

Nitrogen dioxide as a reagent for the detection of aromatic amines on thin-layer chromatograms*

Many important types of organic compounds are aromatic amines: coal tar bases, dyestuff intermediates and sulfonamide antibiotics; and thin-layer chromatography of these substances provides a convenient method of analysis for them. Many of the reagents used to detect these compounds on paper or thin-layer chromatograms lack specificity or sensitivity, or cause diffusion of the substances on the chromatograms. This paper reports the use of the azo coupling reaction for the visualization of aromatic primary amines on thin layer chromatograms.

The strongly acidic aqueous solutions usually used for diazotization cannot be used on thin-layer chromatograms because the free amines and the diazonium salts would be eluted. For this reason, a non-aqueous system is necessary. In an earlier communication¹ a system was reported whereby a solution of dinitrogen trioxide in toluene was used as the diazotizing agent and a solution of β -naphthol and diethylamine in toluene as the coupler. Later experience showed that this method gave erratic results and other non-aqueous reagents were investigated.

One reagent tried was nitrosyl fluoborate (NOBF_4)^{2,3} but although this compound causes rapid diazotization, it is not very useful because it is difficult to prepare and store, and is soluble only in polar solvents such as alcohol. When an alcoholic solution of nitrosyl fluoborate is used to diazotize amines on silica gel G thin layers, the diazonium salts formed are partially eluted and spread.

Attention was then given to nitrogen dioxide as a diazotizing agent. This compound (or rather the dimer, N_2O_4) has been observed to diazotize aromatic amines in non-aqueous systems^{4,5}:



and it was felt that this reaction would proceed with aromatic amines adsorbed on silica gel. When *p*-nitroaniline, adsorbed on a silica gel G thin layer, was exposed to NO_2 and then sprayed with a solution of β -naphthol and triethylamine in benzene, the light yellow amine turned a brilliant red-orange indicating that diazotization and coupling had taken place. Several other aromatic amines were tested as follows:

A solution of the amine in acetone or 95 % ethanol was spotted on a silica gel G thin layer plate so that about 0.2 μg was in the spot. Spots containing larger amounts were also applied by multiple spotting. The plate was exposed to nitrogen dioxide gas for 15 sec at room temperature and then sprayed with a solution of 0.1 *M* β -naphthol and 0.1 *M* triethylamine in benzene. All the primary amines tested gave strongly colored spots. Except for relatively volatile compounds like aniline, the spots remained sharp, no diffusion being observed. The compounds used and results obtained are summarized in Table I. Amides, secondary amines and tertiary amines generally gave very weak colors or no colors at all. Diphenylamine was an exception but the color produced may have been due to impurities (a crude preparation was used). No reason can be advanced for the low sensitivities with the aminonaphthalene sulfonic acids or with 3-aminophthalhydrazide. On exposure to nitrogen dioxide,

* This work was supported by grant number GM 12391 from the National Institutes of Health (U.S.A.).

LE I

EFFECTS OF NO₂ AND β-NAPHTHOL TREATMENT OF VARIOUS AROMATIC AMINES

Compound	Color produced	Smallest detectable amount (μg) (approx.)
anilide	none	—
acetaldiphenylamine	none	—
aniline	none	—
acetophenone	red	0.4
anthraquinone	pink	0.2
benzoic acid	pink	0.2
1,3-naphthalenedisulfonic acid	pink	11
1-naphthalenesulfonic acid	pink	1
2-naphthol-4-sulfonic acid	brown	9
phenol	brown	0.2
phenol	brown	0.1
phthalhydrazide	pink	1
phenol	orange	*
Dimethylaniline	none	—
benzylaminobenzaldehyde	yellow	*
nitroaniline	orange	0.3
nitrodiphenylamine	none	—
phenylamine	orange	*
ethylaniline	yellow	*
phthylamine	violet	0.2
phthylamine	pink	0.2
roaniline	pink	0.3
troaniline	orange	0.2
roaniline	pink	0.2
metidine	violet	0.2
nylenediamine	violet	0.2
nylenediamine	violet	0.2
nilamide	orange	*
idine	tan	0.3
uidine	pink	*

* Sensitivity not determined.

Most of the compounds produced strong colors which frequently remained unchanged after treatment with β-naphthol. This may have been due to intramolecular coupling reactions.

Many solvents used in chromatography react with NO₂ so that chromatograms should be thoroughly dried before the azo coupling reaction is done.

Hood College, Frederick, Md. (U.S.A.)

RONALD S. RATNEY

RATNEY, *J. Chromatog.*, 11 (1963) 111.

Y. YAKOBSON, A. I. D'YACHENKO AND F. A. BEL'CHIKOVA, *Zh. Obshch. Khim.*, 32 (1962) 849.

COPLEY, *Honors Paper*, Hood College, 1966 (unpublished).

HOUSTON AND T. B. JOHNSON, *J. Am. Chem. Soc.*, 47 (1925) 3011.

N. WITT, *Tagebl. Natf.-Vers. Baden-Baden*, (1879) 194; *Chem. Zent.*, 11 (1880) 226; mentioned by HOUSTON AND JOHNSON⁴.

Received June 27th, 1966