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## THIN LAYER CHROMATOGRAPHIC SEPARATION OF CLOSELY RELATED DYES

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### ABSTRACT

A scheme for the TLC separation of twenty five dyes on silica-gel-zinc acetate impregnated plates using a mixture of n-butanol-benzene-ethylacetate as developing system is described. Examples of separations of dyes in mixtures are reported.

### INTRODUCTION

Separation and identification of dyes is of interest to the colour user, since two dyes with same shade may differ substantially in fastness properties. TLC studies on different groups of synthetic dyes have been reported<sup>1,2</sup>. Recently Arsov and coworkers<sup>3</sup> have reported a suitable method for the separation of fat dyes while a review on the TLC of different class of dyes has been given by Venkateraman<sup>6</sup>. However, the use of impregnated layers for the separation of dyes has not received much attention so far<sup>7</sup>. Since dyes are reported to form complexes with metal salts<sup>8</sup> and complexation is reported to improve the separation in many cases<sup>8-12</sup> it was considered necessary to use metal salts as impregnants to study the chromatographic behaviour of dyes. The present paper describes the separation of twenty five dyes on silica Gel layers impregnated with zinc acetate.

### EXPERIMENTAL

The TLC plates (thickness 0.5 mm) were prepared by spreading, by means of a Stahl type applicator, a slurry of 50g of silica gel (BDH) and 50% aq. solution

TABLE - I

$R_f$  Values of Different Dyes on Impregnated and on Plain Silica-Gel Plates

Solvent System-n butanol-benzene-ethylacetate (4C:35:25)

Dye	Colour	$R_f$	
		on plain Silicagel	Silicagel-ZnAc <sub>2</sub>
Acridine orange	Yellow	50	35
Alizarine blue	light pink	35 <sup>T</sup>	30
Auramine O	Lenion Yellow	52 <sup>T</sup>	26
Benzopurpurine 4B	light orange	52 <sup>T</sup>	48
Bismarck brown	orange	76 <sup>T</sup>	53
Bromocresol	Brown	74 <sup>ST</sup>	70
Bromophenol blue	Violet	44 <sup>ST</sup>	34
Bromothynol blue	Dark Yellow	83 <sup>T</sup>	74
Cadion 2B	Brown	95 <sup>T</sup>	94
Crystal Violet	Violet	40 <sup>T</sup>	21
Diamond blue	blue	78 <sup>ST</sup>	71
Dichlorofluorescein	orange	54 <sup>ST</sup>	12
Eosine Yellowish	light orange	50 <sup>T</sup>	42
Erichrome black-T	purple	32 <sup>T</sup>	15
Fuschsinacid	pink	22 <sup>ST</sup>	19
Methyl Red	Red	82 <sup>T</sup>	72
Methyl Violet	Violet	56 <sup>T</sup>	44
Naviline brilliant pink	Violet	96 <sup>T</sup>	90
Naviline Yellow	Pale yellow	97	92
Nigrosin	purple	0	0
Orange G	light orange	20 <sup>ST</sup>	17
Phenol red	Orange red	56 <sup>T</sup>	46
Rhodamine B	pink	56 <sup>T</sup>	04
Roseline hydrochloride	Reddish-pink	70 <sup>ST</sup>	38
Thymol blue	Orange yellow	76 <sup>T</sup>	67

ST = Slight Tailing

T = Tailing

of zinc acetate (100 ml). The coated plates were activated at  $60 \pm 1^\circ\text{C}$  for 24 hrs. 2-3  $\mu\text{l}$  of 0.1 percent alcoholic solution of the dyes was applied to the layers. Chromatograms were developed by ascending technique in a rectangular chamber at a temperature of  $30 \pm 2^\circ\text{C}$  using a mixture of n-butanol-benzene-ethylacetate (40:35:25) as developing system. After development, the dyes were visualized as such.

### RESULTS AND DISCUSSION

For the present studies best separations were obtained with 5% metal salt as impregnant. The most suitable impregnant among the zinc salts found was zinc acetate. n-butanol-benzene-ethylacetate (40:35:25) was found to be the best developing system. The  $hR_f$  values of different dyes in this developing system on 5% zinc acetate impregnated layers are recorded in Table-I along with the  $hR_f$  values on plain silica gel (for comparison).

The difference of  $\pm 3$  units in  $hR_f$  values has been taken as the criterion of satisfactory separation. Some of the typical separations depending upon the difference in  $hR_f$  values were then carried out (Table-II). It is apparent from Table-II that all the dyes can be better separated in three groups  $A_1$ ,  $A_2$  and  $A_3$ . Further it was observed that the  $hR_f$  values did not change when mixtures of dyes were applied.

TABLE - II

Separations of dyes in Mixture on  $\text{ZnAc}_2$  Impregnated plates  
Solvent System-n butanol-benzene-ethylacetate (40:35:25)

$A_1$  Group

Dyes	$hR_f$
Alizarine blue	30
Bismark brown	53
Crystal violet	21
Dichlorofluorescein	12
Eosine Yellowish	94
Methyl Red	72
Orange G	17
Phenol Red	46
Rasoaniline hydrochloride	38
Rhodamine	04
Thymol blue	67

(continued)

TABLE - II (continued)

A <sub>2</sub> Group	
Benzopurpurine 4B	48
Bromophenol blue	34
Cadion 2B	42
Diamond blue	71
Fuschsinacid	19
Navilin brilliant pink	90
Nigrosin	C
A <sub>3</sub> Group	
Acridine orange	35
Auromine O	26
Bromocresol purple	70
Bromothymol blue	74
Erichrome black T	15
Methyl violet	44
Naviline yellow	92

Furthermore, the decrease in  $hR_f$  values on impregnated layers (Table-I) suggested that the complexation between dyes and the metal ion should be an important factor in influencing the chromatographic behaviour of dyes on impregnated layers. Similar view was also put forward by Yasuda<sup>7-10</sup> for the ILC separation of aromatic amines on metal salt impregnated plates. However, this can not be the sole factor because the  $hR_f$  values on zinc acetate and zinc sulphate impregnated layers were found to be different indicating the involvement of anion in influencing the chromatographic behaviour.

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