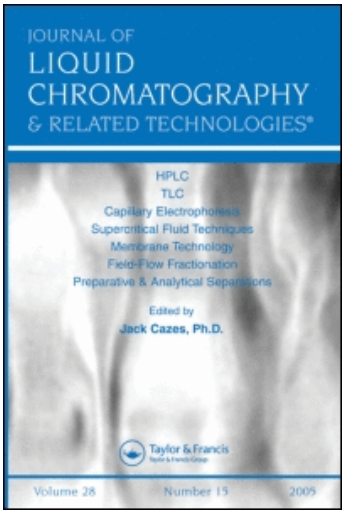

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Effects of Different Stationary Phases and Surfactant or Cyclodextrin Spray Reagents on the Fluorescence Densitometry of Polycyclic Aromatic Hydrocarbons and Dansylated Amino Acids

A. Alak[a]; E. Heilweil[ab]; W. L. Hinze[ac]; H. Oh[a]; D. W. Armstrong[a]
[a] Department of Chemistry, Texas Tech University, Lubbock, TX [b] Whatman Chemical Separation Div., Clifton, N.J. [c] Department of Chemistry, Wake Forest University, Winston-Salem, N.C.

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EFFECTS OF DIFFERENT STATIONARY PHASES AND SURFACTANT OR CYCLODEXTRIN SPRAY REAGENTS ON THE FLUORESCENCE DENSITOMETRY OF POLYCYCLIC AROMATIC HYDROCARBONS AND DANSYLATED AMINO ACIDS

A. Alak, E. Heilweil[†], W. L. Hinze[‡], H. Oh and D. W. Armstrong*

*Contribution from: Department of Chemistry
Texas Tech University, Lubbock, TX 79409

ABSTRACT

The detectable luminescence of twelve dansyl amino acids and four polycyclic aromatic hydrocarbons (PAH's) spotted on five common TLC stationary phases was evaluated. The detectable luminescence varied appreciable for compounds associated with different stationary phases. The use of surfactant and cyclodextrin spray reagents caused luminescence enhancements on some stationary phases but not others. The reagents did not affect all compounds to the same degree indicating that qualitative information could be obtained in some cases. The largest luminescence increase for a compound spotted on silica gel was for pyrene (i.e., 47-fold) sprayed with sodium cholate. The degree to which the plates were dried also affected the luminescence intensity. Possible reasons for the observed effects are discussed.

INTRODUCTION

The detection and quantitation of luminescent compounds in TLC is an important and highly sensitive technique (1). Stationary phase and spray reagent effects can appreciably alter the luminescence behavior of a variety of compounds. Unfortunately there are few analytical studies which consider these effects

[†]Whatman Chemical Separation Div., 9 Bridewell Place, Clifton, N.J.
[‡]Department of Chemistry, Wake Forest University, Winston-Salem, N.C.

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(2-4). Fluorescent probes have been effectively used to examine the surface characteristics of derivatized silica gels (5, 6) and a number of interesting studies have been done on materials used to induce room-temperature phosphorescence on supports such as filter paper and silica gel (7-13).

Solutions of micellar aggregates and cyclodextrin molecules have recently been used to enhance the fluorescence of a variety of compounds (14-16). There have also been reports on the use of micellar mobile phases to improve luminescence detection in LC (17, 18). As yet there have been no systematic studies on the use of surfactant and cyclodextrin spray reagents to enhance the scanning densitometric determination of luminescent compounds on a variety of TLC supports. In this study the luminescence characteristics of several dansyl amino acids and polycyclic aromatic hydrocarbons (PAH's) on five common TLC supports are examined. The modifications of the luminescent intensity of these compounds using different spray reagents are evaluated and discussed.

EXPERIMENTAL

Materials. High purity α,β and γ cyclodextrin was obtained from Advanced Separation Technologies, Inc., 37 Leslie Court, Whippany, NJ 07981. Sodium dodecyl sulfate (SDS) from Bio Rad, cetyltrimethylammonium chloride (CTAC) from Fisher, sodium cholate from Sigma and Zwittergent 3-12 (a zwitterionic surfactant) from Calbiochem were used as received. Silica gel (K6), reverse phase (KC18), alumina (K3) and cellulose (K2) TLC plates were obtained from Whatman. Polyamide -6 TLC plates were obtained

from Brinkmann. HPLC grade water and methanol were obtained from Burdick & Jackson. A Shimadzu CS-910 scanning densitometer coupled to a C-R2AX data station was used for all determinations.

