

Thin layer chromatography of polynitrophenols, nitrosophenols, nitrohydroquinones and related compounds

Polynitrophenols, nitrosophenols, nitrohydroquinones and their esters are useful in the synthesis of high-energy fuels for rockets and also for explosives. Their quick separation and microestimation will be of advantage in the study of the course of chemical reactions during the synthesis of new explosives, for quality control in their manufacture and storage. TLC of phenols, mononitrophenols and hydroquinones has been carried out by different workers¹⁻¹⁷. The colorimetric estimation of some phenols and hydroquinone on TLC using *p*-nitrobenzenediazonium fluoroborate has been reported by SEEBOTH AND GORSCH¹⁸. LIPINA¹⁹ employed diazotized *p*-nitroaniline for the quantitative estimation of some phenols.

In the present work a few polynitrophenols and nitrosophenols, nitrohydroquinones and some of their esters were studied employing thin-layer chromatography. The suitability of various adsorbents for the separation of these compounds is compared. 2,4,6-Trinitroresorcinol, α -nitroso- β -naphthol, β -nitroso- α -naphthol, 2,4-dinitrophenol, 2,4,6-trinitrophenol, nitrosothymol and trinitrothymol were estimated colorimetrically by employing diazotised sulphanic acid as a reagent²⁰. 2,6-Dinitrohydroquinone was estimated by the method of BELYAKOV AND KOMIS²¹ for hydroquinone, employing phloroglucinol and sodium carbonate. The individual compounds after separation and elution were estimated colorimetrically.

Methods and materials

All solvents used were dry and freshly distilled. The temperature of irrigation was $25 \pm 2^\circ$; unless otherwise specified. Kodak photographic glass plates (22 × 22 cm) were used. The distance travelled by the solvent front in each case was 18 cm. A Hilger Spekkar Absorptiometer was employed for the colorimetric measurements.

Adsorbents

1. Silica gel G (200 mesh, B.S.S.).
2. Cellulose powder, ashless (Whatman, 100 mesh, B.S.S.), containing 5 % calcium sulphate (200 mesh).
3. Cellulose acetate, containing 5 % calcium sulphate (200 mesh, B.S.S.). Cellulose acetate was prepared by treating ashless cellulose powder (Whatman) with acetic anhydride for 3 h at room temperature, washing with distilled water, drying at 100° (30 h) and passing through a 200 mesh sieve.
4. Neutral alumina, containing 20 % calcium sulphate (200 mesh, B.S.S.). Neutral alumina was prepared from Brockman alumina by REICHSTEIN AND SHOPPEE'S²² method.
5. Neutral alumina (M. Woelm, 200 mesh, B.S.S.), containing 20 % calcium sulphate (200 mesh).
6. Acidic alumina (M. Woelm, 200 mesh, B.S.S.), containing 20 % calcium sulphate (200 mesh).
7. Amberlite ARI/400/Cl/AR (200 mesh, B.S.S.).
8. Amberlite IR/45/OH/AR (200 mesh, B.S.S.).

Compounds

2-Nitrohydroquinone-4-benzoate (m.p. 96°) was prepared from hydroquinone monobenzoate according to the method of KEHRMANN, SANDOZ AND MONNIER²³. 2,6-Dinitrohydroquinone-4-acetate (m.p. 98°) was prepared from hydroquinone diacetate by following the procedure of NIETZKI²⁴. 2,6-Dinitrohydroquinone (m.p. 136°) was prepared by the hydrolysis of 2,6-dinitrohydroquinone-4-acetate by the procedure of PRIDEAUX AND NUNN²⁵. Nitranilic acid (86–87°, decomposition) was prepared by TOWN'S method²⁶. 2,3,6-Trinitrothymol (m.p. 110°) was prepared by nitration of thymol²⁷, and nitrosothymol (m.p. 163–164°) by following the method of KREMERS, WAKEMAN AND HIXON²⁸. Other phenols were crystallised twice from ethanol. *p*-Toluenesulphonates of 2,6-dinitrohydroquinone, nitrosothymol, and *ortho*-, *meta*-, and *para*-nitrophenols were prepared by heating together nitrohydroquinone or the respective phenol (1 mole), *p*-toluenesulphonyl chloride (1.25 mole) and pyridine (3 mole) in a boiling tube on a water bath for 1.5 h. The mixture was poured into iced water and the resultant crystalline mass was filtered, washed with 5% sodium hydroxide followed by water and repeatedly crystallised from ethyl alcohol.

