NEW INORGANIC AND ORGANIC SPOT TESTS

BY

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ABSTRACT

In the first part of the work a new Schiff base, o-mercaptothenalaniline, obtained from the condensation of thiophene 2-aldehyde and o-aminobenzenethiol is proposed for the sensitive and specific detection of zinc in the presence of other ions. Various procedures are presented for masking the effect of interfering ions.

The identification limit for zinc is 0.05γ using the filter paper technique while the limit is 0.5γ with test tube procedure. Zinc can be detected in various mixtures and in the presence of a very large number of cations.

In the second part of the work, reactions characterizing α - β unsaturated aldehydes are presented.

Specific and sensitive tests are also proposed for a number of organic compounds; it is thus possible to detect 1γ glyoxal, 0.5γ cinnamaldehyde , 0.5γ acetaldehyde, and 1γ ethyl alcohol.

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PART ONE: NEW SPOT TEST FOR ZINC

I. INTRODUCTION

A number of Schiff bases have recently been reported as chelating agents for metal ions. Specific and sensitive spot tests were developed utilizing the colours formed through chelation. The main objective of this work was to synthesize a reagent that would give a colour with zinc ions and thus serve as the basis of a new spot test. Zinc was chosen because there did not seem to be a specific and sensitive spot test for its detection. Most of the tests reported are based on precipitation or oxidation reduction reactions, however, a large number of cations and anions interfere.

Zinc by virtue of its electronic configuration does not tend to form coloured complexes easily. In this connection it must be noted that there are no ligand field stabilization effects in the Zn(II) ion because of the completed d orbitals. Thus as a consequence of the splitting of the d orbitals, the electronic configuration for Zn(II) is $t_{2g}{}^6$ $e_g{}^4$ using the crystal field nomenclature for the d electrons. It was therefore thought that in order to promote the formation of a coloured chelate with zinc, the most favourable conditions have to be satisfied by the chelating agent namely the possibility of forming five-membered rings, resonance, and whenever possible, the availability of sulfur atoms as donor since zinc is a sulfur "loving" element.

These conditions seem to be satisfied by the Schiff base obtained from condensing o-aminobenzenethiol and thiophene 2-aldehyde. The resulting Schiff base reacts with zinc ions in alkaline solution to produce an intense blood red colour. The test proved to be sensitive and fairly selective in the sense that only few cations interfered. However, and as expected, some of the interfering cations, because of their great resemblance to zinc, proved to be difficult to mask. Procedures are proposed for the detection of zinc in the presence of these and other ions.

Compared to the procedures reported, the proposed method presents the following advantages.

1.- Its sensitivity compares very favourably with the most sensitive methods.

2.- It has the least number of interfering cations.

3.- It is not affected by anions unlike many zinc tests based on redox reactions.

4.- The coloured chelate formed is extractable in chloroform.

5.- The procedure is simple and fast.

6.- It is possible to detect zinc in a mixture consisting of a very large number of cations.

7.- The reagent is easily prepared from available chemicals.

II. HISTORICAL

Feigl[1] and Welcher[2] give excellent reviews of the various methods reported for detecting zinc. These reviews are not exhaustive and a search of the literature has shown that a very large number of tests have been reported for detecting zinc.

Essentially the tests for zinc can be classified in three groups:

a) Reactions leading to the formation of a precipitate.

b) Reactions based on redox.

c) Reactions accompanied by the formation of a colour.

When zinc is treated with the thiocyanate of mercuric and trivalent bismuth ions a distinct red colour is formed on a filter paper. The limit of this test is 0.0075 g.-ion/liter.[3] A similar method for detecting zinc involves precipitation with mercury thiocyanate. Cobalt and iron interfere and the limit of identification is 0.2γ in neutral and 0.5γ in acid solution.[4] Furthermore, still with thiocyanates, zinc thiocyanate when treated with methyl violet forms a fine violet crystalline precipitate. Colours are given also by Cd(II), Hg(II), Cu(II), Ag(I), Au(III), Sn(IV), Mo(IV), W(IV), Fe(III), Co(III), Rh(III), Pd(III), Ir(III) and Pt(III) which can all be removed except Fe(III). Also the noble metals, plus Cu(II) and Fe(III) may be removed by reduction. The limiting dilution of zinc for this test is $1:10^7$.[5] Finally in the thiocyanate series, when a solution containing zinc is treated with a reagent composed of pyramidone, potassium thiocyanate, ethyl alcohol and water a white crystalline precipitate is formed.

