

Indian ink and sodium chloride in the mixture with the sulphophthalein, is about 1.56, which indicates adsorption of this dye on Sephadex. The separation of iodophthalein from sodium chloride is quoted as an example.

The initial weight of the mixture was 79.8 mg. The sharp separation during the movement through the column is seen in Fig. 1. The total amount of chloride found by titration with 0.01 *N* AgNO₃ was 43.36 mg; the content of pure dye measured photometrically was found to be 36.1 mg. The extinction value of the dye solution, concentration 1 mg/100 ml 0.1 *N* NaOH, was 0.247 before the treatment and after chromatography 0.545 (measured on Unicam SP 500 in 10 mm cuvettes (580 m μ)). Extinction of chlorosulphophthalein was 0.69 before desalting and 0.975 afterwards, and of bromosulphophthalein 0.40 before desalting and 0.82 afterwards.

Discussion

Our findings showed the possibility of using Sephadex G 25 for the removal of salt from some aromatic dyes. Since the adsorption of some dyes, particularly the phthaleins on ion exchange resins, is so strong that they cannot be eluted, the use of ion exchange resin can sometimes be disadvantageous; this phenomenon has been used e.g. for labelling Dowex with phenolphthalein in the chromatography of acids⁵. It is possible that Sephadex can be used for the desalting of other aromatic compounds.

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Chromatographic separation and quantitative estimation of isomeric dinitrobenzenes

In the present work we were interested in examining the possibilities of applying TLC to the separation of isomeric dinitrobenzenes. At the same time an attempt was made to develop a method for the quantitative estimation of these isomers in a mixture, which might be of interest for the explosives industry.

FRANC¹ in 1955, as well as PRAY AND KABIL² in 1956, succeeded in separating only *ortho*- from *meta*-dinitrobenzene by reversed phase paper chromatography, using an acidic solvent system. ETEL, POSPIŠIL AND DEYL³, in a very comprehensive study of the chromatographic behaviour of nitro compounds, found, however, that

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the results of PRAY AND KABIL were not reproducible. On the other hand, MARCINKIEWICZ AND GREEN⁴ concluded, on the basis of many experiments, that *meta*- and *para*-dinitrobenzene were chromatographically indistinguishable in normal and reversed phase paper chromatography when a partition system consisting of two neutral phases was used.

Nevertheless, we found that by TLC all three dinitrobenzenes can be readily separated, even in a neutral partition system such as benzene-ethylacetate (Table I).

TABLE I

 R_F VALUES OF DINITROBENZENES IN MIXTURES

Compound	$R_F \times 100$
<i>ortho</i> -Dinitrobenzene	64
<i>meta</i> -Dinitrobenzene	72
<i>para</i> -Dinitrobenzene	78

The separation was effected in 40 min and the isomers were visible under U.V. light as dark spots.

For quantitative estimation the spots were scraped off the plate, the scrapings centrifuged with an appropriate solvent, and the resulting supernatants measured spectrophotometrically.

The wavelengths selected for the quantitative determination were: 228 $m\mu$ ($\epsilon = 10.5 \pm 0.7 \cdot 10^3$) in methylene chloride for *ortho*-, 240 $m\mu$ ($\epsilon = 16.3 \pm 0.3 \cdot 10^3$) in chloroform for *meta*-, and 262 $m\mu$ ($\epsilon = 13.3 \pm 0.6 \cdot 10^3$) in chloroform for *para*-dinitrobenzene. The *ortho*-isomer was estimated in methylene chloride on the slope of the absorption curve—in chloroform this isomer has only a slight shoulder at 333 $m\mu$ where the optical densities for the small quantities used were insignificant.

Calibration curves, one for each isomer, were prepared by chromatographing known quantities of the respective isomer, scraping off the spots located by means of U.V. light, and by subsequent treatment as described. From the graphs relating

TABLE II

ANALYSIS OF SYNTHETIC MIXTURE OF DINITROBENZENES

Ratio in mixture	Amount of isomers present ($\mu\text{g/ml}$)			Amount of isomers found ($\mu\text{g/ml}$)			% Error		
	<i>ortho</i>	<i>meta</i>	<i>para</i>	<i>ortho</i>	<i>meta</i>	<i>para</i>	<i>ortho</i>	<i>meta</i>	<i>para</i>
2:3:1	6.3	11.6	2.8	6.6	11.0	2.9	4.8	5.2	3.5
2:1:3	6.3	3.9	8.2	6.7	3.8	8.1	6.3	2.5	1.2
3:1:2	9.4	3.9	5.5	9.9	4.1	5.7	5.3	5.1	4.5
3:2:1	9.4	7.8	2.8	9.8	7.6	2.8	4.3	2.6	1.8
1:3:5	2.1	7.8	9.1	2.2	8.2	9.5	4.8	5.1	4.4
3:5:1	6.3	12.0	1.8	6.5	12.5	1.9	3.2	4.1	5.5
5:1:3	10.5	2.6	5.5	10.4	2.5	5.8	0.9	3.8	5.4
					Average:		4.2	3.9	3.7

optical densities and micrograms of applied isomer, the concentration of individual isomer in a mixture was ascertained. There is a linear relationship between optical densities and concentration in the range from 2 to 15 μg per ml.

By employing a series of different concentrations for examination, the data show that the application of 2–12 μg of each isomer in a mixture was a suitable range for evaluation, giving an average accuracy of 4% (Table II).

Procedure

Ortho-, *meta*- and *para*-dinitrobenzenes were prepared and recrystallized according to the known procedures⁶.

Chromatographic tanks (24 × 16 × 8 cm) with benzene–ethylacetate (50:10, v/v) as solvent mixture were lined with filter paper 1 h in advance, to ensure good saturation. Glass plates (20 × 13 × 0.5 cm) were coated with silica gel G Merck (according to STAHL) and the 0.2 mm layers were activated by heating at 110° for 10 min.

47.07 mg of *ortho*-, 58.28 mg of *meta*- and 41.18 mg of *para*-dinitrobenzene were weighed in 25-ml calibrated flasks and filled up to the mark with chloroform. Different proportions of each solution were taken (Table I, column "Ratio in mixture", where 1 designates 0.3 ml of the solution, 2 designates 0.6 ml etc.), mixed together, and 0.1 ml of the mixture applied to the plate by means of micropipette.

Chromatoplates were developed for 40 min (front 14 cm), dried in a stream of air (3 h) and examined under U.V. light. Spots were marked lightly with a spatula, and scraped into separate centrifugal tubes. The scrapings were centrifuged once with 5 ml, and again with 3 ml each of solvent (15,000 r.p.m. at 15°)—chloroform in the case of *meta*- and *para*-dinitrobenzene, and methylene chloride in the case of the *ortho* derivative. The extracts were each transferred to a 10-ml calibrated flask, made up to the mark with the respective solvent and then determined spectrophotometrically with a Beckman Model DU spectrophotometer.

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