In this way the following compounds were obtained: 2,6-dinitrohydroquinone-4-*p*-toluenesulphonate (m.p. 194–195°; C₁₃H₁₀O₇N₂S; S = 9.32% found, 9.46% calculated), nitrosothymol-*p*-toluenesulphonate (m.p. 87–88°; C₁₇H₁₉O₄NS; S = 9.53% found, 9.60% calculated), *o*-nitrophenol-*p*-toluenesulphonate (m.p. 83°; C₁₃H₁₁O₅NS; S = 10.81% found, 10.92% calculated), *m*-nitrophenol-*p*-toluenesulphonate (m.p. 113°; C₁₃H₁₁O₅NS; S = 10.75% found, 10.92% calculated), and *p*-nitrophenol-*p*-toluenesulphonate (m.p. 97°; C₁₃H₁₁O₅NS; S = 10.88% found, 10.92% calculated).

Preparation of thin layer plates, application of the compounds and irrigation of the plates

A slurry of the adsorbent in a suitable solvent was prepared and poured on to the glass plates. By tilting the plates from side to side a uniformly thin coating was obtained. The plates were left at room temperature (25 ± 2°) overnight and activated or dried at the appropriate temperature in an oven. The plates were weighed before

TABLE I
DETAILS OF TLC COATINGS AND THEIR ACTIVATION

Coating	Wt. of adsorbent (g)	Solvent	Activation temperature (°C) and time (h)	Average coating of adsorbent (mg/cm ²)
Neutral silica gel G	30	Chloroform-methanol (2:1), 100 c.c.	110, 0.5	7.7
Buffered silica gel G	30	0.5 N Oxalic acid, 20 c.c., and methanol, 70 c.c.	110, 2	7.0
Cellulose-CaSO ₄	26.25	Water, 100 c.c.	110, 1	7.6
Cellulose acetate-CaSO ₄	26.25	Water, 100 c.c.	105, 1	7.4
Neutral/acid alumina-CaSO ₄	30	Water, 60 c.c.	180, 0.5	10.7
Amberlite ARI/400/Cl/AR	25	Ethyl acetate, 100 c.c.	60, 1	4.8
Amberlite IR/45/OH/AR	25	Ethyl acetate, 100 c.c.	60, 1	4.8

spotting and the average coating of the adsorbent noted. Table I gives the quantities of adsorbent for five plates (22 × 22 cm) and other details.

An ethanolic solution of each sample (5 μ l containing 5 μ g) was applied on the chromatoplates with a standard capillary. Except when ion exchange resins were used, chromatograms were irrigated by ascending technique.

In the case of Amberlite resins, the plates were kept slanting at 30° angle, solvent was fed through cotton and the descending technique employed. The chromatoplates after irrigation were either viewed under U.V. light or sprayed with a chromogenic reagent (see below under Detection). Table II gives the R_F values of the various compounds.

Solvent systems

- A. Chloroform
- B. Benzene
- C. Chloroform-xylene (4:1)

