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The use of chloroplatinic acid as a chromatographic spray for sulfur compounds

This report describes an innovation on the use of chloroplatinic acid as a chromatographic spray reagent in the detection of some sulfur compounds.

In the course of our studies on the occurrence of bitterness in chopped onions, (*Allium cepa*), we obtained extracts from onions which contained sulfur-bearing compounds other than those responsible for the typical onion odor and flavor. Although these crude extracts have been resolved into a number of different fractions by adsorption chromatography, the identities of the sulfur compounds have not yet been completed. The proof of the presence of these sulfur compounds, however, has been established by elemental analyses.

In order to monitor the eluates from the separation columns, we found it expedient to use thin-layer chromatography (TLC) because of the advantage of speed. This was accomplished by ascending solvent techniques on $\frac{1}{2}$ in. \times 8 in. glass strips coated with silicic acid containing 5 % rice starch as binder. Demonstration of the presence of the sulfur compounds was done by spraying with a sulfur-indicating reagent.

Because of the unidentified status of these compounds, one could not readily select a particular sulfur-reactive reagent with any degree of certainty that the desired result would be obtained. A number of reagents including FEIGL's reagent¹, its variation by CARSON AND WONG², the iodine-azide method of CHARGAFF *et al.*³, as well as the iodoplatinate procedure of WINEGARD *et al.*⁴ and the variation described by TOENNIES AND KOLB⁵ were tried.

In our hands, the reagent of TOENNIES AND KOLB⁵ showed the most promise. The spray reagent finally adopted for use was made up according to the following formula: 14.5 mg Chloroplatinic acid, 311 mg KI, 2.8 ml 2 N HCl and absolute ethanol to 500 ml volume. The reagent is stable for several months if kept refrigerated. On the TLC strips, the presence of the sulfur compounds was indicated by a rarefied or faintly bleached area against a tan to light brown background. While the results showed consistency they were often difficult to observe, especially if the concentrations of the unknowns were low. Fuming with HCl vapors as suggested by WINEGARD *et al.*⁴ and TOENNIES AND KOLB⁵ did not improve the contrast between the background and reaction sites.

However, a striking change takes place if the TLC strips, after having been sprayed with the chloroplatinic acid reagent, are dried and then re-sprayed with distilled water. The brownish background quickly turns to a purple color, and the here-to-fore bleached areas of low contrast become white in sharp relief. We make no attempt to explain the phenomenon. However, the presence of the starch binder in the TLC coating is necessary for this change to occur. This sharpness in contrast is maximal after the TLC coating dries. The color effect is stable for several days; but once faded, it can be restored by a light re-spraying of distilled water. Fig. 1 shows the degree of this contrast between the water sprayed and the untreated TLC strips developed with the chloroplatinic acid reagent.

The sensitivity obtained by use of this procedure on some sulfur-bearing compounds is shown in Table I. It may be noted here that in comparison with some of the sulfur reagents mentioned earlier in this paper, particularly with regard to cysteine, cystine, and methionine; the iodine-azide method of CHARGAFF *et al.*³ requires a larger sample (5 ×) and also considerable development time (up to 60 min for methionine). On the other hand, while the color development of the iodoplatinate reagent of WINEGARD *et al.*⁴ is in the order of rapidity of the method described in this paper, quantities necessary for detection are reported to be approximately twelve times the amounts detectable with the chloroplatinic acid; and, in the case of methionine sulfoxide and methionine sulfone, even higher concentrations are necessary.

