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# FLUORESCENCE ENHANCEMENT IN THIN-LAYER CHROMATOGRAPHY BY SPRAYING VISCOUS ORGANIC SOLVENTS

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## SUMMARY

Spraying of the fluorescence spot on a thin-layer plate with a non-volatile and viscous organic solvent after development enhanced the fluorescence. The elucidation of the physicochemical factors that affect such an enhancing effect was investigated. The effect was not related to the transmissibility of light through the thin layer but to the properties of the spraying reagent (polarity, viscosity and acidity).

The appropriate reagents for 5-dimethylamino-1-naphthalenesulphonamides (DANS-amines) are non-polar, viscous and non-acidic solvents such as a mixture of liquid paraffin and *n*-hexane, which enhanced the fluorescence intensity 10-fold. With benzo(*a*)pyrene, the use of a solvent such as this liquid paraffin mixture enhanced the fluorescence intensity 35-fold.

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## INTRODUCTION

A method for enhancing the fluorescence of spots on thin-layer (TLC) plates has been devised[7], based on the fact that the uniform spraying of some fluorescent spots with a non-volatile and viscous organic solvent after development resulted in a considerable increase in the fluorescence intensities and the enhanced intensities were maintained for a long period[8].

This present paper describes the elucidation of the physicochemical factors of the spraying reagents that affect the fluorescence-enhancing action using 5-dimethylamino-1-naphthalenesulphonamides (DANS-amines) as test compounds. The selection of a suitable reagent for some other compounds was also investigated.

## EXPERIMENTAL

### *Materials*

All chemicals and organic solvents were of reagent grade. 5-Dimethylamino-1-naphthalenesulphonyl chloride (DANS-Cl) was purchased from Seikagaku Industries. 5-Dimethylamino-1-naphthalenesulphendimethylamide (DANS-DMA) was prepared by Seiler's method[9]; yellow needles, m.p. 103–104°; mass spectrum, *m/e* calculated for

$C_{14}H_{18}O_2N_2S$ 278.1073 ($M^+$), found 278.1059. A silica gel TLC plate (Merck) was used, the layer thickness pre-coated on the glass plate being 0.25 mm.

### *Apparatus*

The fluorescence spectra were recorded with a Hitachi Model MPF-3A spectrofluorimeter. The fluorescence and transmittance scans were measured with an Ozumz Model SD-92 spectrophotodensitometer, the excitation wavelength being 365 nm and the whole emission being measured. The mass spectrum of DANS-DMA was measured with a JEOL Model JMS-OISG-2 mass spectrometer. The viscosities of the solvents were measured in centipoise with an Ubbelohde viscometer. The dielectric constants of the solvents were measured with a Shibayama Model SS-208 dielectric constant meter.

### *Spraying equipment*

The equipment consisted of a sprayer with a gas pressure regulator, a belt conveyer to carry a plate and a plastic spray cabinet with a ventilation flue[8]. It was possible to spray a reagent uniformly at the position of a particular $R_F$ value. When DANS-DMA was used as a fluorescent compound, the coefficients of variation of the fluorescence intensities on a plate before and after spraying ($n = 7$) were 2.53 and 2.83%, respectively.

### *Simplified determination of volatile amines*

The determination was carried out as follows[5]. Ammonia and volatile amines, extracted from a sample with 1 ml of 4% trichloroacetic acid, were added to the external cell of Conway's microdiffusion apparatus and were transferred to 1 ml of 0.05 *N* sulphuric acid in the centre well by addition of concentrated potassium hydroxide solution to the external cell. To 0.2 ml of the sulphuric acid solution was added 0.2 ml of a 1% solution of DANS-Cl in acetone and the mixture was made alkaline with 0.2 ml of 0.2 *M* sodium hydrogen carbonate solution. A 5-$\mu$l volume of the mixture was spotted on to a plate. The fluorescence intensity of each spot was measured after development.

### *Spraying procedure with a fluorescence-enhancing reagent*

After development and standing for 1 h, the plate was sprayed with the appropriate reagent and then allowed to stand for a further 1 h in the dark. The fluorescence intensity of the spot was then measured.

### *Fluorescence-enhancing reagent*

Two types of reagents were used: (a) a polar solvent such as a mixture of glycerol and ethanol or of glycerol and methanol and (b) a non-polar solvent such as a mixture of liquid paraffin (LP) and toluene or LP and *n*-hexane, or of LP and benzene. In addition, a low-viscosity solvent was added to each mixture in order to ensure smooth spraying.

