamine in acetone, 0.1 M borax and 4 M HCl in doubly distilled water were prepared.

Procedure

NE solution (15 ml) was transferred to a 100-ml separatory funnel and 15 ml water, 5 ml borax solution and 2 ml fluorescamine were added. After shaking the mixture for 30 seconds, 30 ml dichloromethane and 2 ml HCl 4 M were added immediately. After extraction, the organic layer was separated and appropriate volumes of dichloromethane and the second organic solvent were added according to Table I.

Results and Discussion

Effect of Solvents on the Emission Spectrum

Table II shows the maximum excitation and emission wavelengths of the pyrrolinone derivative (fluorescamine derivative of NE) in pure dichloromethane, ACN, THF, dioxane and 1:1 dichloromethane with ACN, THF or

Table 1. Preparation of pyrrolinones in mixed solvents

	Solution 1	Solution 2	Solution 3
Volume of extract (mL)	2	2	2
Volume of dichloromethane (mL)	6	3	0
Volume of second solvent (mL)	2	5	8
NE concentration (mg/L)	1	1	1
Fraction of 2nd solvent (%)	20	50	80

Table 2. Emission and excitation of the pyrrolinone derivative in different media

Wavelength(nm)	CH_2Cl_2	THF	Dioxane	ACN	CH_2Cl_2: THF (50:50)	CH_2Cl_2: Dioxane (50:50)	CH_2Cl_2: ACN (50:50)
Emission	480	471	472	475	475	478	479
Excitation	397	395	399	391	397	395	394

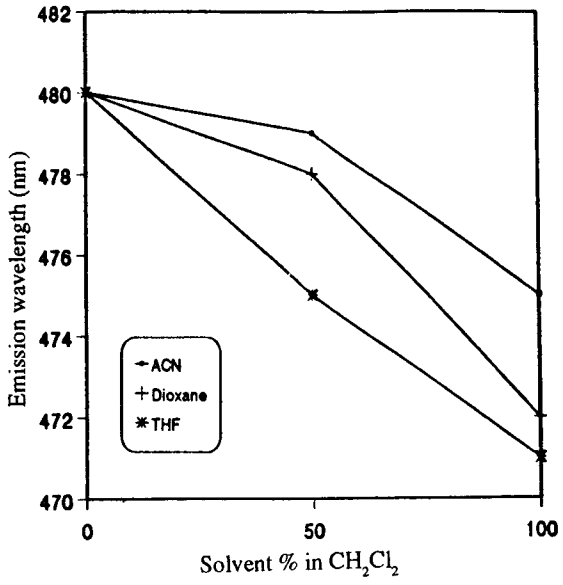

Figure 1. The variation of emission maxima vs. the fraction of THF, ACN and dioxane

dioxane. Figure 1 illustrates the emission maxima versus the fraction of THF, ACN and dioxane in dichloromethane. From the results obtained, a small blue emission shift was observed by the addition of ACN, THF or dioxane, with their increasing fractions. The dependence of fluorescence intensity on the nature of organic solvents, the shape of variation toward solvent characteristics such as proton acceptance (a measure of hydrogen bonding tendency as a Lewis base), proton donation (a measure of hydrogen bonding tendency as Lewis acid) and dipole parameter of the solvents [22] were subsequently studied. Consideration of the results given in Figures 2,3 and 4 shows that regular decrease only with respect to proton acceptance parameter of the solvents was observed. These observations are in good agreement with the results obtained by Matega and co-workers [23] relating emission maxima with the hydrogen bonding effect of solvents.

Effect of Solvents on Fluorescence Intensity

Fluorescence intensity of the pyrrolinone derivative was measured in a mixture of dichloromethane plus a second solvent in various proportions. The results are shown in Figure 5. From these results, a relative increase for emission quantum by increasing ACN, THF and dioxane is observed. In contrast, the fluorescence intensity fades in the presence of methanol and acetic acid. This was attributed to the reaction of the pyrrolinone derivative with these solvents and is in agreement with previous results indicating that water or other molecules having hydroxyl groups are capable of interacting with pyrrolinone derivative to form non-fluorescent compounds [23].

