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# New chromogenic reagent for carboxylic acids on thin-layer plates

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Thin-layer chromatography (TLC) has proved to be of general applicability for rapid separation and identification of non-volatile carboxylic acids. Several chromogenic reagents have been reported for detection of these acids. They are mostly pH indicators giving light coloured spots against a dark background[1,2]. An opposite coloration is established by means of the ammonium molybdate reagent, spraying being followed by UV irradiation which gives blue spots against a pale background[3]. Rasmussen[4] has developed a thin-layer method with incorporation of dichlorofluorescein into the organic layer of the solvent to aid UV detection of some acids on the developed plates. Recently, some new chromogenic reagents for carboxylic acids have been described which necessitate the preparation of convenient derivatives, such as hydroxamates, prior to TLC, thus being tedious and unsuitable for quick identification[5,6]. Some chromogenic reagents based on the formation of complexes between $Fe^{3+}$ and organic acids[7,8] have also been reported. All the above methods have been utilized for qualitative identification of carboxylic acids, but because of some disadvantages, especially unevenness of the background, spot instability and lack of reproducibility, they are not convenient for quantitative densitometric evaluation.

In this paper a new chromogenic reagent for detection of carboxylic acids by TLC is proposed which is appropriate also for densitometric quantitation. This reagent yields blue spots on a very pale blue and uniform background owing to the formation of a complex between some polybasic acids and $Cu^{2+}$ ions on the silica gel thin layer.

A similar reaction was used by Wieland and Feld[9] for retention analysis of hydroxy dicarboxylic acids on paper chromatograms. Several other authors estimated fatty acids as copper salts after treatment with potassium ferrocyanide[10–12] or dithiooxamide[13]. The use of cupric ions in spectrophotometric titration for determination of citrate and oxalate ions has also been reported[14].

## EXPERIMENTAL

### *Materials*

Silica gel 60 $F_{254}$ DC-Alufolien (20 × 20 cm, layer thickness 0.20 mm) and DC-Fertigplatten (20 × 20 cm, layer thickness 0.25 mm) thin-layer plates were obtained from E. Merck, Darmstadt, G.F.R. The following general purpose reagent-grade organic acids were obtained from Merck, Riedel de Haën (Hannover, G.F.R.) and Fluka (Buchs, Switzerland); oxalic, malonic, succinic, methylsuccinic, adipic, pimelic, suberic, sebacic, fumaric, maleic, citraconic, mesaconic, itaconic, glutaconic, *cis*- and *trans*-aconitic, phthalic, terephthalic, *cis*-hexahydrophthalic, benzene-1,2,4-tri-carboxylic, $\alpha$-ketoglutaric, oleic, levulinic, glycolic, lactic, malic, tartaric, citric and ascorbic. Standard solutions were prepared by dissolving these acids in distilled water or 98% ethanol, and terephthalic acid in 0.1 *N* NaOH. For separation and detection of organic acids, reagent-grade isopropyl ether, formic acid, methanol and $CuSO_4 \cdot 5\,H_2O$ (Merck) were used.

*Thin-layer chromatography*

Samples of organic acids were applied to a TLC plate with a distance of 1.5 cm between the spots and to the sides of the plate. Micro-pipettes (2 $\mu$l) were used for application of standard solutions. After introducing the last sample, the plates were air dried for 30 min and subsequently developed in the solvent system[2] diisopropyl ether–formic acid–water (90:7:3). The development was carried out in a saturated tank, with an elution time of 40–45 min and an elution distance of 10 cm. After TLC the plates were air dried for 30 min, heated in an oven for 10 min at 110° to drive off the formic acid and left in the stream of air until the next day.

Spots were detected by means of the new chromogenic reagent, which was always freshly prepared by dissolving 300 mg of $CuSO_4 \cdot 5H_2O$ in 20 ml of methanol. Thin layer chromatograms were evenly sprayed with the reagent and allowed to stand in the stream of air until they were dry. Typical blue coloured spots for succinic acid and some other acids appeared rapidly on a pale blue and uniform background.

*Spectrodensitometry*

Quantification of the thin-layer chromatograms was carried out with the Opton Model PMQ II chromatogram spectrophotometer in the reflectance mode, using tungsten light, filter FL 39, and a 0.05 × 8 mm slot at the wavelength of 690 nm. Adjustments were made so as to obtain *ca.* 80–90% full scale deflection on the recorder (Varian A 25) when the most intense spot on the plate was being scanned.

## RESULTS AND DISCUSSION

The results summarized in Table I indicate that the new copper salt reagent is convenient for detecting numerous carboxylic acids on TLC. From the given data, the structures of acids and their ability to give a blue colour reaction can be correlated In a series of monocarboxylic acids the proposed $CuSO_4$ reagent gives no typical colour reaction, perhaps because of the formation of a complex only between $Cu^{2+}$ and polycarboxylic acids. The best results with this reagent have been achieved in the detection of saturated dicarboxylic acids ,such as succinic, methylsuccinic, pimelic, suberic and sebacic acids. Detection is also satisfactory for a series of unsaturated dicarboxylic and tricarboxylic acids and acids with –COOH groups attached to the benzene or cyclohexane ring. It is interesting that hydroxycarboxylic acids are not well detectable by means of this reagent or require greater amounts of acids to be introduced to the thin layer per spot.

