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DETERMINATION OF FORMIC ACID IN DILUTE AQUEOUS SOLUTION AND SEPARATION AND DETERMINATION OF ANILINE AND TOLUIDINE ISOMERS BY GAS CHROMATOGRAPHY OF FORMANILIDE AND FORMYL TOLUIDIDES

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SUMMARY

The determination of formic acid in various aqueous conditions (as free acid and as a salt in the presence of mineral or other carboxylic acids) by the gas chromatography of the formyl derivatives of aniline and *o*-, *m*- and *p*-toluidine is described. The analysis for these four amines and N-methyl aniline and N-methyl *p*-toluidine in aqueous and non-aqueous mixtures by the gas chromatography of either their formyl or their unsubstituted acetyl derivatives is also described. Columns of sodium dodecylbenzenesulphonate coated on Chromosorb G are used for the chromatography in both cases.

INTRODUCTION

Electro-organic synthesis has received a great deal of interest in recent years. One of the major problems associated with this type of study is the analysis for the organic compounds in aqueous media in the presence of supporting electrolytes which may be mineral acids and salts, or organic quarternary bases and salts. The present work deals with two examples of this situation:

(a) The quantitative determination of formic acid (present as free acid or as an alkali metal or quarternary ammonium salt) in dilute aqueous solution and in the presence of mineral and other low-molecular-weight (C_2–C_8) carboxylic acids without prior separation, by the formation and gas chromatography of the formyl derivative of any one of the following compounds: aniline and *o*-, *m*- and *p*-toluidine.

(b) The quantitative determination of aniline, *o*-, *m*- and *p*-toluidine, N-methyl aniline and N-methyl *p*-toluidine in aqueous and non-aqueous media by the gas chromatography of their formyl and acetyl derivatives.

The determination of formic acid by gas chromatography has largely involved the direct injection of the free acid, often in a mixture with other low-molecular-weight aliphatic carboxylic acids, into the instrument. Special stationary phase

combinations are formulated in order to avoid among other things the decomposition of formic acid[1-3]. Formic acid in cigarette smoke has been analysed by the gas chromatography of its methyl ester on 20% Carbowax 20 M coated on 60/80 mesh Chromosorb P[4]. The formyl derivatives of each of the amines mentioned above are stable, non-volatile and easy to prepare in all the possible aqueous conditions where formic acid or formate may be present.

Toluidines and certain other aromatic amines have been directly chromatographed on stationary phases coated on base-loaded supports[5-7]. However, the use of the derivatives rather than the free compounds often improves the quantitative gas chromatographic separations of most of these amines. The behaviour of the common derivatives on some selected stationary phases has been studied[8]. DOVE[9] described a method for the separation and determination of aniline and toluidine and other related amines via the gas chromatography of their trifluoroacetyl derivatives on a stationary phase mixture of 9.5% (w/w) Apiezon L and 3.5% (w/w) Carbowax 20 M coated on 80/100 mesh Aeropak 30. In the present work the formyl and unsubstituted acetyl derivatives are shown to facilitate the separation and determination of aniline and toluidine isomers on a single stationary phase coated on non-base-loaded Chromosorb G. The acetyl derivatives are also shown to be separable to a certain extent on the same stationary phase coated on the same support 1% (w/w) base-loaded with potassium hydroxide.

EXPERIMENTAL

A Perkin Elmer Model F 11 gas chromatograph equipped with dual flame ionisation detector was used for the analysis, and nitrogen served as the carrier gas.

Column preparation

2.5 g of sodium dodecylbenzenesulphonate (DBS) were dissolved in water–methanol (1:1) and the solution was added to 97.5 g of NAW 60/80 mesh Chromosorb G. After mixing by stirring the solvent was evaporated in the oven at 100° with occasional stirring until the solid appeared dry. It was cooled and packed by suction into 6 ft. (A) and 12 ft. (B) by 3 mm I.D. glass columns.