Effect of Solvents on the Fluorescence Stability

Two important factors in an analytical method are reproducibility and precision, while an important factor in fluorimetric analysis is the stability of fluorescence. The greater the stability and the lower the quenching effect, the higher the reproducibility. For this reason, the quenching of the pyrrolinone derivative was studied in two manners. The fluorescence intensity was measured in a 1:1 ACN:dichloromethane ratio by 1) continuous

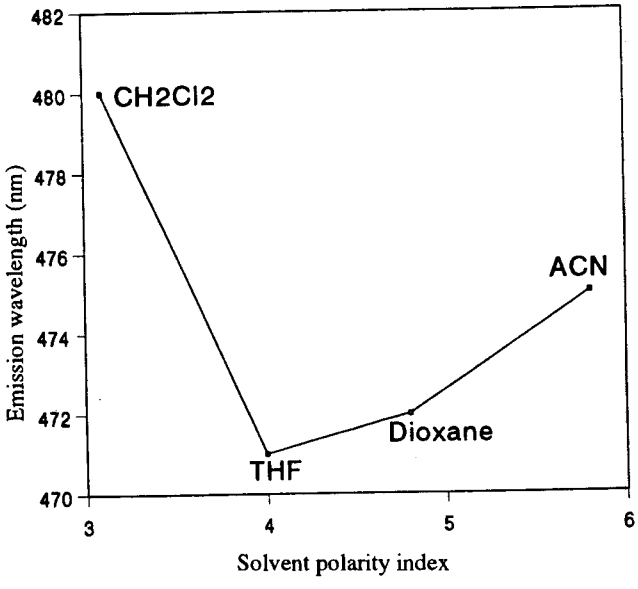

Figure 2. The variation of emission maxima vs. the solvent polarity index

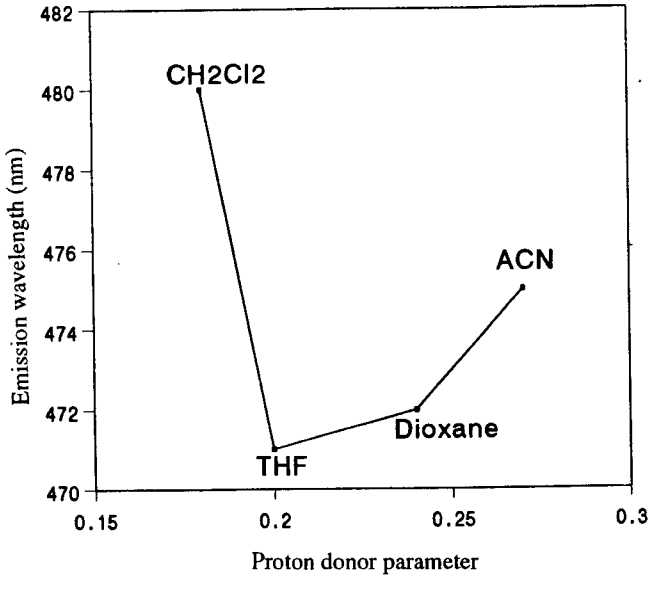

Figure 3. The variation of emission maxima vs. the proton donor parameter

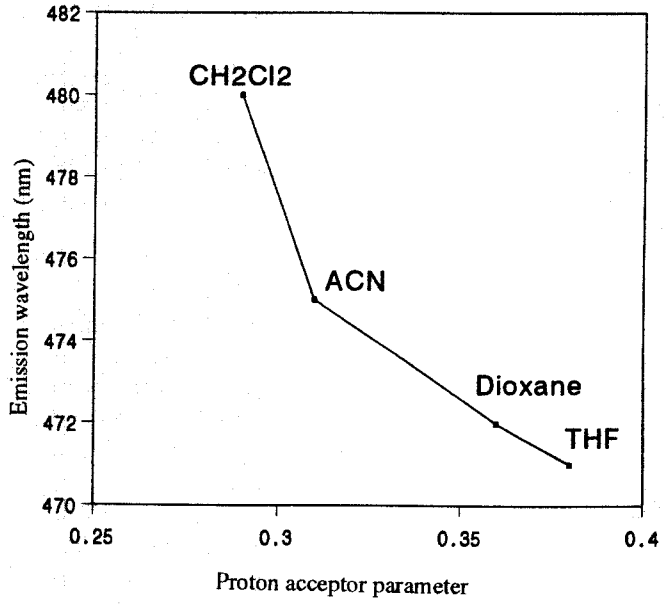

Figure 4. The variation of emission maxima vs. the proton acceptor parameter

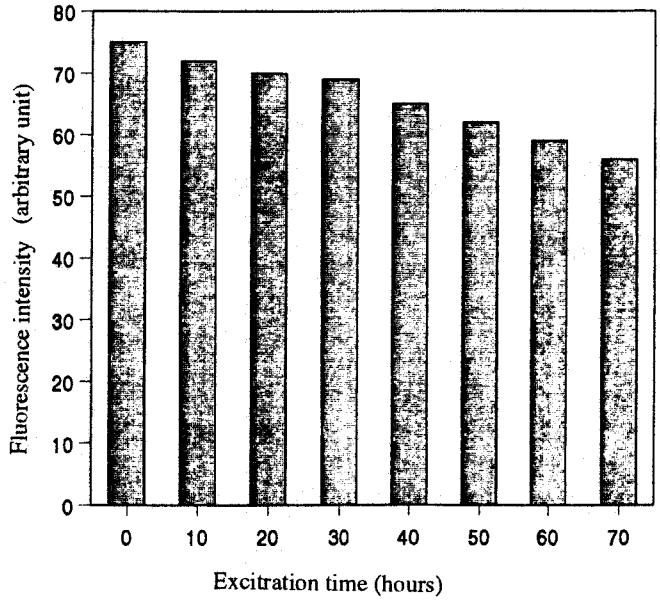

Figure 6. Fluorescence intensity of the pyrrolinone derivative in a mixture of dichloromethane: ACN (1:1) by continuous excitation