Sensitivity of the proposed copper salt reagent is also given in Table I. Each

TABLE I

DETECTION OF CARBOXYLIC ACIDS BY COPPER SALT REAGENT ON TLC SILICA GEL ALU-SHEETS

| *Free acid* | $R_F$ | *Colour* | *UV fluorescence (254 nm)* | *Sensitivity (μg)* |
|---|---|---|---|---|
| Oxalic | 0.00 | hardly detectable | + | 30 |
| Malonic | 0.29 | faint blue | + (faint) | 20 |
| Succinic | 0.45 | blue | — | 5 |
| Methylsuccinic | 0.58 | blue | — | 10 |
| Adipic | 0.56 | blue | — | 15 |
| Pimelic | 0.63 | blue | — | 5 |
| Suberic | 0.70 | blue | — | 5 |
| Sebacic | 0.78 | blue | — | 5 |
| Fumaric | 0.82 | faint blue | + | 10 |
| Maleic | 0.15 | blue | + | 10 |
| Citraconic | 0.34 | faint blue | + | 20 |
| Mesaconic | 0.83 | blue | + | 5 |
| Itaconic | 0.57 | blue | + | 10 |
| Glutaconic | 0.56 | blue | + | 15 |
| *cis*-Aconitic | 0.45 | blue | + | 10 |
| *trans*-Aconitic | 0.46 | blue | + | 10 |
| Phthalic | 0.57 | faint blue | + | 30 |
| Terephthalic | 0.00 | blue | + | 15 |
| *cis*-Hexahydrophthalic | 0.67 | blue | — | 5 |
| Benzene-1,2,4-tricarboxylic | 0.38 | blue | + | 15 |
| α-Ketoglutaric | 0.09 | faint blue | + | 25 |
| Oleic | — | no colour reaction | — | — |
| Levulinic | — | no colour reaction | — | — |
| Glycolic | 0.15 | no colour reaction | + (faint) | — |
| Lactic | 0.28 | no colour reaction | + (faint) | — |
| Malic | 0.04 | hardly detectable | — | 30 |
| Tartaric | 0.00 | hardly detectable | — | 30 |
| Citric | 0.00 | hardly detectable | — | 30 |
| Ascorbic | 0.00 | no colour reaction | + | — |

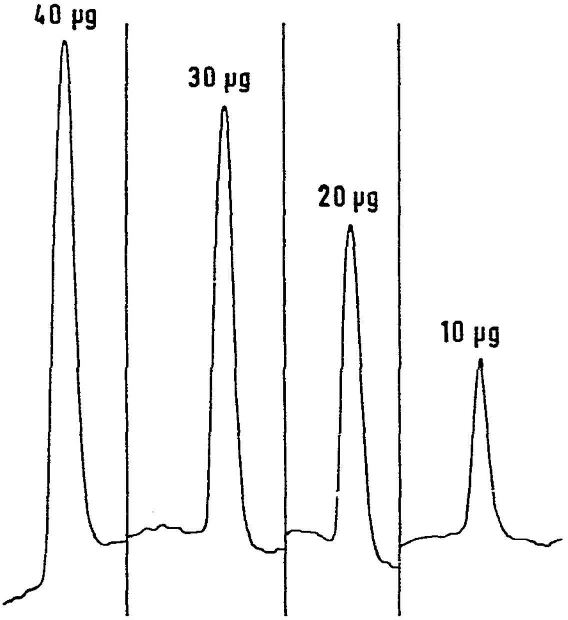
acid was tested with solvent development at levels of 2–30 μg per spot. The limits of detection on TLC alu-sheets are in the range of 5–30 μg. By testing two kinds of thin layers, silica gel plates and silica gel alu-sheets, it has been found that the magnitude of a single spot depends on the layer thickness. TLC alu-sheets with a layer thickness of 0.2 mm have proved to be more useful for detection of small concentrations.

The Knappe–Rohdewald solvent system[1] enables good separation of carboxylic acids, as seen from Table I. When there was no colour reaction the $R_F$ values were obtained by observing dark spots in the UV light at 254 nm.

An advantage of the proposed copper salt reagent for detecting carboxylic acids, especially compared with classical reagents, is the excellent colour stability: storage of developed and sprayed plates for up to several weeks changes neither the colour of spots nor the background, even if the TLC plates are not stored in the dark.

Homogeneity of spots, uniformity of the background and colour stability are the factors that permit this copper reagent to be used for quantitation of some polycarboxylic acids.

A typical densitogram for quantitative evaluation of succinic acid is shown in Fig. 1. The functional dependence between spot area or appropriate peak area on the densitogram against concentration was found to be linear in the concentration range from 10 to 40 $\mu$g of succinic acid (Fig. 2).



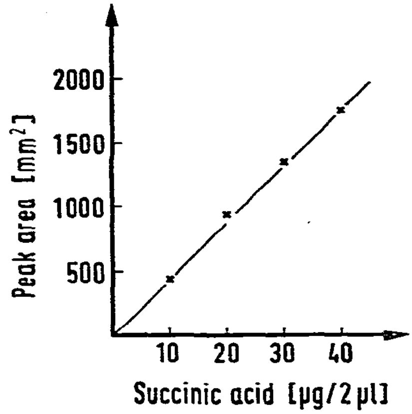


Fig. 1. Typical densitogram of 40, 30, 20 and 10 $\mu$g of succinic acid; 2 $\mu$l of standard solutions were applied to the TLC plate.

Fig. 2. Standard curve describing the relationship between densitogram peak area and succinic acid concentration.

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