### *Relative fluorescence quantum number*

The relative fluorescence quantum number (RFQN) of the whole fluorescence was taken as the ratio (area shown in a specific solution)/(area shown in a hexane solution) under the compensated fluorescence spectrum, which was measured at an excitation wavelength of 350 nm, a temperature of 20° and a slit width of 10 nm. The RFQN of DANS-DMA in *n*-hexane solution was assigned a value of 1.0.

## RESULTS AND DISCUSSION

### *Transmissibility of light through a thin layer*

There was a possibility that the fluorescence-enhancing action obtained by spraying the reagent was due to an increase in the transmissibility of light through the thin layer. In order to study this point, a silica gel plate on which the same amount of DANS-DMA had been spotted at regular intervals was sprayed with a mixture of LP and *n*-hexane (2:1). As shown in Fig. 1, the transmittance at an excitation wavelength of 365 nm decreased when the plate was sprayed once or twice but increased rapidly when the surface of the thin layer became wet and semi-permeable. The transmittance at emission wavelengths of 450–550 nm showed the same effect. On the other hand, the fluorescence intensities of the spots increased with spraying and became constant when the spraying was repeated twice, irrespective of the transmissibility of light. The same phenomena were also observed with polar reagents.

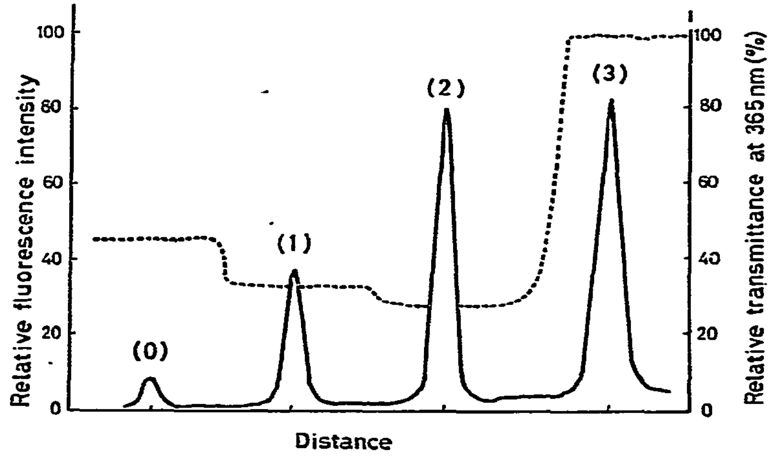

Fig. 1. Relationship between fluorescence intensity of 5 nmole of DANS-DMA per spot and transmissibility of light through a silica gel plate sprayed with liquid paraffin–*n*-hexane (2:1). The numbers in parentheses indicate the number of times spraying was carried out. The broken line shows the relative transmittance of light for a plate sprayed three times, which is apparently semi-permeable.

The results suggest that the fluorescence enhancement was not related directly to the transmissibility of light through the thin layer but to a solvent effect of the spraying reagent, DANS-DMA being dissolved in the layer on the plate.

### *Fluorescence spectra in a solvent*

The fluorescence spectra of DANS-DMA dissolved in polar and non-polar solvents were measured with a fluorescence spectrophotometer. Fig. 2(a) indicates that the spectra in non-polar solvent systems showed increased fluorescence intensities with the addition of a viscous solvent such as LP. Fig. 2(b) indicates that the spectra in polar solvent systems had the same maximal excitation wavelength as in non-polar systems but the maximal emission wavelengths shifted to higher values and the fluorescence intensities decreased with the addition of a viscous solvent such as glycerol. The results suggest that the polarity of the solvent has a greater effect than the viscosity on the fluorescence enhancement of DANS-DMA.