Methods. One microliter of 10^{-3}M solutions of twelve dansyl amino acids and four polycyclic aromatic hydrocarbons (Table 1) was individually spotted (Drummond micropipet) on five types of TLC support (i.e., silica gel, alumina, C_{18}-reversed phase, cellulose and polyamide). The spots were scanned and quantitated with a Shimadzu CS-910 densitometer in the fluorescence mode. The excitation wavelength was 256 nm for the PAH's and 320 nm for the dansyl amino acids. A "UV-D_2" cut-off filter was placed in front of the detector. The chromatographic plates were then sprayed with a 1% solution of either SDS, CTAC, sodium cholate, Zwittergent or β-cyclodextrin for 10 seconds at a distance of 2 feet. The spots were then scanned at 2 minutes (wet plates) and 75 minutes (dry plates) after spraying. Blanks (i.e., prespotted plates sprayed with water containing no reagent) were run in all cases.

Spotting reproducibility was ±2.3% as determined from the analysis of 10 identical experiments. The variation in fluorescence caused by plate to plate inconsistencies (within a given lot) was ±3.2%.

RESULTS AND DISCUSSION

A compound's fluorescence can vary tremendously when in contact with different TLC matrices. Consequently, stationary phase effects on luminescence detection and quantitation can be appreciable. Figure 1 illustrates the magnitude of this effect

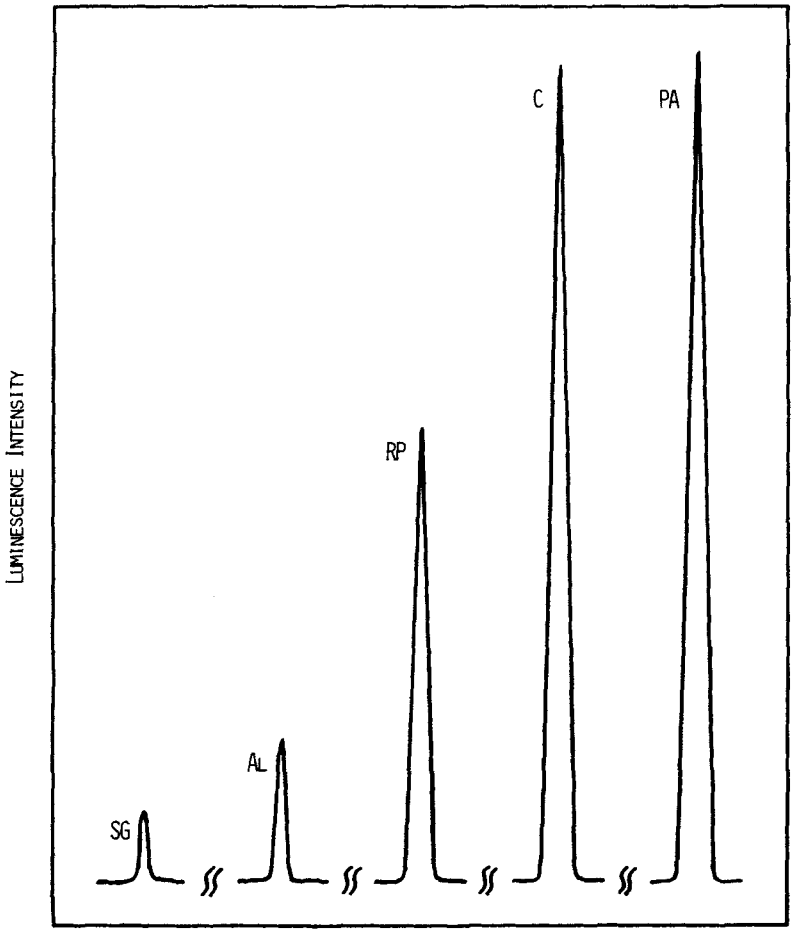

Figure 1. A comparison of the stationary phase effect on the detectable luminescence of one nanogram of dansyl glycine spotted on each plate. SG = silica gel, Al = alumina, RP = dimethyloctadecylsilanated reversed phase, C = cellulose, PA = polyamide. The detectable luminescence was 18 to 25 greater on polyamide and cellulose then on silica gel. The detectable luminescence on C_{18} reversed phase was 10 to 15 times greater than that on silica gel while alumina rarely produced more than a 2-fold increase over silica gel.