TABLE II
 R_F VALUES OF THE VARIOUS COMPOUNDS IN VARIOUS SOLVENT SYSTEMS AND ON VARIOUS ADSORBENTS

Adsorbents		Silica gel G				Buffered silica gel G				Cellulose-CaSO ₄			
Sample No.	Compound	A*	B	C	D	A	B	C	D	E	F	G	H
1	2-Nitrohydroquinone-4-benzoate	0.85	0.91	0.74	0.68	0.91	0.92	0.64	0.64	0.37	0.53	0.81	0.49
2	2,6-Dinitrohydroquinone-4-acetate	0.68	0.37	0.51	0.16	0.35	0.43	0.32	0.27	0.31	0.40	0.72	0.94
3	2,6-Dinitrohydroquinone	0.48	0.00	0.13	0.06	0.33	0.27	0.07	0.05	0.23	0.36	0.64	0.75
4	2,4,6-Trinitroresorcinol	0.10	0.34	0.20	0.05	0.69	0.55	0.28	0.28	0.36	0.18	0.33	0.81
5	α -Nitroso- β -naphthol	0.85	0.52	0.53	0.27	0.92	0.41	0.32	0.29	0.95	0.89	0.77	0.48
6	β -Nitroso- α -naphthol	—	—	0.20	0.04	0.48	0.15	0.13	0.61	0.81	0.95	0.84	0.51
7	4-(<i>p</i> -Nitrosophenylazo)-resorcinol	0.00	0.04	0.14	0.08	0.33	0.06	0.31	0.04	0.92	0.95	0.78	0.04
8	2,4-Dinitrophenol	0.85	0.86	0.62	0.48	0.94	0.98	0.56	0.53	0.79	0.74	0.77	0.76
9	Nitranilic acid	—	—	—	—	—	—	—	—	—	—	—	—
10	Nitrosothymol	0.56	0.08	0.22	0.16	0.38	0.15	0.13	0.10	0.91	0.91	0.92	0.53
11	Trinitrothymol	0.28	0.15	0.16	0.06	0.93	0.58	0.49	0.54	0.87	0.91	0.89	0.71
12	Picramic acid	0.49	0.33	0.37	0.18	0.65	0.24	0.18	0.16	0.67	0.64	0.75	0.61
13	2,4,6-Trinitrophenol	0.00	0.19	0.03	0.11	0.97	0.09	0.41	0.10	0.96	0.80	0.86	0.82
14	2,6-Dinitrohydroquinone-4- <i>p</i> -toluenesulphonate	0.98	0.80	0.00	0.03	1.00	0.71	—	0.40	0.98	0.94	0.89	0.84
15	Nitrosothymol- <i>p</i> -toluenesulphonate	0.99	0.56	0.59	0.30	1.00	0.40	0.41	0.61	0.98	0.95	0.92	0.00
16	<i>o</i> -Nitrophenol- <i>p</i> -toluenesulphonate	0.97	0.77	0.56	0.36	1.00	0.67	0.45	0.41	0.98	0.94	0.92	0.00
17	<i>m</i> -Nitrophenol- <i>p</i> -toluenesulphonate	0.99	0.84	0.63	0.44	1.00	0.75	0.52	0.52	0.97	0.93	0.91	0.00
18	<i>p</i> -Nitrophenol- <i>p</i> -toluenesulphonate	0.98	0.89	0.67	0.50	1.00	0.83	0.57	0.52	0.87	0.53	0.91	0.00

* For key to solvents, see text.

- D. Chloroform-xylene (1:1)
 E. *n*-Butanol saturated with 5 *N* ammonium hydroxide
 F. *n*-Butanol-3 *N* ammonium carbonate-3 *N* ammonium hydroxide (4:3:3)
 G. *n*-Butanol-ethanol-3 *N* ammonium carbonate (40:11:19)
 H. 3 % sodium chloride solution
 I. *n*-Butanol-water (86:14)
 J. *n*-Butanol saturated with water
 K. Ethanol-water (2:3)
 L. Acetone-water (2:3)
 M. Petroleum ether (b.p. 40-60°)-ether (1:1) (irrigation was done at 0°).
 N. *n*-Butanol-water-acetic acid (6:2:1).
 O. Cyclohexane-ethyl acetate-acetic acid (5:1:1).

Detection

The spots could be detected by: (1) use of an ultraviolet lamp "Chromatolite"

<i>Cellulose acetate-CaSO₄</i>				<i>Alumina (neutral)-CaSO₄</i>			<i>Alumina (neutral)-CaSO₄</i>			<i>Alumina (acidic)-CaSO₄</i>			<i>Amberlite ARI/400/-Cl/AR</i>	<i>Amberlite 45/OH/AR</i>
<i>I</i>	<i>J</i>	<i>K</i>	<i>L</i>	<i>M</i>	<i>B</i>	<i>C</i>	<i>M</i>	<i>B</i>	<i>C</i>	<i>M</i>	<i>B</i>	<i>C</i>	<i>N</i>	<i>O</i>
0.74	0.91	0.79	0.91	0.00	0.11	0.00	0.32	0.03	0.16	0.42	0.13	0.46	0.02	0.08
0.86	0.63	0.88	0.93	0.07	0.00	0.05	0.02	0.00	0.00	0.15	0.00	0.00	0.04	0.00
0.83	0.61	0.86	0.91	0.06	0.00	0.05	0.02	0.00	0.00	0.20	0.00	0.00	0.23	0.00
0.64	0.48	0.91	0.94	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.83	0.87	0.79	0.73	0.06	0.00	0.06	0.13	0.00	0.06	0.11	0.06	0.05	0.37	0.00
0.87	0.96	0.71	0.85	0.05	0.00	0.00	0.02	0.00	0.00	0.10	0.00	0.05	0.42	0.00
0.86	0.89	0.56	0.46	0.04	0.00	0.29	0.00	0.00	0.08	0.09	0.00	0.00	0.42	0.00
0.82	0.74	0.91	0.91	0.02	0.06	0.06	0.03	0.00	0.00	0.04	0.04	0.06	0.16	0.00
—	—	0.97	0.55	0.00	0.00	0.00	0.03	0.00	0.00	—	0.06	0.00	—	—
0.92	0.91	0.89	0.74	0.75	0.16	0.22	0.77	0.04	0.18	0.86	0.09	0.30	0.59	0.00
0.66	0.70	0.92	0.91	0.00	0.00	0.93	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.12
0.84	0.72	0.83	0.83	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00
0.71	0.74	0.86	0.84	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06
0.79	0.90	0.84	0.88	0.00	0.00	0.00	0.00	0.00	0.16	0.00	0.00	0.00	0.62	0.13
0.87	0.96	0.60	0.60	0.68	0.70	0.86	0.92	0.72	0.72	0.53	0.57	0.86	1.00	0.00
0.87	0.95	0.78	0.68	0.69	0.69	0.87	0.82	0.76	0.68	0.48	0.58	0.78	0.71	0.00
0.87	0.91	0.78	0.65	0.81	0.79	0.93	0.88	0.84	0.72	0.63	0.67	0.80	0.85	0.00
0.87	0.86	0.78	0.56	0.88	0.79	0.93	0.96	0.85	0.78	0.86	0.68	0.83	—	0.37

