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Thin-layer chromatographic analysis of some phenothiazine sulphones

The direct nitration of phenothiazine with nitric acid is associated with the oxidation of the sulphur atom, since nitric acid in most concentrations is not only a nitrant but also an oxidant[1]. In our previous publications[3–5], we reported the synthesis via the Smiles rearrangement and the thin-layer chromatography (TLC) on silica gel[2–5] of some nitrophenothiazines. In this paper the sulphones have been prepared by oxidising the phenothiazines using $KMnO_4$ or 30% H_2O_2 in glacial acetic acid. In the former case both sulphoxides and sulphones were obtained, making their purification difficult. Oxidation of the phenothiazines using excess 30% H_2O_2 in acetone or an aqueous solution gave only a low yield of the sulphones. However, by carrying out the oxidation using excess 30% H_2O_2 in glacial acetic acid, sulphones were obtained in almost quantitative yields.

Although the literature is replete with publications on thin-layer chromatographic analysis of phenothiazines[6–11], to date there has been no attempt to organise TLC studies of sulphones of this class. In the present investigation, a method is described for the separation and detection of sulphones in submicrogram quantities on thin layers of silica gel using nonaqueous developing solvent systems. Considering the minimum amount of sulphone that can be detected when chromatographed together with large quantities of phenothiazine, we found that in a 100-μg sample of phenothiazine, 0.1 μg of sulphone could be made visible.

Experimental

Apparatus and reagents. Standard thin-layer chromatographic equipment (plates, atomizers, applicator with a fixed thickness and developing tanks) was obtained from M/s Adair Dutt and Co. (New Delhi). Carbon tetrachloride, methanol and acetone were of analytical grade, while benzene and *n*-hexane were dried over sodium wire. Silica Gel G was from Merck. Phenothiazine was purified by sublimation under reduced pressure.

Preparation of phenothiazine sulphones. The sulphones listed in Table II were prepared by the following procedure.

To a 0.02 *M* solution of phenothiazine in 100 ml of glacial acetic acid were added 5 ml of 30% H_2O_2 at room temperature. After heating at 60–70° for 15 min, the colour of the solution turned yellow. At this time another 5 ml of 30% H_2O_2 were added, and the solution was heated and refluxed for 2–4 h. A major portion of the solvent was then removed by distillation under reduced pressure, and the solution was poured into cold water. The yellow residue which separated was filtered and crystallised from alcohol to give the desired product.

Chromatographic procedure. Five 20 × 20 cm glass plates were coated (0.25 mm thick) with a well-stirred mixture of 30 g of Silica Gel G and 60 ml of distilled water. The plates were air dried and activated by heating in an oven at 120° for 1 h. The substances were dissolved in acetone, and aliquots of the solutions containing the mixture of the phenothiazine and related sulphone were spotted at 1-cm intervals on the starting line of the chromatogram. The solutions were added in increments by means of a micropipette so as to keep the spot size less than 3 mm in diameter.

The plates were then placed in developing tanks to which 120 ml of solvent had been added at least 1 h prior to use. The ends of the tanks were lined with strips of filter paper freshly saturated with the solvent, and the lids of the tanks were sealed with vacuum grease. Development was allowed to proceed until the solvent front had risen 10–12 cm beyond the original spots. The plates were removed from the tanks and, after marking the solvent front, were allowed to dry in air. The spots were visible in daylight. Phenothiazine itself was located as a green spot by exposing it to iodine vapours. Standard R_F data for phenothiazines and their sulphones were obtained using this procedure for all three solvent systems. Phenothiazine was included on every plate as a standard so that the relative R_F values could be calculated.

Results and discussion

Many solvent combinations were examined for developing the chromatograms on silica gel; the best of these were found to be: (A) benzene–*n*-hexane (80:20); (B) carbon tetrachloride–acetone (90:10); (C) Carbon tetrachloride–methanol (95:5). It was noted that carbon tetrachloride, hexane, acetone and various systems of methanol with or without water did not yield good separations. This was due either to tailing of the spots or to the lower R_F values obtained that resulted in little or no separation. It was particularly noticeable that the phenothiazine sulphones had lower R_F values than the corresponding phenothiazines in all the solvent systems studied. It was also observed that phenothiazine sulphone had lower R_F values than the 1-nitro-substituted phenothiazine sulphones which exhibited a six-membered chelate of high stability through strong N–H---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 IR spectral studies. The spectra of the phenothiazine sulphone exhibited a sharp N–H stretching peak near 3450 cm^{-1}, indicating a free N–H group, while the corresponding band in the spectra of 1-nitro derivatives is observed at 3270–3350 cm^{-1}, suggesting mainly O–H---N intramolecular hydrogen bonding.

It is difficult to obtain reproducible R_F values in TLC[12] when working with activated layers and nonaqueous solvent systems. For this reason phenothiazine was selected as a standard and spotted with the other compounds on all plates in a series. The mean R_F of phenothiazine was calculated from the values obtained on many different plates; this mean value was regarded as fixed. The difference between the R_F value of phenothiazine observed on an arbitrary plate and the fixed value was used as a correction factor. The correction factor which could be positive or negative was added to the R_F values of the compounds on that plate. In this manner corrected R_F values were obtained with a reproducibility of ± 0.02 R_F units*. The R_F values given in Tables I and II are the mean of at least six corrected R_F values obtained on different plates.