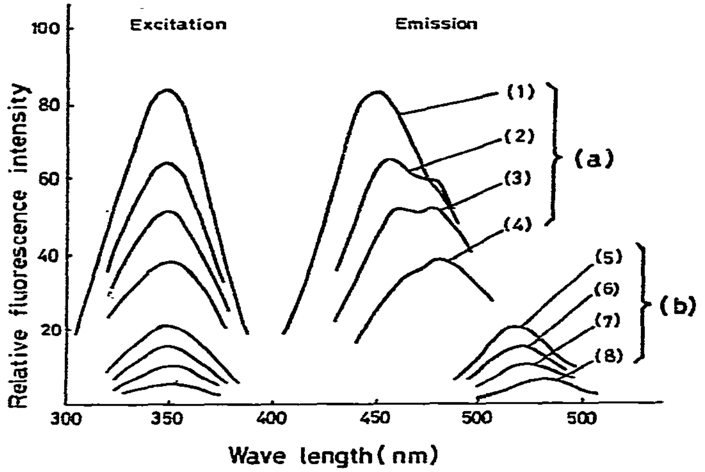

Fig. 2. Fluorescence spectra of $1.54 \cdot 10^{-5}$ *M* of DANS-DMA in various solvents. (a) Non-polar solvent system: (1) liquid paraffin (LP); (2) LP–*n*-hexane (4:1); (3) LP–*n*-hexane (3:2); (4) *n*-hexane. (b) polar solvent system: (5) ethanol; (6) glycerol–ethanol (3:2); (7) glycerol–ethanol (4:1); (8) glycerol.

*Polarity of solvent*

The polarities of the solvents were assessed in terms of their dielectric constants. As the fluorescence intensity on a plate measured with a fluorodensitometer was equal to RFQN in the range of the whole wavelengths, an accurate comparison of fluorescence intensities was substituted for that of RFQNs.

As shown in Fig. 3, the RFQNs of DANS-DMA, dissolved in various solvents, decreased linearly with increase in the dielectric constant of the solvent. This indicated that the fluorescence enhancement occurred effectively in a non-polar solvent.

*Acidity of solvent*

Pyridine (a basic solvent) was located just on the line in Fig. 3, but acetic acid (an acidic solvent) was far from the line and also had a small RFQN. This result suggested that the relationship between the dielectric constant of the solvent and the RFQN might be valid in basic and neutral solvents but could not apply to an acidic solvent.

*Viscosity of solvent*

From the fact that the LP solution enhanced the fluorescence intensity of DANS-DMA considerably, as shown in Fig. 3, the relationship between the viscosity of the solvent and the RFQN was investigated with *n*-alkanes that had almost the

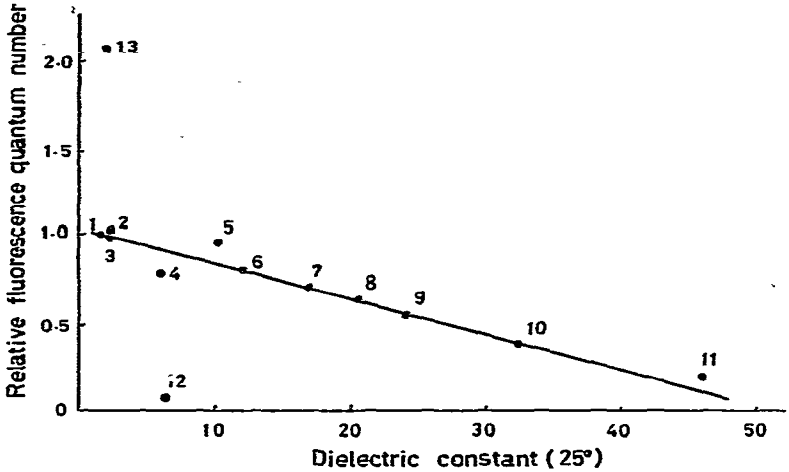

Fig. 3. Relationship between dielectric constant of solvent and relative fluorescence quantum number of DANS-DMA dissolved in the solvent. 1 = *n*-Hexane; 2 = benzene; 3 = toluene; 4 = ethyl acetate; 5 = 1,2-dichloroethane; 6 = pyridine; 7 = 1-butanol; 8 = acetone; 9 = ethanol; 10 = methanol; 11 = glycerol; 12 = acetic acid; 13 = liquid paraffin.

same dielectric constant as LP. The RFQN was proportional to the viscosity, as shown in Fig. 4, which suggested that spraying with a non-polar and viscous solvent is very effective in enhancing the fluorescence intensity on a plate. In other words, the greatest effect was expected when the LP solution mixed with toluene, benzene or *n*-hexane was sprayed. Of these organic solvents, *n*-hexane, which had no absorption or fluorescence itself, is the preferred solvent for diluting LP.

The RFQN increased with the addition of LP and became constant at a viscosity of 13.4 cP with a 4:1 mixture of LP and *n*-hexane. This mixture, however, is too viscous to be sprayed easily and a 2:1 mixture is recommended for practical use.