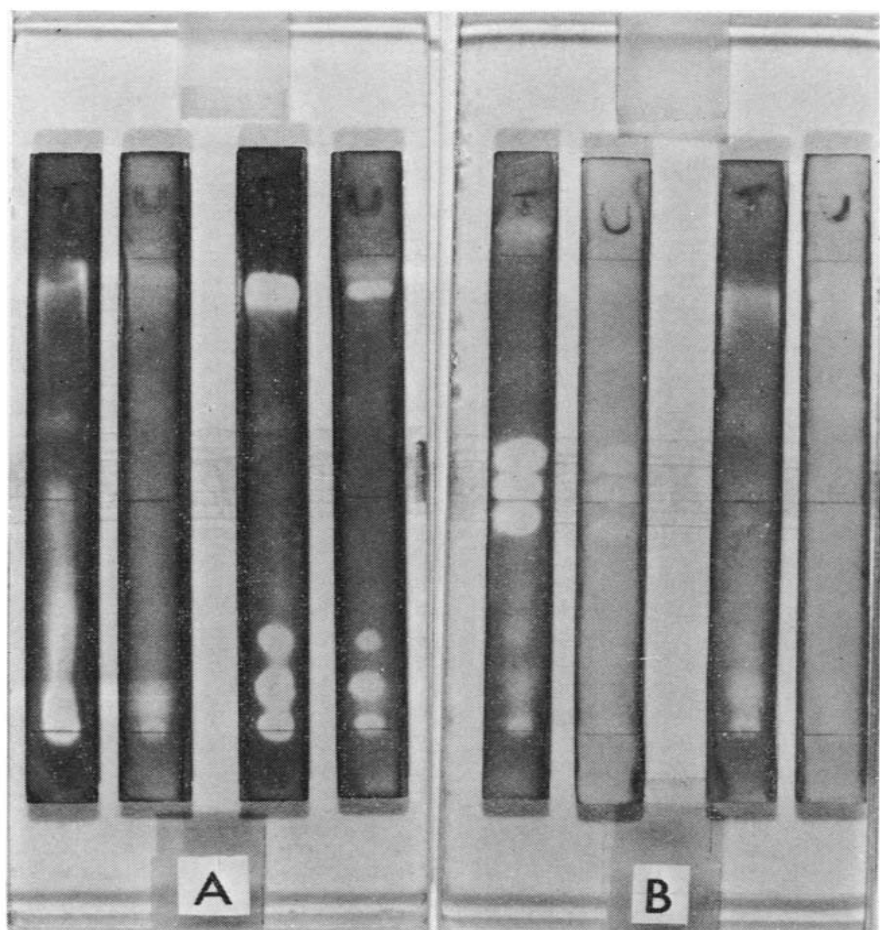


Fig. 1. Contrast obtained with TLC strips (left strip of each pair) after re-spraying with water. (A) Shortly after re-spraying, (B) 24 h later.

The effectiveness of the novel use of the water spray is also applicable to paper chromatograms of these sulfur compounds, providing that a starch-treated paper is used for the chromatography. We have found that dipping papers such as Whatman No. 1 or S & S No. 589 Blue Ribbon, for example, in a commercial soluble starch solution diluted with four parts water and air-dried in a fume hood prior to use was

convenient. After development of the chromatograms, these were handled in the same manner as the TLC strips. With the papers, the change in background color is from a pale pink to a deep violet.

TABLE I

SENSITIVITIES OBTAINED^a USING CHLOROPLATINIC ACID REAGENT ALONE AND WITH RESPRAYING OF TLC STRIPS

<i>Sulfur compound</i>	<i>Reagent only</i>	<i>Re-sprayed</i>	<i>Sulfur compound</i>	<i>Reagent only</i>	<i>Re-sprayed</i>
Cysteine · HCl · H ₂ O	1-3	0.5	Methionine sulfone	5	1
L-Cystine	1	1	S-Benzyl-L-cysteine	1	0.5 ^b
Methionine	1-2	1 ^b	Cycloalliin	0.5	0.5
Methionine sulfoxide	1-3	0.5 ^b	6-Mercapto succinic acid	0.5	0.5
S- <i>n</i> -Propyl cysteine-sulfoxide	0.5	0.5 ^b	Thiourea	0.25	0.25

^a Sensitivity expressed as p.p.m. (wt./vol.).

^b Dark purple or purple spot with white halo against background.

TABLE II

RESULTS OBTAINED BY RE-SPRAYING WITH WATER, CHROMATOGRAMS DEVELOPED WITH CHLOROPLATINIC ACID REAGENT

<i>Sulfur compound</i>	<i>TLC strips^a</i>	<i>Starch treated paper^b</i>
Cysteine · HCl · H ₂ O	Very strong white	Strong white
L-Cystine	Very strong white	Strong white
Methionine	Purple spot with white halo	Light purple ^c
Methionine sulfoxide	Purple spot with white halo	Weak white ^c
S- <i>n</i> -Propyl cysteine-sulfoxide	Purple spot with white halo	Weak white ^c
Methionine sulfone	Pale white	Pale white
S-Benzyl-L-cysteine	Purple spot with white halo	Weak purple ^c
Cycloalliin	Purple spot with white halo	Weak purple ^c
6-Mercapto succinic acid	Very strong white	Very strong white
Thiourea	Very strong white	Very strong white
Methyl sulfide	Strong white	Strong white
<i>n</i> -Propyl sulfide	Strong white	Faint purple ^c
Methyl disulfide	Weak white	Weak purple ^c
<i>n</i> -Propyl disulfide	Strong white	Weak purple ^c

^a Background color is deep purple.

^b Background color is deep violet.

^c Fades on standing, but quickly restored by light re-spraying with water.

This procedure has been tested with some known sulfur-bearing compounds on both TLC strips and paper chromatograms. The results obtained are shown in Table II. In most of the examples showing weak responses, the compounds being tested either showed no observable reaction or were barely discernible in the media not re-sprayed with water. The aliphatic mono- and disulfides were included as spot tests to show the effectiveness of this method with these very volatile compounds.

Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

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