Column C. 1 g of potassium hydroxide was dissolved in 50 ml of methanol and added with more methanol to 99 g of NAW Chromosorb G. After mixing by stirring the solvent was removed. Then 97.5 g of the dry solid were added to 2.5 g of DBS dissolved in water–methanol, mixed thoroughly and the solvent removed as above in the oven. The solid was cooled and packed in a 6 ft. × 3 mm I.D. glass column (C).

The three packed columns A, B and C were conditioned at 220° for 18 h.

Instrument analytical conditions

The instrument conditions and the columns employed for the various separations and determinations are listed in Table I.

Materials

The chemicals were used as obtained from BDH without further purification. The formyl and acetyl derivatives of the anilines and toluidines were prepared as described by OPENSHAW[10] for retention time determinations. Dilute aqueous solutions

TABLE I

INSTRUMENTAL CONDITIONS FOR THE ANALYTICAL SEPARATIONS

Columns: (A) 6 ft. × 3 mm I.D. glass column packed with 2.5% (w/w) sodium dodecylbenzenesulphonate (DBS) on NAW 60/80 mesh Chromosorb G; (B) the 12 ft. × 3 mm I.D. version of column A; (C) 6 ft. × 3 mm I.D. glass column packed with 2.5% (w/w) DBS on NAW 60/80 mesh 1% (w/w) base-loaded Chromosorb G.
The oxygen and hydrogen inlet pressures and the injection port temperature were the same for all the separations, *viz.* 26/18 lb./in.^{2} and 250°, respectively.

Chromatography of	*Instrumental conditions*
Formanilide (or any formyl derivative of any of the toluidines) for formic acid determination using methyl stearate as internal standard	Columm A; oven temperature, 200°; nitrogen inlet pressure, 10 lb./in.^{2}
Formyl derivatives of aniline, *o*-, *m*- and *p*-toluidine, N-methyl aniline, and N-methyl *p*-toluidine using methyl stearate as internal standard	Column B; oven temperature, 210°; nitrogen inlet pressure, 15 lb./in.^{2}
Acetyl derivatives of *o*-, *m*- and *p*-toluidine, N-methyl aniline, and N-methyl *p*-toluidine	Column B; oven temperature, 200°; nitrogen inlet pressure, 12 lb./in.^{2}

of formic acid were standardised by direct titration with standard sodium hydroxide solution using phenolphthalein as indicator. Standard formate solutions were made by treating measured aliquots of the standardised formic acid solution with little excess of sodium or tetramethyl- (or ethyl-) ammonium hydroxide in a volumetric flask and making up to the mark with distilled water, keeping the pH at 8–10.

PROCEDURE

Preparation of formic acid derivatives and chromatography

(a) Free formic acid only. 2 ml of 0.1 *M* aqueous solution of formic acid were pipetted into a 50-ml stoppered flask. 1 ml of 1 *M* hydrochloric acid, 0.2 g of amine and one small glass bead were added. The mixture was boiled for 10 min under reflux over a hot plate and cooled for 5 min in an ice bath. 1 ml of 2 *M* sodium bicarbonate solution was added and the mixture shaken. 5 ml of ethyl acetate containing 0.2% (w/w) methyl stearate were added and the flask stoppered and shaken vigorously for 1 min. After the two layers separated 2 μl of the ethyl acetate layer were taken with a hypodermic syringe and injected into the instrument (column A).

(b) Formic acid present as sodium/potassium or tetraalkylammonium formate. 2 ml of 0.5 *M* formate solution were placed into the stoppered flask. Drops of 1 *M* hydrochloric acid were added (*ca.* 1 ml) to bring the pH to 1. 1 g of amine and a glass bead was added and the mixture boiled under reflux for 10 min, cooled and treated with 1 ml of 2 *M* sodium bicarbonate solution and extracted with 5 ml ethyl acetate containing 0.2% (w/w) methyl stearate and injected into the instrument as in (a).

(c) Formic acid in the presence of mineral acids and/or other straight-chain C_2–C_8 carboxylic acids. 2 ml methanolic solution 1 *M* in each of formic and the carboxylic acids, and 1 ml of 2 *M* sulphuric acid were taken in the conical flask. Solid sodium bicarbonate was added in excess keeping the pH at 8–9. The pH of the solution was then brought down to 1 with drops of hydrochloric acid solution. 2 g of

amine were added and the mixture boiled under reflux, cooled and extracted as above (but using methyl myristate as internal standard) and injected into the instrument (column A or C).