These crystals probably are $(C_{13}H_{17}ON_3)_2H_2[Zn(CNS)_4]$. By this method 7$\gamma$ of zinc can be detected in the presence of Ba(II), Ca(II), Mg(II), Sr(II), Al(III), Cr(III), Mn(II), As(III) and As(V) up to 0.5 N concentrations.[6]

Sodium diethyldithiocarbamate reacts with neutral solutions of zinc to give a white turbidity or precipitate. A faint opalescence will be observed in the presence of 0.0006 mg of Zn(II). The reaction will not distinguish zinc from silver, lead, mercury, cadmium, antimony, tin and aluminium.[7]

Quite a number of tests for Zn(II) have been developed making use of ferricyanides and ferrocyanides. The addition of zinc to potassium nickel ferrocyanide, precipitates potassium zinc ferrocyanide, liberating nickel which when treated with dimethylglyoxime produces the characteristic red colour of nickel dimethylglyoxime. The test is based on the displacement of nickel from potassium nickel ferrocyanide by zinc. Cadmium interferes.[8]

Morell has reported that of the many cations that give precipitates with ferrocyanides zinc might be distinguished from the rest since only zinc ferrocyanide is soluble in NaOH and insoluble in HCl. However, Pb(II), Sn(II), Cu(II), colloidal sulfur and strong oxidizing agents interfere.[9]

The fact that zinc ferrocyanide is a precipitate, helps in the increase of the potential of the system $Fe^{+3} \rightarrow Fe^{+2}$, when zinc and ferrocyanide ions are present. This makes possible the oxidation of certain organic compounds which would have been oxidized slowly or not at all in the presence of ferricyanide alone. Most of these organic compounds are highly coloured in their oxidized form and thus they tint the white zinc ferrocyanide. Numerous tests based on this principle are described.[10] Some of the indicators used are p-phenetidine or diphenyl amine; however more sensitive results are obtained with naphthidine and 3,3 dimethyl naphthidine. Limits of identification are 1 ug. using naphthidine and 0.1 ug. with 3,3 dimethyl naphthidine.[10] Ions that give coloured precipitates with ferrocyanides such as Fe(II), Fe(III), Cu(II), Mn(II), Co(II), Ni(II) interfere. Anions such as MnO_4^-, $Cr_2O_7^{--}$, IO_3^-, $S_2O_8^{--}$ etc. which themselves oxidize the organic reagents also interfere and must be removed.[11,12]

When potassium ferricyanide is added to zinc the resulting mixture gives a rose colour with adrenaline, violet with diphenylamine, blue-violet with benzidine and blue with starch and potassium iodide. Ni(II), Co(II), Cu(II), Mn(II) interfere but they give different colours from zinc and their interferences might be removed.[13]

The zinc urate oxidation reaction provides a selective test for zinc. When uric acid is treated with a zinc solution, in the presence of NaOH, and then oxidized by the gradual addition of iodine in potassium iodide a blue colour is formed indicating Zn(II). The test is delicate since it does not work in alkaline solutions at pH values below 12. Colours formed by Cu(II), Co(II), Ni(II) are reported to be of different categories and are apparently formed before adding any oxidizing agent. Ag(I) and Mn(II) also gave colours involving reduction when alkaline urate is added. 1:10000 Zn(II) is detected by this method.[14]

When a reagent composed of pentacyanoammine ferroate and p-nitrosodimethylaniline is added to an acidic solution containing zinc, a blue colour is formed. Identification limit: 0.05 γ zinc. Hg(II), Cu(II), Cd(II), Co(II), Ni(II), Fe(III) and U(VI) interfere and must be removed.[15]

A recent method developed by Platte and Marcy provides a successful procedure for determining zinc and specially for removing the interferences. In testing for zinc with zincon the interfering effects of the heavy metals are removed by adding chloral hydrate, which frees the zinc without destroying the other cyanide complexes formed when cyanide is added to complex all the heavy metals. By this method of cyanide-chloral hydrate, zinc can be accurately determined in the presence of many heavy metal ions. However, cadmium interferes even with this modification; so does Mn(II), but the error introduced by the latter can be decreased by the addition of sodium ascorbate.[16]

Marion and Zlochower have developed individual spot tests for all the Group III cations. In their work they mention that the tests have been chosen such that each Group III cation may be detected in the presence of all the others provided that they are in equal concentrations. For zinc a drop of dithizone in CCl_4 is added to an alkaline test solution; if zinc is present a raspberry red colour is formed.[17] Dithizone forms insoluble coloured inner complex salts with the heavy metals. The test is not specific since as mentioned the rest of the heavy metals give similar coloured complexes extractable in organic solvents. Limit of identification: 5γ Zn(II).[18]