excitation (Fig. 6) and 2) instantaneous excitation (Fig. 7). It was observed that by instantaneous excitation, the fluorescence intensity remains constant for more than one day in these media. However, when continuously exposed to near UV radiation, the fluorescence intensity was quenched considerably signifying the energetic decomposition of the fluorogenic compound(s) in the presence of UV radiation.

The data show that dichloromethane+ACN, THF or dioxane mixtures can be useful solvents for the fluorimetric determination of fluram derivatives with compounds carrying a primary amino group. In order to study the effect of variation of the ACN, THF or dioxane fraction on the lifetime and stability of pyrrolinone derivative and so

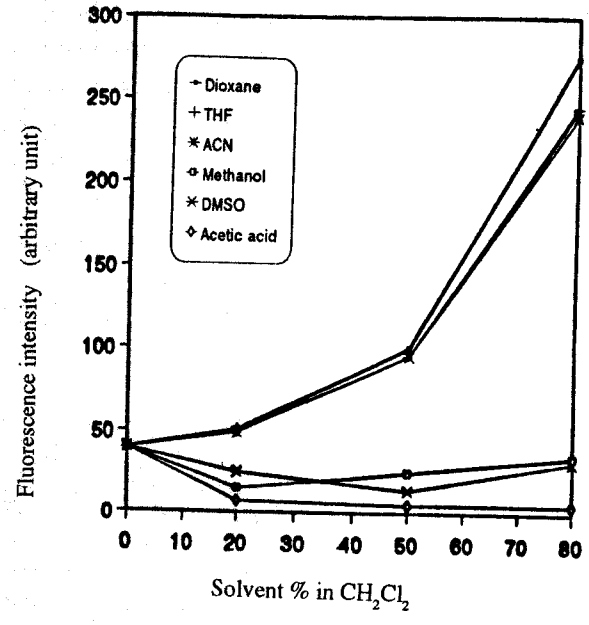

Figure 5. Fluorescence intensity of the pyrrolinone derivative in a mixture of dichloromethane and a second solvent with various fractions