for dansyl glycine. Analogous results were obtained for the other compounds in this study. The magnitude of detectable luminescence varied on different stationary phases as follows: cellulose ≈ polyamide > C_{18}-reversed phase > alumina > silica gel. The detectable luminescence of dansyl amino acids on cellulose or polyamide plates was as much as 25 times greater than on silica gel. The detectable luminescence on alumina was only slightly better than on silica gel (about 2-fold) while it was as much as 15 times greater on C_{18} reversed phase plates. Possible reasons for these effects will be discussed at the end of this section.

The effect of surfactant and cyclodextrin spray reagents on the luminescence of dansyl amino acids and PAH's varies considerably for different stationary phases. In fact, significant enhancements were only observed on silica gel and alumina (Tables I and II). The spray reagent effect on luminescence on reversed phase, cellulose and polyamide plates was much less pronounced and was as likely to cause modest decreases in luminescence as increases (Tables III, IV and V). It is apparent that the analytical usefulness of these particular spray reagents is mainly limited to silica gel and alumina. It is also interesting that the stationary phases on which these reagents produce their greatest effect are those which seem to "inhibit" the fluorescence of dansyl amino acids and PAHs the most (i.e., silica gel and alumina, in Figure I).

A closer look at the data in Tables I and II reveals several interesting trends as well as significant differences in spray reagents. The usefulness of these reagents in lowering detection limits and increasing the linear dynamic range is illustrated in

Table I. A List of the Fluorescence Enhancements for a Variety of Dansylated Amino Acids and Polycyclic Aromatic Hydrocarbons (on Silica Gel) Resulting from Surfactant or Cyclodextrin Spray Reagents.[a]

	Spray Reagent									
	β-Cyclodextrin		CTAC		SDS		Sodium Cholate		Zwittergent	
Compound[b]	wet[c]	dry[d]	wet[c]	dry[d]	wet[c]	dry[d]	wet[c]	dry[d]	wet[c]	dry[d]
α-aminobuteric acid	2.2	5.4	9.9	5.8	2.6	1.9	3.1	1.5	1.9	1.0
norleucine	1.9	4.0	8.9	4.2	3.1	2.2	3.4	1.4	2.4	1.2
leucine	2.5	3.9	7.8	4.5	2.2	2.0	7.2	1.1	10	1.5
norvaline	3.0	4.9	9.3	5.1	3.0	2.0	4.7	1.9	2.9	1.3
phenylalanine	4.7	5.4	9.2	5.9	3.1	1.8	8.0	1.0	3.7	1.3
methionine	2.4	4.7	8.1	5.7	1.9	1.0	3.5	1.0	1.2	1.1
glutamic acid	3.1	7.0	12	5.7	4.9	1.3	4.7	1.0	2.5	1.0
serine	2.1	6.0	9.3	5.0	2.8	1.3	6.1	1.1	3.1	1.4
glycine	5.0	8.4	11	5.6	1.3	2.5	3.1	1.3	4.0	1.2
threonine	2.4	5.2	10	5.7	3.7	1.0	4.0	1.2	2.7	1.3
tryptophan	2.9	5.7	13	7.4			7.3	1.4	2.1	1.2
aspartic acid	3.4	5.5	12	5.7	5.7	1.0	3.6	1.5	1.2	1.1
pyrene	19	16	20	6.9	27	7.0	47	27	47	5.8
benzanthracene	2.1	14	4.5	3.4	7.6	3.1	10	6.2	10	4.7
benzo (α) pyrene	1.9	5.5	7.5	3.4	5.4	2.0	6.5	5.0	6.5	5.1
benzo (e) pyrene	1.0	6.4	10	3.0	5.9	2.3	5.1	3.0	6.6	5.0

[a] The spray reagents consisted of 1% of the surfactant or cyclodextrin in water. The enhancements listed are taken as the fluorescence peak area (after spraying) divided by the peak area of the dry, unsprayed fluorescent spot.

[b] All listed amino acids are dansylated, therefore the fluorescence enhancement is for the attached fluorophor.

[c] Fluorescence was measured two minutes after spraying.