A test of secondary importance is resorcinol with ammoniacal zinc. The product is coloured blue. Limit: 2γ Zn(II).[19]

p-dimethylaminostyryl β-naphthiazole methiodide is a purple to black crystalline powder that dissolves in alcohol to give a deep red solution with a magenta fluorescence. When this solution is added to a water containing mineral acid the red colour is discharged and the resulting solution becomes yellow. If the acid solution contains a salt of Zn(II) intense colours are formed. This method was applied to spectrophotometric determination of zinc in the range of 0 - 15 ug., but a very large number of cations interfere particularly if they are at concentrations equal to or higher than zinc.[20]

III. EXPERIMENTAL

A. Preparation of the Reagent:

1.12 gr. of thiophene 2-aldehyde is added to 1.25 gr. of o-aminobenzenethiol in a 15 ml centrifuge tube. The mixture is well shaken, 4 mls of distilled water added and the test tube placed in a water bath for 30 minutes. The mixture is then centrifuged for three minutes and allowed to stand overnight. The clear supernatant liquid is pipetted off and the condensed product, spread on a watch glass, dries to a yellow solid upon exposure to atmosphere. The yield at this stage is 94.7% and the melting point ranges between 45 - 60°C.

The Schiff base is recrystallized from petroleum ether 50 - 70, the dark yellow oily residue being discarded. Bright yellow crystals are obtained which are dried in a vacuum desiccator. Repeated crystallization yields a pale yellow crystalline product, the melting point of which is 96 - 97°C.

The purified Schiff base is stored in a dark glass bottle kept in a refrigerator.

A saturated solution of the reagent is prepared by dissolving 1.3 gr. of the Schiff base in 3 mls of $CHCl_3$. The reagent is kept in a dark brown dropper bottle.

The thiophene 2-aldehyde was obtained from Fluka while the o-aminobenzenethiol was obtained from Eastman Kodak Co.

B. Detection of Zinc

Two procedures were investigated for the detection of zinc with the reagent: the filter paper method and the test tube method. The filter paper method presented the advantage of having a lower limit of identification while the test tube method allowed the extraction of the red colour in chloroform. Droppers delivering drops of 0.05 ml were used all through the work.

1.- Filter paper method

Procedure: An Ederol filter paper No. 13 is spotted with a drop of a saturated chloroform solution of the reagent and then with a drop of the test solution. The filter paper is then exposed to ammonia fumes obtained from heating fresh concentrated ammonium hydroxide. According to the zinc content a dark red-orange stain is obtained. Even with minutes amounts of zinc a positive reaction is easily distinguished against the yellow colour of the reagent. Concentrations above 5γ Zn(II) gave an intense red stain, 0.5γ Zn(II) gives a definite red colouration which can be compared with a blank. 0.05γZn(II) gives a faint red ring while the blank leaves the colour of the reagent unchanged.

Limit of identification: 0.05γ zinc.

Limit of dilution: 1:1,000,000.

Reagents: Saturated solution of the reagent in chloroform.

Fresh concentrated ammonium hydroxide.

An interesting observation was made while determining the limit of identification of zinc on filter paper. Although various filter paper types were used only Ederol filter paper No. 13 gave a positive response with 0.05γ zinc. Thus Whatman No. 1 and ashless filter paper No. 42 responded only to 0.5γ zinc. This seems to confirm the fact that reaction on a filter paper is the result of a complicated interplay of capillary spreading, diffusion, swelling and adsorption.[21] West and Hamilton had reported that the identification limits of some sensitive tests conducted on filter paper and carried out under identical conditions, may vary by powers of ten according to the type of paper used.[22]

Effect of other ions

The following table shows the colours obtained when the Ederol filter paper treated with the reagent is spotted with a drop of test solution containing 500γ of cation. The procedure is the same as described above.