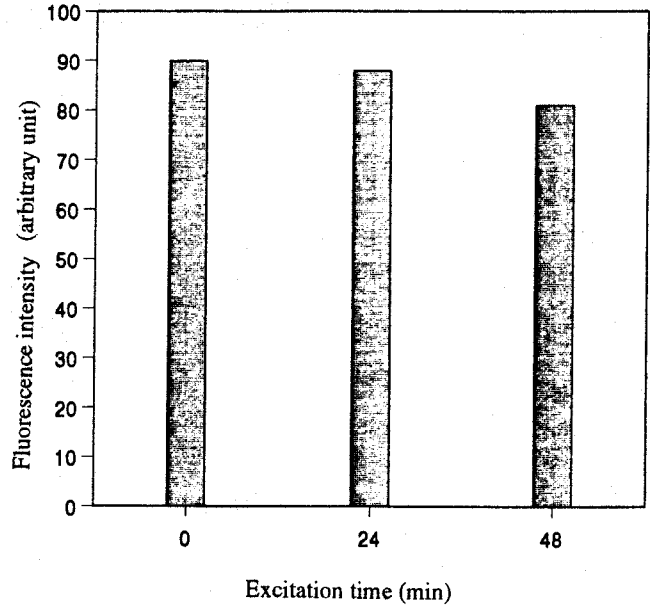

Figure 7. Fluorescence intensity of the pyrrolinone derivative in a mixture of dichloromethane:ACN (1:1) by instantaneous excitation

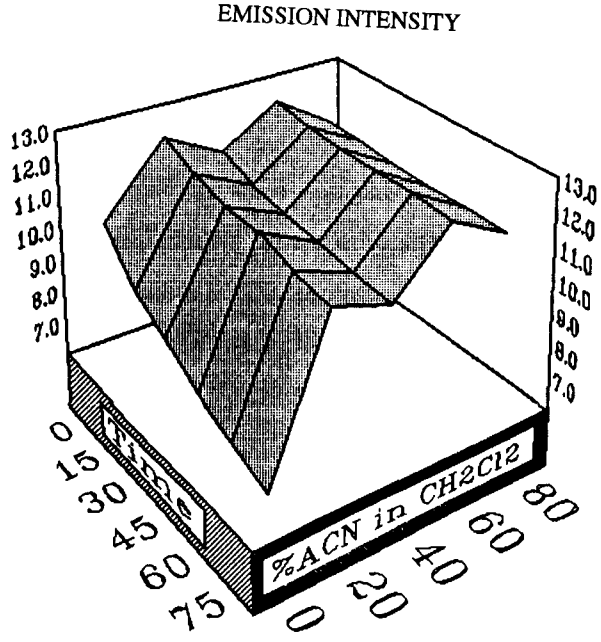

Figure 8. Variation of fluorescence intensity at 10 min intervals in different fractions of ACN

as to determine optimal condition, variation of fluorescence intensity was measured at 10 minute intervals in different fractions of ACN, THF or dioxane in dichloromethane. From the results obtained (Figs. 8,9 and 10) only a small increase of fluorescence intensity with increasing solvent fractions could be observed. These data suggest that dichloromethane containing quantities of ACN, THF or dioxane as chromatographic modifiers may be useful as mobile phases for assays involving gradient or isocratic elution of fluorescamine derivatives.

Correlation between Fluorescence Intensity and Analyte Concentration

Since the fluorescence intensity is nearly constant in different ranges of the studied solvent fractions and so as to prevent sample dilution, the calibration graph of NE in dichloromethane:dioxane (4:1) was raised over the 0.1-1 mg/l range at 10 minute intervals after the extraction of the pyrrolinone derivative with dichloromethane. The results are presented in Table 3 and show that the calibration graphs are thoroughly similar and relative standard deviation for concept and slope are 1.88 and 0.43, respectively. RSD% is 0.82 for five replicates.