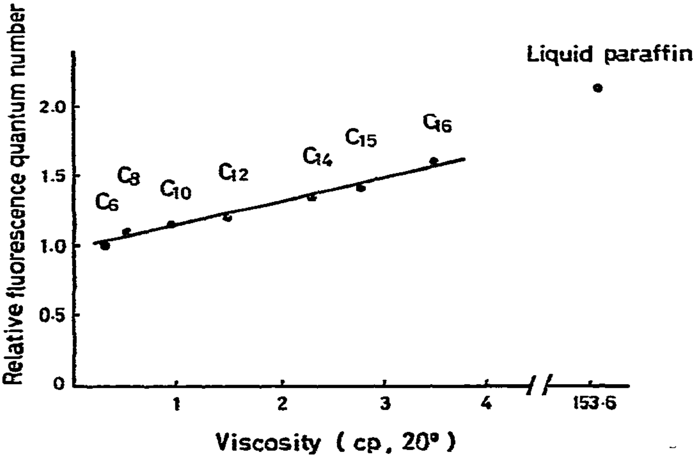

Fig. 4. Influence of viscosity of $C_6$–$C_{16}$ *n*-alkanes as solvents on the relative fluorescence quantum number of DANS-DMA.

### *Fluorescence enhancement on a plate*

Seiler used monoethanolamine as a fluorescence enhancing reagent for DANS-amines[7,9]. Fig. 5 showed a comparison of LP–*n*-hexane and glycerol–ethanol mixtures and monoethanolamine as spray reagents for DANS-DMA spots on a plate.

Monoethanolamine gave a fluorescence intensity as high as that with the LP mixture at first, but the enhanced intensity decreased and the fluorescent spot became extended with time. On the other hand, the LP mixture retained a high fluorescence intensity and a constant spot shape for a long period. The glycerol solution did not give as high an intensity and the spot became extended. Hence the LP mixture was the most suitable reagent for enhancing the fluorescence. It is assumed that monoethanolamine gave a high initial fluorescence intensity owing to the good solvent properties of monoethanolamine for DANS-DMA or to its high permeability in the silica gel layer.

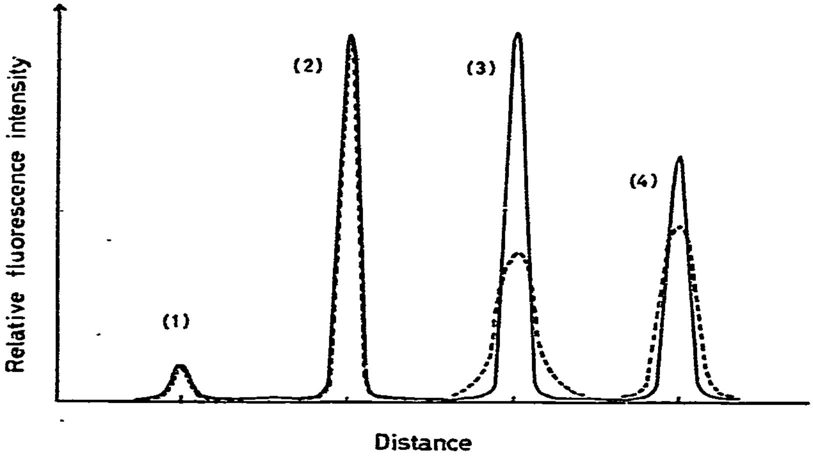

Fig. 5. Comparison of spray reagent with fluorescence enhancement of DANS-DMA (5 nmole per spot) on a silica gel plate. The broken line represents the peaks after 10 h. Peaks: (1) not sprayed; (2) sprayed with liquid paraffin–*n*-hexane (2:1); (3) sprayed with monoethanolamine; (4) sprayed with glycerol–ethanol (1:1).

### *Determination of ammonia and volatile amines*

The use of the LP mixture decreased the detection limit of dimethylamine (DMA), monomethylamine (MMA) and ammonia from $1 \cdot 10^{-4}$ to $1 \cdot 10^{-5}$ *M*, as reported previously[8]. Fig. 6 shows the fluorodensitogram obtained from the TLC of DANS-ammonia and DANS-volatile amines derived from the degradation of trimethylamine oxide by $\gamma$-irradiation. As shown in Fig. 6(b), the fluorescence intensity of each spot sprayed with the LP mixture was enhanced effectively.

### *Other fluorescent compounds*

This fluorescence-enhancing method with a viscous organic solvent was useful not only with DANS-amines but also with other fluorescent compounds. In selecting suitable spray reagents, it was necessary to investigate the effects of the polarity and acidity of the reagent in addition to viscosity, on fluorescence enhancement.

