

ringerten Menge, chromatographiert. Das Fließmittel ist in beiden Fällen dasselbe.

Der Zusatz von Rhodamin B zum jeweiligen Fluoreszenzindikator lässt, wie ersichtlich, die Flecken schärfer vom Fond hervorstechen. Das nachweisbare Minimum an Lipiden beträgt 0.5 bis 1.0  $\mu$ . Die Menge des zugesetzten Rhodamins B kann innerhalb der Grenzen 15 bis 40 mg je 1 g Fluoreszenzindikator schwanken, ohne dass der scharfe Kontrast der Flecken zum Fond beeinträchtigt wird. Werden diese Grenzen jedoch über- bzw. unterschritten, so bewirkt das eine wesentliche Einschränkung der Indikatorempfindlichkeit.

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### Thin layer chromatographic studies on some new nitrophenothiazines

Phenothiazines have been used extensively in medicine and industry as anti-helmintics, antihistamines, dyes and antioxidants. The rapid separation and identification of nuclear substituted phenothiazines thus merits further investigation.

Although a number of references deal with the thin layer chromatographic separation of 10-substituted phenothiazines<sup>1-8</sup>, there is no report in the literature with regard to the thin layer chromatography of nitrophenothiazines. In the present investigation we successfully applied this technique to the qualitative analysis of a number of nitrophenothiazines and nitrodiphenyl sulphides. Various solvent systems have been established that permit the detection and differentiation of these compounds.

The U.V. absorption maxima of the compounds are also reported, which may be of interest in their characterisation.

Nitrophenothiazines and diphenyl sulphides were obtained by treatment of reactive halogenonitro compounds with substituted *o*-aminothiophenols under alkaline conditions. Halogenonitrobenzenes having both positions *ortho* to the activated halogen atom substituted either by two nitro groups or by one nitro group and one halogen atom, provided the nitrophenothiazines directly. On the other hand, halogenonitrobenzenes having only one nitro group *ortho* to the activated halogen atom provided the diphenyl sulphides, which on formylation followed by SMILES rearrangement gave the respective nitrophenothiazines.

The plates (25 mm  $\times$  25 mm) were coated with Silica Gel G (10% CaSO<sub>4</sub>), and dried and activated in the usual manner.

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The plate was spotted with a micropipet 0.5 cm from one end with 1–2  $\mu$ l of a benzene solution containing 1–10  $\mu$ g/ml test solution. The plate was then placed in a chamber saturated with the solvent, and developed by the ascending method until the solvent front had travelled 10–15 cm. The solvent was allowed to evaporate, and the colour of the spots was observed before and after exposure to iodine vapour and their locations were compared with a reference chromatogram.

It was found that silica gel washed with chloroform gave much better and reproducible  $R_F$  values than when unwashed. In a benzene–hexane system, a higher benzene content tended to increase the  $R_F$  values and a higher hexane content to decrease the  $R_F$  values. It was also observed that 3-methyl-7-nitro-, 3-chloro-7-nitro-, and 2-chloro-7-nitro-phenothiazines had lower  $R_F$  values than the 1-nitro substituted phenothiazines which exhibit a six membered chelate of high stability through strong  $-N-H \cdots O-N-$  bonding between the hydrogen atom of the secondary amino group and the oxygen atom of the nitro group at position 1. The presence of the hydrogen bond in 1-nitrophenothiazines was also established by I.R. spectral studies. It was noted that benzene–acetone, benzene–methanol, acetone and various systems of methanol, with or without water, did not yield good separations.

The U.V. absorption maxima of nitrophenothiazines and nitrodiphenyl sulphides are also listed in Tables I and II. These data can be profitably used for the qualitative determination of such compounds with a minimum amount of the sample,

TABLE I

 $R_F$  VALUES AND U.V. CHARACTERISTICS OF SOME NEW NITROPHENOTHIAZINES

Phenothiazine	$R_F$ value ( $\times 100$ ) for solvent*				M.p. $^{\circ}C$	Spot colour	Absorption max. ( $m\mu$ )
	A	B	C	D			
1-Nitro-7-methyl-	75	67	50	40	141	violet	250, 315
1-Nitro-7-bromo-	72	64	48	35	219	violet	250, 315
1-Nitro-7-chloro-	78	68	52	42	217	light violet	250, 315
1,3-Dinitro-7-chloro-	81	72	58	45	217	red-violet	240, 300
1,3-Dinitro-8-chloro-	75	64	53	41	265	red-violet	240, 296
1,3-Dinitro-7-bromo-	70	59	50	37	210	red-violet	242, 299
1,3-Dinitro-7-methoxy-	83	73	62	53	203	violet	241, 300
1,3-Dinitro-7-methyl-	79	67	55	48	223	violet	240, 300
1-Nitro-3-chloro-7-bromo-	88	78	62	58	261	violet	251, 318
1-Nitro-3-chloro-7-methyl-	80	69	53	47	208	violet	250, 315
1-Nitro-3,7-dichloro-	70	58	42	39	203	violet	250, 315
1-Nitro-3,8-dichloro-	63	50	35	30	303	violet	255, 315
1-Nitro-3-chloro-	77	64	52	45	250	violet	250, 313
1,3-Dinitro-	69	58	43	35	148	violet	240, 295
3-Chloro-7-nitro-	47	35	25	16	268	pink	250, 285
2-Chloro-7-nitro-	44	33	22	14	244	yellow	245, 305
3-Methyl-7-nitro-	42	31	19	10	232	pink	246, 310
Phenothiazine	73	65	52	(41)	182	green**	253, 320

\* Solvent systems: A = benzene; B = benzene–hexane, 70:30; C = benzene–hexane, 50:50; D = pyridine–hexane, 10:90.

\*\* Turns green after exposure to iodine vapours.

TABLE II

 $R_F$  VALUES AND U.V. CHARACTERISTICS OF SOME NEW NITRODIPHENYL SULPHIDES

Diphenyl sulphide	$R_F$ values ( $\times 100$ ) for solvent*				M.p. $^{\circ}\text{C}$	Spot colour	Absorption max. ( $m\mu$ )
	A	B	C	D			
2-Amino-5-chloro-2',4'-dinitro-	46	33	25	16	168	yellow	244, 320, 394
2-Amino-4-chloro-2',4'-dinitro-	52	39	30	21	186	yellow	245, 320, 395
2-Amino-5-methyl-2',4'-dinitro-	49	35	27	17	166	yellow	245, 320, 394

\* Solvent systems: A = benzene; B = benzene-hexane, 70:30; C = benzene-hexane, 50:50; D = pyridine-hexane, 10:90.

because their spectra are quite distinct and the intense absorptions are detectable even at a very low concentration (0.0080 to 0.0165 mg/ml). All the phenothiazines exhibited two sharp peaks in the ranges of 240–255  $m\mu$  and 285–318  $m\mu$ . The sulphides exhibited a sharp peak in the visible region at 395  $m\mu$  in addition to two sharp bands in the U.V. region at 245  $m\mu$  and 320  $m\mu$ .

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