Conclusion

From the results obtained, it may be concluded that the addition of different fractions of ACN, THF and dioxane to the dichloromethane solvent increases fluorescence stability and intensity of the pyrrolinone derivative. Hence, the proposed method may be considered reproducible (RSD% = 0.82). On the other hand, spectrofluorimetry is inherently sensitive and can be used for analysis of biological amines in low concentrations. With little variation, the mentioned mixture of solvents can also be used in liquid chromatography as a mobile phase for the separation of amino compounds in complex matrices.

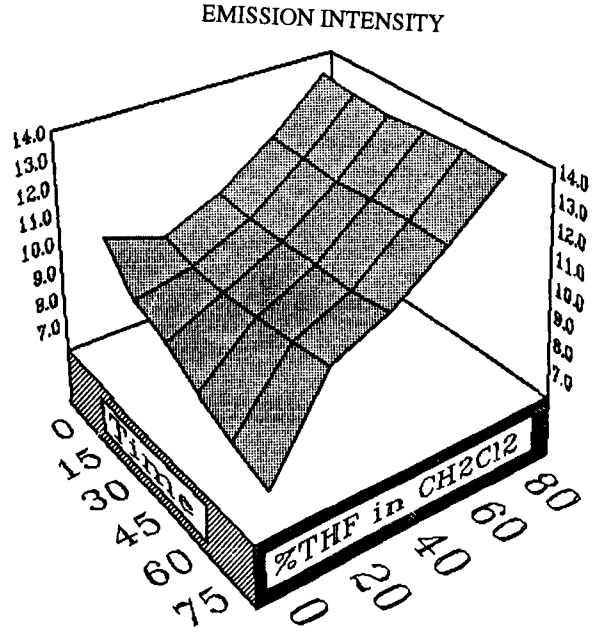

Figure 9. Variation of fluorescence intensity at 10 min intervals in different fractions of THF

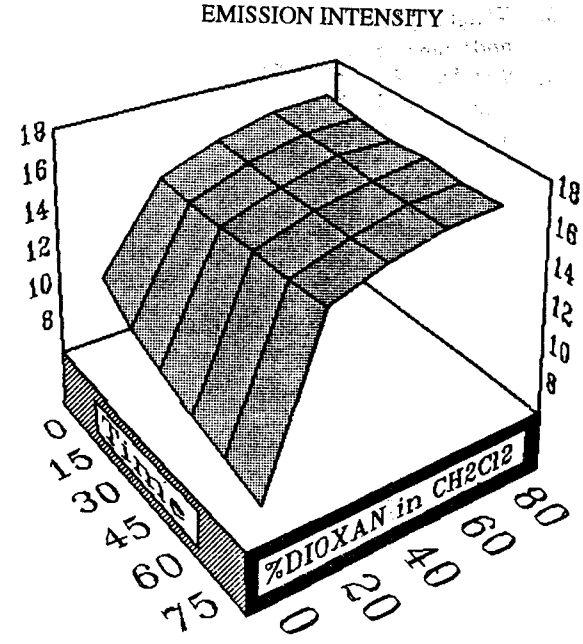

Figure 10. Variation of fluorescence intensity at 10 min intervals in different fractions of dioxane

Table 3. Calibration equation of NE at 10 min intervals in CH_2Cl_2: dioxane (80:20)

Time (min)	Calibration graph equation	r[¥]	Mean ± standard deviation	
			Intercept	Slope
0	I= 2.68 + 16.13C[¢]	0.997	2.66 ± 0.05	16.08 ± 0.07
10	I= 2.61 + 16.02C	0.997	(RSD = 1.88%)	(RSD = 0.43%)
20	I= 2.61 + 16.02C	0.997		
30	I= 2.61 + 16.06C	0.997		
40	I= 2.67 + 16.07C	0.998		
50	I= 2.74 + 16.05C	0.997		
60	I= 2.71 + 16.21C	0.998		

[¥]Correlation coefficient
[¢]I and C are fluorescence intensity and concentration, respectively

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