Table I

Colours Obtained with Various Cations (Filter Paper)

Ions	Colour obtained	Ions	Colour obtained
Li(I)	yellow	Sn(II)	orange
Na(I)	yellow	As(V)	yellow
K(I)	Yellow	Sb(III)	orange
Be(II)	yellow	Bi(III)	orange
Mg(II)	yellow	Ce(IV)	yellow
Ca(II)	yellow	U(VI)	yellow
Sr(II)	yellow	Ag(I)	orange-yellow
Ba(II)	yellow	Pb(II)	orange-yellow
Ti(III)	pale orange	Tl(I)	orange
Zr(IV)	yellow	Hg(I)	yellow-green
V(V)	yellow	Hg(II)	yellow
Cr(III)	yellow	Co(II)	black
Mo(VI)	green black	Ni(II)	brown
Mn(II)	yellow	Pd(II)	brown
Fe(III)	green black	Cu(II)	black
Fe(II)	pale brown	Cd(II)	orange-yellow
Al(III)	yellow	Zn(II)	red
Pt(II)	yellow		

It is apparent, under these conditions of operation and concentration, that only zinc produces a red stain. Apart from the black forming sulfide cations most metal ions give an orange or yellow colour. Surprisingly, cadmium and mercury(II) give yellow and yellow-orange stains. In view of their position in the Periodic Table a stronger interference would have been expected.

2.- Test tube method

Procedure: To one drop of the test solution add one drop of 10% NaOH and two drops of the saturated chloroform solution of the reagent. The test tube is shaken and placed in a water bath for one minute. Three mls of distilled water and five or six drops of chloroform are added. An intense red colour depending on the concentration of the zinc is obtained in the chloroform layer. Addition of distilled water was found to increase the extraction of the red colour in the non-aqueous solvent.

Using the above procedure it was possible to detect 0.5γ zinc which imparts an orange colour to the chloroform bead formed at the bottom of the test tube. A blank test carried with a drop of distilled water gave a yellow colour in the chloroform layer.

Limit of identification: 0.5 γ zinc.

Limit of dilution: 1:100,000.

Reagents: Saturated solution of the reagent in chloroform.
10% NaOH.
Chloroform.

Effect of other ions

The following table shows the colours obtained when the procedure described above is applied to various cations. In each case a drop of the test solution containing 500γ of the cation is used.

Table 2

Colours obtained with Various Cations (Test Tube)

Ions	Colour obtained	Ions	Colour obtained
Li(I)	yellow	Sn(II)	yellow
Na(I)	yellow	As(V)	yellow
K(I)	yellow	Sb(III)	yellow
Be(II)	yellow	Bi(III)	yellow
Mg(II)	yellow	Ce(IV)	yellow
Ca(II)	yellow	U(VI)	yellow
Sr(II)	yellow	Ag(I)	yellow
Ba(II)	yellow	Pb(II)	orange
Ti(III)	yellow	Tl(I)	orange
Zr(IV)	yellow	Hg(I)	yellow
V(V)	yellow	Hg(II)	yellow
Cr(III)	yellow	Co(II)	red
Mo(VI)	yellow	Ni(II)	dark red
Mn(II)	yellow	Pd(II)	red
Fe(II)	yellow	Cu(II)	brown
Fe(III)	yellow	Cd(II)	orange
Al(III)	yellow	Zn(II)	red
Pt(II)	yellow		

It is apparent from the above results that only Co(II), Ni(II), Pd(II), Tl(I), Cu(II), Pb(II), Cd(II) interfere. The first three only giving red colours, the others producing colours ranging from brown to orange.

The interference of Tl(I) and Pb(II) is of no consequence since an HCl solution would not contain these ions.

Although the filter paper method is more sensitive than the test tube procedure, it was found preferable to develop and further investigate the latter.

The reasons behind this choice are the following:

a.- The extraction of the colour in chloroform is of particular interest.

b.- The effect of interfering ions can be controlled and neutralized without greatly affecting the dilution of the test solution. Thus copper for instance interferes on the filter paper method by forming a black sulfide. Addition of reagents to complex it will dilute the solution so that when a drop is eventually taken the zinc would have been appreciably diluted.

c.- The possibility of converting the procedure into a quantitative method for determining zinc in the presence of other ions without preliminary separation.

The greater sensitivity of the filter paper method is in agreement with reported and expected cases. The dimethylglyoxime test for nickel will reveal as a test tube reaction 2.8γ nickel in 2 mlsof solution. Dilution limit: 1:700,000. As a filter paper reaction a drop (0.05 ml) will reveal as little as 0.015γ . Dilution limit: 1:3,300,000.

Analogous results are obtained with the Prussian blue test for ferrocyanide. This increase in sensitivity has various reasons.[23]

1.- The reaction apparently occurs in the plane of the paper.

2.- Coloured products are held near the site of their production by the capillaries of the paper and are more readily seen because of the white background of the paper.