Preparation of derivatives of aniline, o-, m- and p-toluidine, N-methyl aniline and N-methyl p-toluidine in a mixture and chromatography

(a) As formyl derivatives. 1 ml of the methanolic solution 0.5 *M* in each of the above amines was taken in the conical flask. 0.3 g of formic acid and 4 ml of water were added. The mixture was boiled under reflux for 10 min, cooled in an ice bath and shaken with 4 ml of 2 *M* sodium bicarbonate solution, and then extracted with 10 ml of ethyl acetate containing 0.2% (w/w) methyl stearate. The ethyl acetate layer was then injected into the instrument (column B) (Fig. 6).

(b) As acetyl derivatives. This method applies only to the toluidines and N-methyl aniline since *o*-toluidine and aniline derivatives were not separated on the available column. To a 1-ml aliquot of the methanolic solution 0.5 *M* in each of the amines 0.3 g of acetic anhydride was added in a 25-ml tube and the tube was placed in a hot water bath (75°) for 10 min and then cooled in an ice bath. 10 ml of ethyl acetate and 5 ml of 2 *M* sodium bicarbonate solution were added and the tube was shaken vigorously for 1 min. The ethyl acetate layer was injected into the instrument (columns B and C) (Figs. 7a and b).

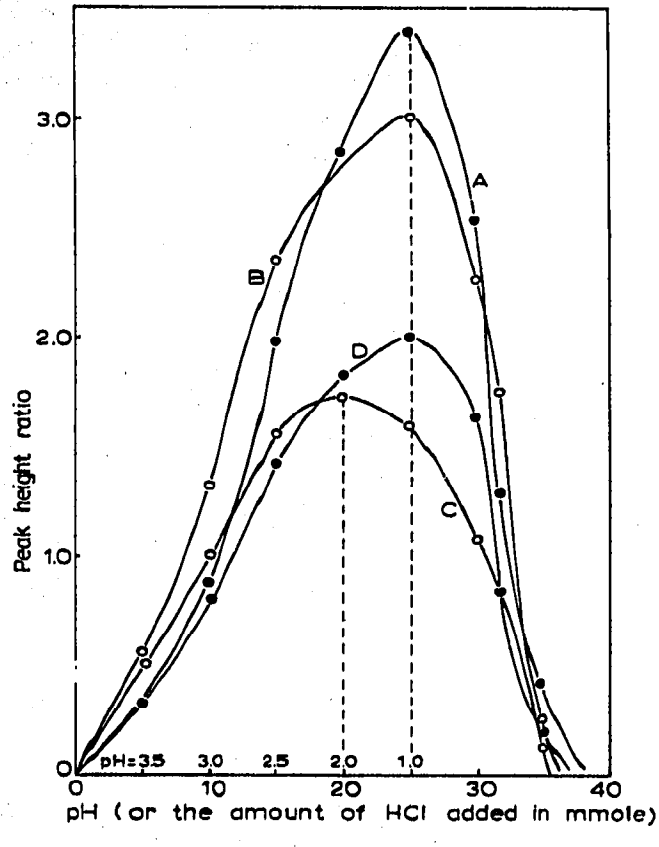

Fig. 1. Effect of the pH on the amount of the formyl derivatives of aniline and *o*-, *m*- and *p*-toluidine formed. (A) = *o*-toluidine; (B) = *m*-toluidine; (C) = *p*-toluidine; (D) = aniline.