[d] Fluorescence was measured 75 minutes after spraying.

Table II. A List of Average Fluorescence Enhancements for Twelve Dansyl Amino Acids and Individual Enhancements for the Polycyclic Aromatic Hydrocarbons on Alumina.[a]

	Spray Reagent									
	ß-cyclodextrin		CTAC		SDS		Sodium Cholate		Zwittergent	
Compound	wet[b]	dry[c]	wet[b]	dry[c]	wet[b]	dry[c]	wet[b]	dry[c]	wet[b]	dry[c]
dansylamino acids	2.3	3.4	1.0	3.4	3.7	1.9	3.4	0.8	3.9	0.8
pyrene	20	8.9	5.6	4.9	39	2.1	30	15	27	12
benzanthracene	6.0	4.2	7.9	2.8	9.1	2.3	9.5	5.1	9.9	4.8
benzo (α) pyrene	3.1	3.0	4.5	2.9	14	3.1	7.0	4.3	5.8	4.0
benzo (e) pyrene	0.5	2.0	4.0	2.5	4.2	2.0	5.2	3.3	5.2	3.7

[a]The spray reagents consisted of 1% of the surfactant or cyclodextrin in water. The enhancements listed are taken as the fluorescence peak area (after spraying) divided by the peak area of the dry, unsprayed fluorescent spot.

[b]Fluorescence was measured two minutes after spraying.

[c]Fluorescence was measured 75 minutes after spraying.

Table III. Average Fluorescence Changes for Twelve Dansyl Amino Acids and Four Polycyclic Aromatic Hydrocarbons (PAH) on C_{18}-Reversed Phase.[a]

	Spray Reagents									
	β-cyclodextrin		CTAC		SDS		Sodium Cholate		Zwittergent	
Compounds	wet[b]	dry[c]	wet[b]	dry[c]	wet[b]	dry[c]	wet[b]	dry[b]	wet[b]	dry[c]
Dansyl amino acids	0.6	1.0	1.6	2.2	0.6	1.2	1.2	1.7	1.2	1.3
PAH	0.8	1.0	0.8	0.8	1.2	1.0	1.3	1.1	1.1	1.0

[a]The spray reagents consisted of 1% of the surfactant or cyclodextrin in water. The enhancements listed are taken as the fluorescence peak area (after spraying) divided by the peak area of the dry, unsprayed fluorescent spot.

[b]Fluorescence was measured two minutes after spraying.

[c]Fluorescence was measured 75 minutes after spraying.

Table IV. Average Fluorescence Changes for Twelve Dansyl Amino Acids and Four Polycyclic Aromatic Hydrocarbons (PAH) on Cellulose.

	Spray Reagents									
	ß-cyclodextrin		CTAC		SDS		Sodium Cholate		Zwittergent	
Compounds	wet[b]	dry[c]	wet[b]	dry[c]	wet[b]	dry[c]	wet[b]	dry[c]	wet[b]	dry[c]
Dansyl amino acids	0.8	1.9	0.5	1.8	0.1	0.3	0.2	0.4	0.3	0.8
PAH	1.2	1.9	1.2	2.0	1.4	2.2	1.0	1.1	1.1	1.0

[a]The spray reagents consisted of 1% of the surfactant or cyclodextrin in water. The enhancements listed are taken as the fluorescence peak area (after spraying) divided by the peak area of the dry, unsprayed fluorescent spot.

[b]Fluorescence was measured two minutes after spraying.

[c]Fluorescence was measured 75 minutes after spraying.

Table V. Average Fluorescence Changes for Twelve Dansyl Amino Acids and Four Polycyclic Aromatic Hydrocarbons (PAH) on Polyamide.[a]

	Spray Reagent									
	ß-cyclodextrin		CTAC		SDS		Sodium Cholate		Zwittergent	
Compound	wet[b]	dry[c]	wet[b]	dry[c]	wet[b]	dry[c]	wet[b]	dry[c]	wet[b]	dry[c]
Dansyl amino acid	0.5	1.0	0.3	0.8	0.8	1.0	0.5	0.7	0.8	0.6
PAH	1.7	1.5	1.3	0.9	2.1	1.2	1.2	1.1	1.0	0.9

[a]The spray reagents consisted of 1% of the surfactant or cyclodextrin in water. The enhancements listed are taken as the fluorescence peak area (after spraying) idvided by the peak area of the dry, unsprayed fluorescent spot.