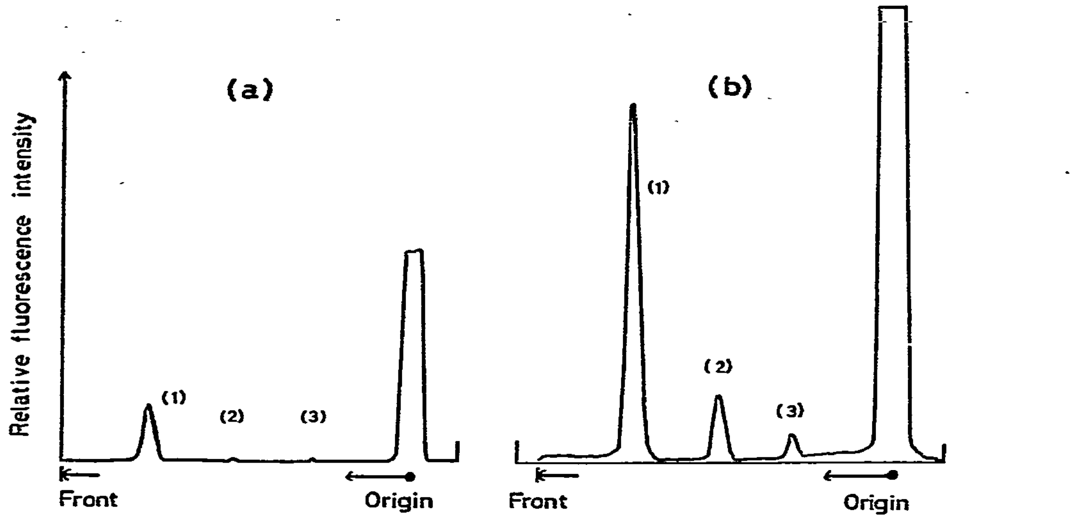

Fig. 6. Thin-layer chromatography–fluorodensitometry on DANS-ammonia and DANS-volatile amines produced from trimethylamine oxide by $\gamma$-irradiation. (a) Not sprayed; (b) sprayed with liquid paraffin–*n*-hexane (2:1). Peaks: (1) DANS-DMA; (2) DANS-MMA; (3) DANS-ammonia.

The use of a non-polar and viscous solvent such as the LP mixture with the carcinogen benzo(*a*)pyrene enhanced its fluorescence intensity 35-fold. With aflatoxin $B_1$, one of the mycotoxins, the use of a non-polar and viscous solvent enhanced its fluorescence 2.5-fold. An alkaline solvent such as monoethanolamine, however, had no effect. With ochratoxin, another mycotoxin, a suitable polar and viscous solvent was a mixture of glycerol and ethanol (1:1), which enhanced the fluorescence intensity 2-fold.

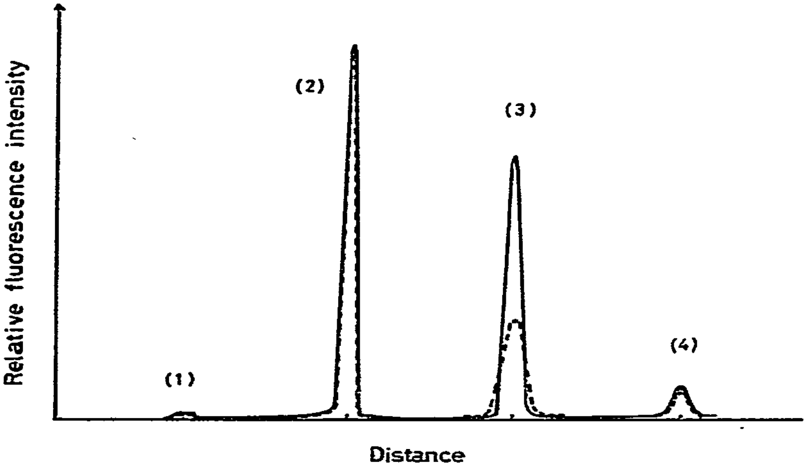

Fig. 7. Comparison of spray reagent with fluorescence enhancement of benzo(*a*)pyrene (80 ng per spot) on a silica gel plate. The broken line represents the peaks after 10 h. Peaks: (1) not sprayed; (2) sprayed with liquid paraffin–*n*-hexane (2:1); (3) sprayed with monoethanolamine; (4) sprayed with glycerol–ethanol (1:1).

Fig. 7 shows the change in the fluorescence intensity of benzo(*a*)pyrene when the plate was sprayed with the LP mixture, monoethanolamine or a mixture of glycerol and ethanol, and was then allowed to stand in the dark.

This fluorescence enhancement with DANS-DMA and benzo(*a*)pyrene was also observed on other adsorbents such as polyamide and cellulose.

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