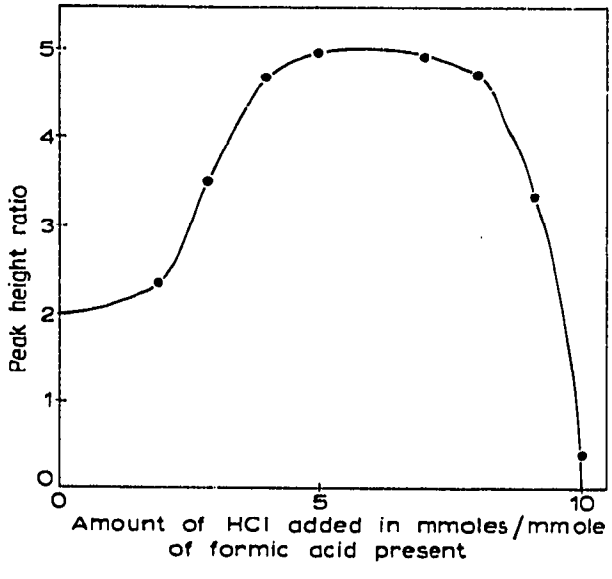

Fig. 2. Effect of the amount of hydrochloric acid added to fixed amounts of formic acid and aniline on the amount of formanilide formed.

In the absence of N-methyl aniline, methyl myristate was used as the internal standard.

RESULTS AND DISCUSSION

Formic acid

The quantitative formation of the formyl derivative of each of the amines for formic acid determination was found to be dependent on three major factors, namely, the pH of the solution when dealing with a formate solution, the formic acid/hydrochloric acid molar ratio when dealing with free formic acid solution, and the amine/formic acid molar ratio.

The amount of derivative formed from a given quantity of a formate solution varied with the pH (Fig. 1), the maximum amount being formed at pH 1. A given quantity of free formic acid solution gave some derivative when reacted with any of the amines, but the amount of derivative formed with a fixed quantity of amine greatly increased with the addition of hydrochloric acid. Since free formic acid aqueous solution was found to exert a large buffer action when increasing amounts of hydrochloric acid were added to it, the effect of the presence of mineral acid on the amount of derivative formed from fixed quantities of formic acid and amine was investigated in terms of formic acid/hydrochloric acid molar ratio. The maximum amount of derivative was formed when the molar ratio of the two acids was 1:5 (Fig. 2). No significant loss in sensitivity and precision was observed when 4–8 mmoles hydrochloric acid per mmole of formic acid were used in derivative preparation.

At the correct pH or formic acid/hydrochloric acid molar ratio, the amount of derivative formed from a given amount of formate or formic acid also varied with amine/formic acid molar ratio (Fig. 3). The use of 6–10 mmoles of amine for each mmole of formic acid present proved quantitatively satisfactory.

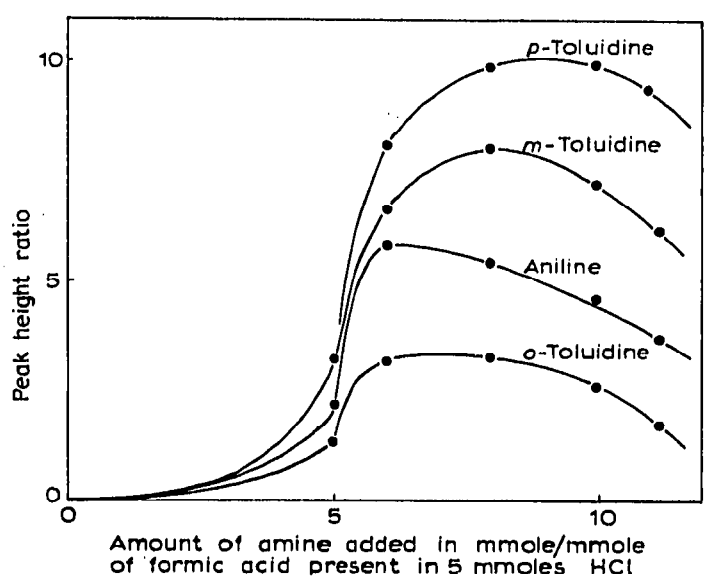

Fig. 3. Effect of the amine/formic acid molar ratio on the amount of derivative formed.