[b]Fluorescence was measured two minutes after spraying.

[c]Fluorescence was measured 75 minutes after spraying.

Figure 2 for dansyl glycine. β-Cyclodextrin and CTAC have a much greater effect on the luminescence of the dansyl amino acids than the other surfactants (Table I). It is interesting that CTAC has its greatest effect when the plate is freshly sprayed (i.e., still wet) whereas β-cyclodextrin has a greater effect when dry. Nearly all of the surfactant spray reagents produce a greater effect on silica gel and alumina when freshly sprayed. This could be a result of the presence of aggregational structures (e.g., micelles or bilayers) in the wet media. A given spray reagent tends to affect all the dansylated amino acids by about the same amount (for a given stationary phase) although there are small variations (Tables I-V). The polycyclic aromatic hydrocarbons (PAH) tended to show greater variation on silica gel and alumina but very little on the other stationary phases. Pyrene gave the largest fluorescence enhancements of any compound tested (Figure 3). Enhancements of 20 times or more (Table I, II and Figure 3) were not unusual for any freshly sprayed chromatogram. Sodium cholate produced the greatest overall luminescence increases for the PAH's although β-cyclodextrin and Zwittergent were also effective. On the other hand, CTAC was the most effective reagent for the dansyl amino acids, closely followed by β-cyclodextrin. Sodium cholate and Zwittergent produced little effect on the dansyl amino acids in the dry state. It seems that there is a certain amount of reagent selectivity in the luminescence enhancements of different compounds. This could be potentially useful in the identification of certain substances.

A complete elucidation of the mechanism(s) through which the luminescence is controlled and reasons for the variations in

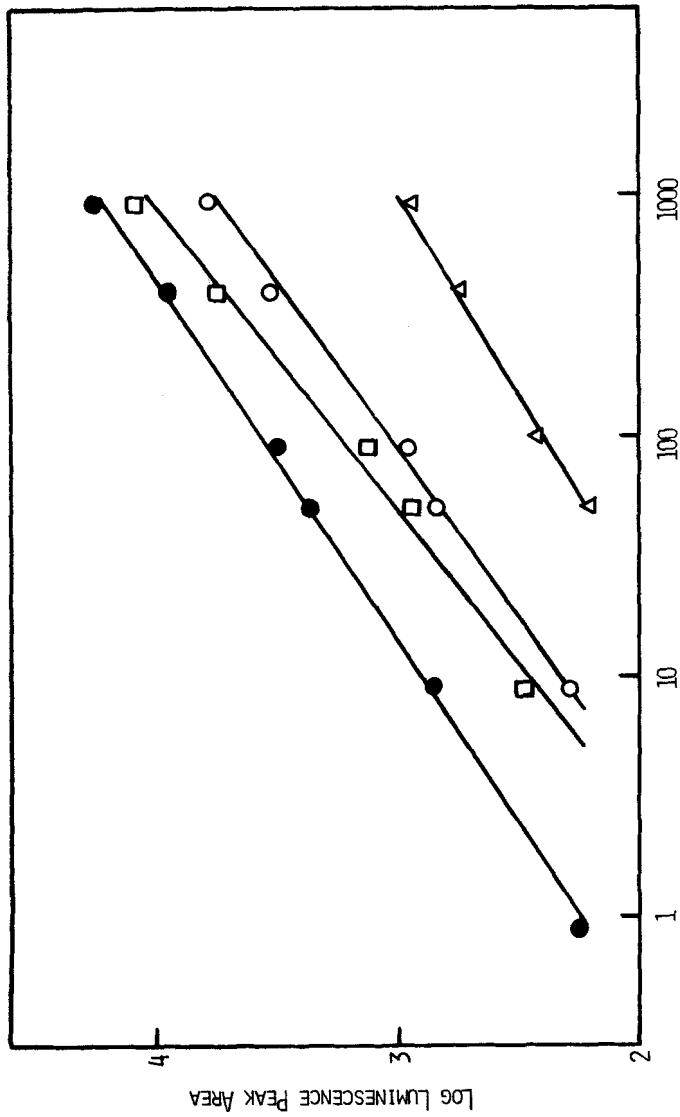

Figure 2. Plots showing the effect of different spray reagents on the linear dynamic range of dansyl glycine on silica gel. Δ = no spray reagent, O = CTAC sprayed and dried, □ = β-cyclodextrin sprayed and dried, O = CTAC freshly sprayed (i.e., the luminescence was measured 2 min. after spraying while it was still wet).