for short-wave (2537 Å) radiation, which revealed all the compounds; (2) spraying the plates with 2 % phosphomolybdic acid solution and then treating with ammonia vapour; and (3) spraying with sulphanilic acid reagent (solution A, followed by solution B). Solution A is an 0.5 % solution of sulphanilic acid in *N* HCl and solution B a 5 % solution of sodium nitrite.

Estimation of phenols

Cellulose-CaSO₄ plates were used in all cases. The spotting as well as the irrigation in the case of nitrosothymol and trinitrothymol were carried out at $0 \pm 2^\circ$. A number of spots of each compound (5 to 20 μ l) having different amounts were spotted and irrigated with *n*-butanol-3 *N* ammonium hydroxide-3 *N* ammonium carbonate (4:3:3). After observing the spots under U.V. light they were scraped off, extracted with 50 % ethanol (5 c.c., 1 h) and filtered. At 0° 2 c.c. of 0.2 % sulphanilic acid containing 0.7 c.c. concentrated hydrochloric acid, 1 c.c. of 0.2 % sodium nitrite solution, and 2.5 c.c. of 0.25 % sodium hydroxide solution were added to the filtrate. Before addition all solutions were kept at 0° , and the mixture also maintained at the same temperature. After 3 min the colour intensities were read in the colorimeter using a suitable filter (see Table III) and compared with the standard curves previously plotted for the individual compounds.

Estimation of 2,6-dinitrohydroquinone

2,6-Dinitrohydroquinone, after spotting, irrigation and detection under U.V., as in the case of phenols, was extracted from the adsorbent with 50 % ethanol (5 c.c., 1 h). The extract was filtered, and the filtrate added to a mixture of 0.05 % phloroglucinol (2 c.c.) and 1.5 % sodium carbonate (4 c.c.). After leaving the solution for 1 h the colour intensities (percentage transmission) were noted using the appropriate filter (see Table III).

TABLE III

LIMITS OF ESTIMATIONS OF THE COMPOUNDS AND THE FILTERS EMPLOYED

Sample No.	Compound	Filter ($m\mu$)	Lower limit of estimation (μ g)
1	2,6-Dinitrohydroquinone	540	5
2	2,4,6-Trinitroresorcinol	425	3.3
3	α -Nitroso- β -naphthol	425	10
4	β -Nitroso- α -naphthol	425	3.3
5	2,4-Dinitrophenol	425	5
6	2,4,6-Trinitrophenol	425	5.5
7	Nitrosothymol	425	6
8	Trinitrothymol	425	16

Discussion

The technique of coating the glass plates with various adsorbents by pouring a known volume of the slurry on to the plates and then tilting them from side to side produced thinner coatings compared to other conventional methods. It was observed that the thinner the coating, the quicker and better is the resolution of the com-

pounds. The coatings of Amberlite resins were found to crack if dried for periods longer than 1 h at 60°.

Buffering of silica gel G with 0.5 N oxalic acid had a marked effect on the R_F values of some of the polynitrophenols, *viz.* 2,4,6-trinitroresorcinol, 2,4-dinitrophenol, trinitrothymol, and 2,4,6-trinitrophenol.