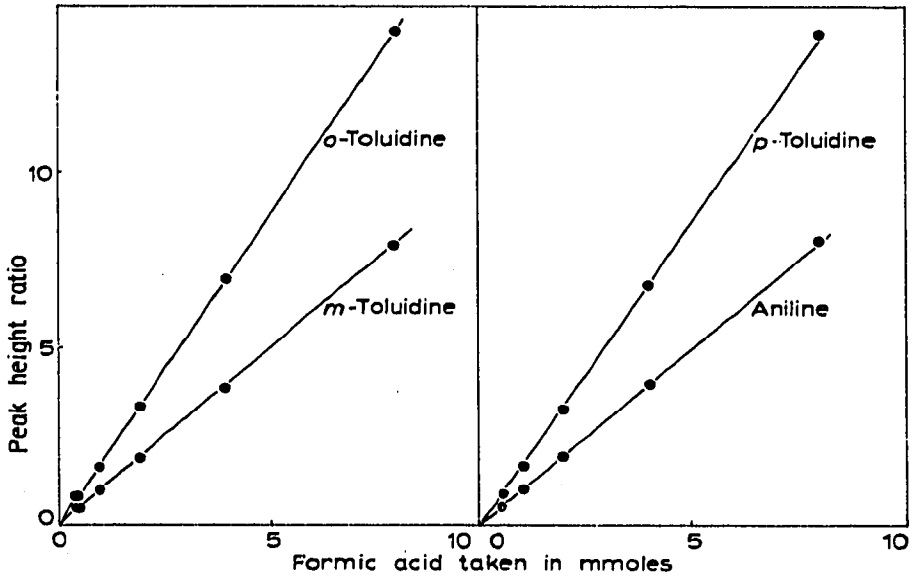

Fig. 4. Calibration curve for the formyl derivatives of aniline and *o*-, *m*-, and *p*-toluidine.

The calibration curves obtained (Fig. 4) by taking increasing aliquots of aqueous formic acid and proportionate amounts of hydrochloric acid and the amines indicated a linear relationship between the amounts of derivatives present and the formic acid taken. Up to 0.1 mg of formic acid may be determined by this method using any of the amines.

Amines

In the determination of the amines by formylation the formic acid/amine molar ratio has also been shown to affect the amount of derivative formed (Fig. 5). A reasonable excess of aqueous formic acid was necessary and the use of 2–6 mmoles of formic acid per mmole of amine present was found satisfactory.

The formylation method is useful when working in an aqueous medium and

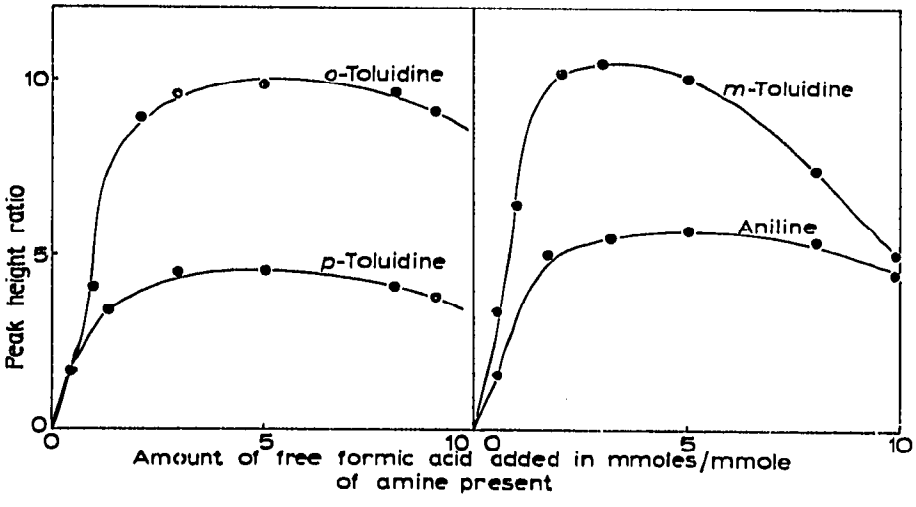

Fig. 5. Effect of the formic acid/amine molar ratio on the amount of derivative formed.