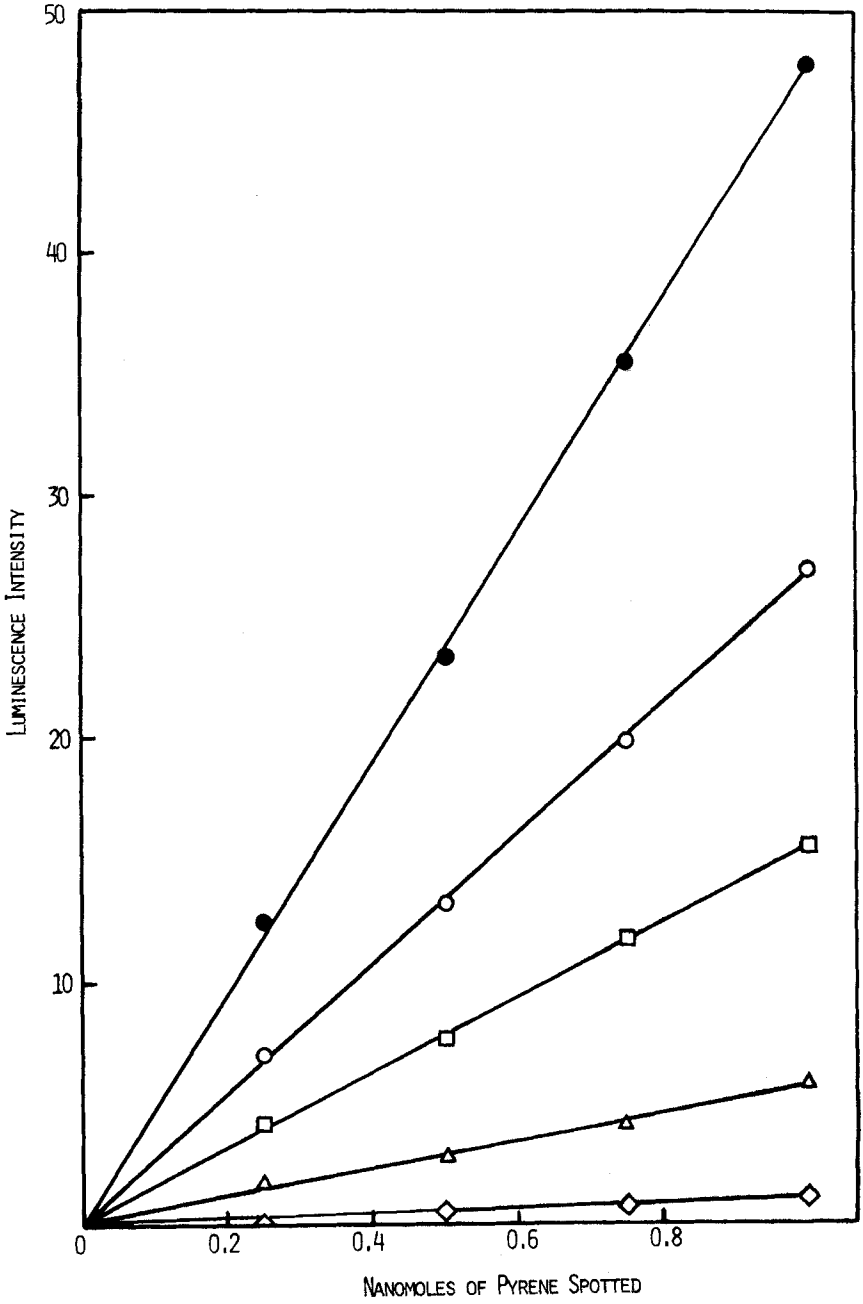

Figure 3. Plots showing the effect of different spray reagents on the calibration curve of pyrene on silica gel. ◇ = no spray reagent, Δ = Zwittergent sprayed and dried, □ = β -cyclodextrin sprayed and dried, 0 = sodium cholate sprayed and dried, 0 = sodium cholate freshly sprayed.