On cellulose and cellulose acetate plates the use of 2% starch instead of calcium sulphate as binder resulted in major tailing of the compounds.

A reversed phase system using cellulose acetate-calcium sulphate produced better resolutions compared to cellulose-calcium sulphate in the case of nitrohydroquinone and its esters, 2,4,6-trinitroresorcinol, 2,4-dinitrophenol, picramic acid and *p*-toluenesulphonates.

Neutral alumina-calcium sulphate coatings in general gave low R_F values compared to silica gel G. However, alumina was found to be the best adsorbent for the resolution of *p*-toluene sulphonates. In general, the R_F values of phenols on acid alumina (Woelm) were higher than on neutral alumina (Woelm). On the other hand, acid alumina (Woelm) lowered the R_F values of *p*-toluene sulphonates compared to neutral alumina (Woelm).

Basic ion exchange resins had a powerful binding effect on polynitrophenols.

In view of their slow vaporisation from chromatoplates the spotting, irrigation and elution of nitrothymol and trinitrothymol had to be carried out at low temperature ($0 \pm 2^\circ$).

In the estimation of phenols with sulphanilic acid reagent, a time of 3 min gave maximum colour development and was rigidly adhered to; longer periods produced erroneous results. All the compounds were found to observe linearity in their colours developed with the chromogenic reagent with respect to their weights. The lower limits of detection are given in Table III.

Acknowledgements

Thanks are due to Dr. KARTAR SINGH, Director, Defence Science Laboratory, Delhi and Dr. V. RANGANATHAN, Deputy Chief Scientist, Research and Development Organisation, Ministry of Defence, New Delhi, for their encouragement.

Defence Science Laboratory, Metcalfe House,
Delhi (India)

D. B. PARIHAR
S. P. SHARMA
K. C. TEWARI

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Received February 22nd, 1966

J. Chromatog., 24 (1966) 230-236

A method for quantitative thin-layer chromatography

In this communication, a convenient method is described for the determination of substances on thin-layer chromatograms by means of a gas-chromatographic technique of elementary analysis.

Fig. 1 shows a sketch of an apparatus consisting of a combustion furnace and gas chromatograph. After separation of a compound containing nitrogen from a sample of known volume by thin-layer chromatography, the spot is scraped off with the adsorbent (aluminum oxide or silica gel) into a platinum boat. As soon as the

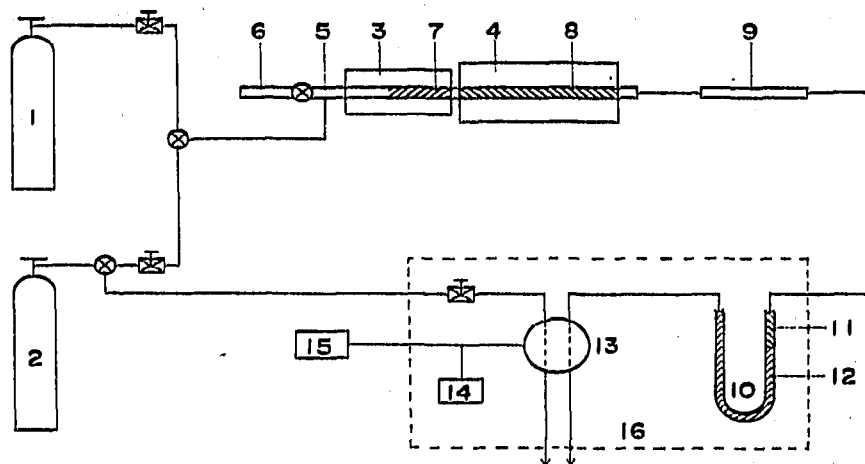


Fig. 1. Outline of apparatus. 1 = He containing 2% O₂; 2 = He; 3 = electric furnace I (900°); 4 = electric furnace II (500°); 5 = combustion tube; 6 = preparation chamber; 7 = cupric oxide; 8 = reduced copper; 9 = anhydron; 10 = U-type column (ø 4 mm × 75 cm); 11 = MnO₂; 12 = activated carbon; 13 = T.C. detector; 14 = recorder; 15 = electronic integrator; 16 = gas chromatograph. Gas-chromatographic conditions: He flow rate, 30 ml/min; column temperature, 100°; detector temperature, 100°.

J. Chromatog., 24 (1966) 236-238