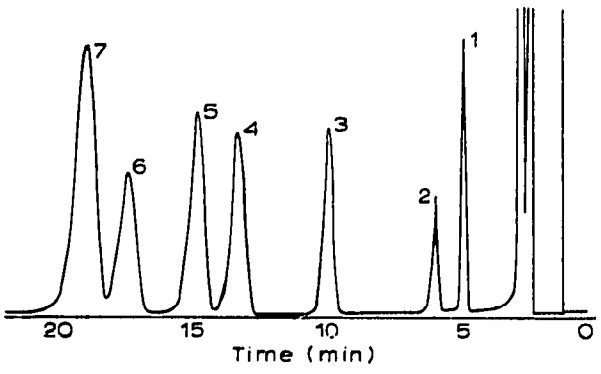

Fig. 6. Separation of formyl derivatives of N-methyl aniline (1), N-methyl *o*-toluidine (2), *o*-toluidine (4), aniline (5), *m*-toluidine (6) and *p*-toluidine (7) on a 12 ft. 2.5% DBS on Chromosorb G column, using methyl stearate (3) as internal standard.

with mixtures containing aniline and the toluidines. Acetylation on the other hand is useful in a non-aqueous medium and where only the toluidines and N-methyl aniline are present.

The formyl derivatives of the amines used here were quantitatively separated on column B but not on column C (Fig. 6). The acetyl derivatives on the other hand were separated on both columns though *m*- and *p*-toluidines were not quantitatively separated on column C (Figs. 7a and b).

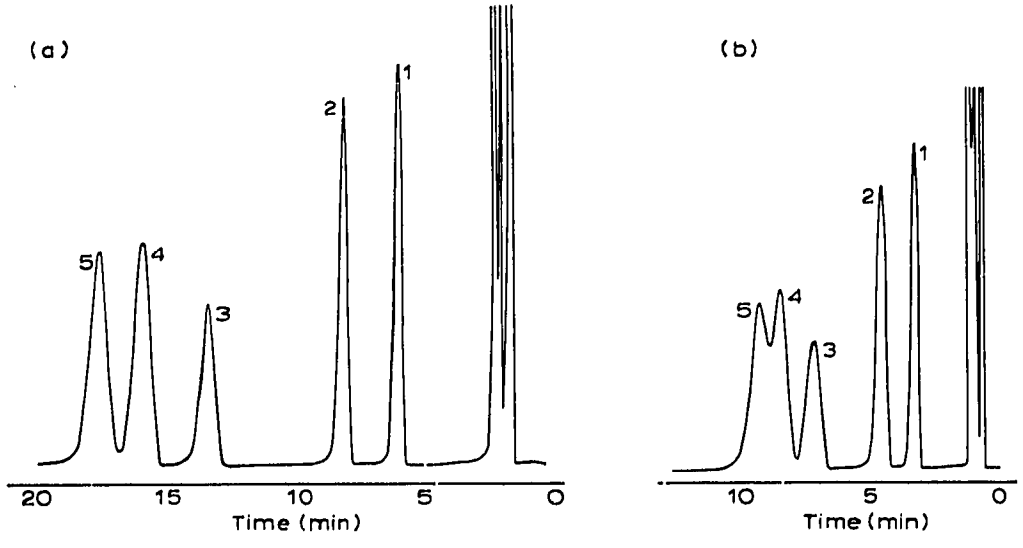

Fig. 7 (a) Separation of acetyl derivatives of *o*-, *m*- and *p*-toluidines, N-methyl aniline, and N-methyl *p*-toluidine on a 12-ft. 2.5% DBS on Chromosorb G column; 1 = N-methyl aniline, 2 = N-methyl *p*-toluidine, 3 = *o*-toluidine, 4 = *m*-toluidine, and 5 = *p*-toluidine. (b) Separation of acetyl derivatives of N-methyl aniline (1), N-methyl *p*-toluidine (2), *o*-toluidine (3), *m*-toluidine (4) and *p*-toluidine (5) on a 6-ft. 2.5% DBS on Chromosorb G column coated 1% with potassium hydroxide.

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