it are beyond the scope of this paper. However, some general statements can be made concerning the role of the stationary phase and spray reagents in luminescence detection on TLC plates. Firstly, the observed enhancements due to the spray reagents were not the result of increased solubilization of the fluorophor on the surface of the stationary phase or to internal reflections from a liquid surface layer. Spray "reagent" blanks (e.g., pure water, H_2O/methanol, etc) consistently caused reduced luminescence on all stationary phases. Secondly, when luminescent compounds are spotted on the more strongly adsorbing media (i.e., silica gel and alumina) they produce substantially less fluorescence (Figure 1). The spray reagents, however, produce their greatest effects on these same media. It has been reported that strong absorption can increase the nonradiative rate constant of pyrene (19) which leads to a decrease in fluorescence. It was further reported that the addition of certain compounds (such as long chain alcohols or glycerol) would preferentially interact with the strong adsorption sites thereby allowing pyrene "to be adsorbed in areas of weaker interactions" (19). This tended to diminish the probability of static quenching of tightly bound pyrene as well as enhance the possibility of dynamic eximer formation (due to the greater mobility of pyrene (19)). Certainly many of these phenomena are possible in the present system as all of the spray reagents strongly adsorb to silica gel. In the case of β-cyclodextrin, formation of stable inclusion complexes with the fluorophor would be another important factor (4). Furthermore, it is well known that micellar aggregates can enhance fluorescence (15,16) and this could be a factor in the freshly sprayed chro-

matograms. The aggregational structure would be destroyed upon drying, however. Spray reagents have essentially no effect on luminescence of compounds spotted on C_{18}-reversed phase plates. In this case the adsorption sites have been silanized and the relatively nonpolar environment of any existing aggregates is no better than that which already exists on the stationary phase. Surfactants also adsorb somewhat to cellulose and polyamide. However, if the environment provided by the surfactant or cyclodextrin is less hospitable to the luminescent specie than the stationary phase, then one observes decreased luminescence (Tables II, IV and V).

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REFERENCES

1. Touchstone, J. C. and Dobbins, M.F., Practice of Thin Layer Chromatography, 2nd Edition, John Wiley & Sons, New York (1983).

2. Kinoshita, T., Iinuma, F., Atsumi, K., Kanada, Y. and Tsuji, A., Chem. Phar. Bull., 23, 5, 1166 (1975).

3. Uchiyama, S. and Uchiyama, M., J. Chromatogr., 153, 135 (1978).

4. Hinze, W. L., Sep. Purif. Methods, 10, 159 (1981).

5. Lochmüller, C. H., Marshall; D. B. and Wilder, D. R., Anal. Chim. Acta, 130, 31 (1981).

6. Lochmüller, C. H. and Marshall, D. B., Anal. Chim. Acta, 142, 63 (1982).

7. Paynter, R. A., Wellons, S. L. and Winefordner, J. D., Anal. Chem., 51, 736 (1974).

8. Parker, R. T., Freelander, R. S., Schulman, E. M., Dunlap, R. B., Anal. Chem., 51, 1921 (1979).

9. Vo-Dinh, T., Hooyman, J. R., Anal. Chem., 51, 1915 (1979).

10. Meyers, M. L. Seybold, P. G., Anal. Chem., 51, 1609 (1979).

11. Ford, C. D., Hurtubise, R. J., Anal. Chem., 52, 656 (1980).

12. Hurtubise, R. J., Solid Surface Luminescence Analysis: Theory, Instrumentation, Applications, Marcel Dekker, New York (1981).

13. Ramasamy, S. M., Hurtubise, R. J., Anal. Chem., 54, 1642 (1982).

14. Hinze, W. L., Solution Chemistry of Surfactants, K. L. Mittal, Ed., Plenum Press, New York, 1979, Vol. 1.

15. Singh, H. and Hinze, W. H., Anal. Lett., 15, A3, 221 (1982).

16. Singh, H. and Hinze, W. L., Analyst, 107, 1073 (1982).

17. Armstrong, D. W., Hinze, W. L., Bui, K. H. and Singh, H. N., Anal. Lett., 14, A19, 1659 (1981).

18. Weinberger, R., Yarmchuk, P., Cline Love, L. J., Anal. Chem., 54, 1552 (1982).

19. Bauer, R. K., de Mayo, P., Ware, W. R., Wu, K. C., J. Phys. Chem., 86, 3781 (1982).