Solvent B had to be changed after developing every four plates since R_F values were lower on subsequent plates. This change in R_F values is probably caused by selective evaporation of acetone. The developing tanks containing solvents A and C were used satisfactorily for 10 plates. Because the R_F values of many of the sulphones are similar in each system, the use of an R_F value as an aid in identification was

* Editor's note: Correction factors should be calculated from R_M values rather than from R_F values.

TABLE I

THIN-LAYER CHROMATOGRAPHIC DATA FOR PHENOTHIAZINES

Phenothiazine	*System A*		*System B*		*System C*		*M.P.* (°C)	*Spot colour*
	R_F	*Rel.* R_F	R_F	*Rel.* R_F	R_F	*Rel.* R_F		
1,3-Dinitro-	0.61	0.88	0.68	1.04	0.64	1.10	148	violet
1,3-Dinitro-7-chloro-	0.78	1.13	0.65	1.00	0.56	0.96	217	red-violet
1,3-Dinitro-8-chloro-	0.70	1.01	0.80	1.23	0.84	1.44	265	red-violet
1,3-Dinitro-7-methyl-	0.72	1.04	0.56	0.86	0.46	0.79	223	violet
1-Nitro-3-chloro-	0.74	1.07	0.65	1.00	0.56	0.96	250	violet
1-Nitro-3,7-dichloro-	0.66	0.95	0.83	1.27	0.80	1.37	203	violet
1-Nitro-3-chloro-7-bromo-	0.80	1.15	0.72	1.10	0.59	1.01	261	violet
1-Nitro-3-chloro-7-methyl-	0.77	1.11	0.60	0.92	0.55	0.94	208	violet
1-Nitro-7-chloro-	0.72	1.04	0.62	0.95	0.57	0.98	217	light violet
1-Nitro-7-bromo-	0.64	0.92	0.60	0.92	0.50	0.86	219	violet
1-Nitro-7-methyl-	0.75	1.08	0.57	0.87	0.52	0.89	141	violet
1-Nitro-2,4-dibromo-7-methyl-	0.58	0.84	0.65	1.00	0.60	1.03	102	violet
3-Nitro-	0.26	0.37	0.48	0.73	0.39	0.67	218	violet
3,7-Dinitro-	0.19	0.27	0.43	0.66	0.33	0.56	276	violet
Phenothiazine	0.69	1.00	0.65	1.00	0.58	1.00	182	green[a]

[a] Turns green after exposure to iodine vapours.

TABLE II

THIN-LAYER CHROMATOGRAPHIC DATA FOR PHENOTHIAZINE SULPHONES

Phenothiazine sulphone	*System A*		*System B*		*System C*		*M.P.* (°C)	*Spot colour*
	R_F	*Rel.* R_F	R_F	*Rel.* R_F	R_F	*Rel.* R_F		
1,3-Dinitro-	0.49	0.71	0.56	0.86	0.51	0.87	>360	red
1,3-Dinitro-7-chloro-	0.63	0.91	0.49	0.75	0.44	0.75	312	yellow
1,3-Dinitro-8-chloro-	0.54	0.78	0.66	1.01	0.72	1.24	>360	buff
1,3-Dinitro-7-methyl-	0.50	0.72	0.44	0.67	0.36	0.62	>360	yellow
1-Nitro-3-chloro-	0.48	0.69	0.47	0.72	0.45	0.77	>360	yellow
1-Nitro-3,7-dichloro-	0.46	0.66	0.73	1.12	0.67	1.15	>360	buff
1-Nitro-3-chloro-7-bromo-	0.68	0.98	0.68	1.04	0.43	0.74	>360	greenish yellow
1-Nitro-3-chloro-7-methyl-	0.57	0.82	0.51	0.78	0.44	0.75	275	orange
1-Nitro-7-chloro-	0.54	0.78	0.48	0.73	0.40	0.68	303	yellow
1-Nitro-7-bromo-	0.43	0.62	0.49	0.75	0.34	0.58	295	greenish yellow
1-Nitro-7-methyl-	0.59	0.85	0.43	0.66	0.37	0.63	258	yellow
1-Nitro-2,4-dibromo-7-methyl-	0.37	0.53	0.47	0.72	0.41	0.70	233	brown
3-Nitro-	0.17	0.24	0.33	0.50	0.21	0.36	347	brown
3,7-Dinitro-	0.12	0.17	0.36	0.55	0.29	0.50	>360	yellow
Phenothiazine sulphone	0.23	0.33	0.34	0.52	0.25	0.43	258	dirty yellow
Phenothiazine	0.69	1.00	0.65	1.00	0.58	1.00	182	green[a]

[a] Turns green after exposure to iodine vapours.

dependent on its reproducibility. The most crucial factor in attaining reproducibility was securing a constant degree of saturation in the developing tanks prior to inserting the plates. The described procedure has proved satisfactory.

All R_F values reported in Tables I and II were obtained on plates conditioned and developed as described in the procedure. Plates stored in the laboratory and developed at 22–28° have not produced reproducible R_F values. The range of R_F values obtained from the six plates used for the data in Tables I and II were compared with those from six plates developed in unsaturated tanks. It was noted that plates developed in unsaturated tanks did not give reproducible R_F values, whereas plates developed in saturated tanks gave values with the normally accepted tolerance ($\pm$0.02 R_F units) for work on TLC.

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