3.- Filter paper not only brings about a rapid uniform spreading of liquids through its coarse capillaries, but also the fine porosity of the cellulose fibers produces an effective adsorption medium due to the great surface exposed.

4.- The diffusion and dilution which invariably accompany the mixing of the reagent and test solution is avoided.

Effect of anions

Anions have been reported to interfere with a number of tests for zinc.[24] These are permanganate, chromate, dichromate, vanadate, persulfate, iodate. They usually oxidize the organic reagent and have to be absent when the detection of zinc is performed.

It was therefore decided to investigate the effect of various anions on the zinc test developed in this work. Two sets of experiments were carried out.

0.1 M. test solutions of the various anions shown in the table below were prepared. In the first set a drop of each of these test solutions was treated with one drop of 10% NaOH and two drops of the reagent, as in the procedure described above.

The results are summarized in table 3 below.

Table 3

Colours Obtained with Anions (Test Tube)

Ions	Colour obtained	Ions	Colour obtained
Cl^-	yellow	CO_3^{--}	yellow
Br^-	yellow	$C_2O_4^{--}$	yellow
I^-	yellow	$Cr_2O_7^{--}$	yellow
NO_3^-	yellow	PO_4^{---}	yellow
SO_4^{--}	yellow	CH_3COO^-	yellow
$S_2O_3^{--}$	yellow	$Fe(CN)_6^{-4}$	yellow
$S_2O_8^{--}$	yellow	$Fe(CN)_6^{-3}$	yellow
S^{--}	yellow	CNS^-	yellow
MnO_4^-	yellow		

It is apparent that none of the anions tried reacted with the reagent to produce a colour that might interfere with the zinc colour.

In the second set of experiments the test for zinc is carried in the presence of each of these anions. In each case 1 drop of 0.1 M solution of the anion is added to 1 drop containing 500γ of zinc. The usual test tube procedure described above is then applied. Results are shown in the following table.

Table 4

Colours Obtained with Zinc in the Presence of various Anions (Test tube)

Ions	Colour obtained	Ions	Colour obtained
Cl^- + Zn(II)	red	CO_3^{--} + Zn(II)	red
Br^- + Zn(II)	red	$C_2O_4^{--}$ + Zn(II)	red
I^- + Zn(II)	red	$Cr_2O_7^{--}$ + Zn(II)	red
NO_3^- + Zn(II)	red	PO_4^{---} + Zn(II)	red
SO_4^{--} + Zn(II)	red	CH_3COO^- + Zn(II)	red
$S_2O_3^{--}$ + Zn(II)	red	$Fe(CN)_6^{-4}$ + Zn(II)	red
$S_2O_8^{--}$ + Zn(II)	red	$Fe(CN)_6^{-3}$ + Zn(II)	red
S^{--} + Zn(II)	red	CNS^- + Zn(II)	red
MnO_4^- + Zn(II)	red		

N.B. 50γ Zn(II) still responds in the presence of 0.1 M ferrocyanide and sulfide.

It thus appears that none of the anions that were tried affected the test for zinc. This is rather surprising considering for example the very high insolubility of zinc ferrocyanide $K_{s.p.}$ 10^{-95}. This would confirm an observation of Feigl[25] that certain sensitive tests can be carried on the precipitate of highly insoluble substances. The obvious advantage of the method described in this work is the fact that oxidizing and reducing anions do not affect the test. Thus chromate, permanganate, vanadate, iodate, persulfate, etc.. which oxidize 3,3'-dimethylnaphthidine and diethylaniline are without effect on the reagent proposed in this work. This is reasonable since the reagent is not a redox indicator.

C. Detection of Zinc in the Presence of Interfering Ions

Examination of table 2 shows that of the cations that were tried only Co(II), Cu(II), Ni(II), Pd(II) and Cd(II) interfere by forming a red or orange colour of their own. The elimination of all these ions by a simple procedure did not prove to be successful, for whatever reagent used to neutralize them did in fact eliminate the zinc. It seemed that the conditioning of the zinc reaction could not be accomplished by the simple experiments normally adopted in spot techniques. Various methods were tried for "masking" the interfering ions. Precipitants such as carbonates, sulfides, phosphates eliminated the zinc; complexing agents such as oxalates, fluorides, citrates, tartrates, thiosulfates, cyanides, E.D.T.A. either failed to inhibit the interfering ions or complexed the zinc itself. Since all these ions form fairly stable ammonia complexes it was thought that control of pH with ammonia instead of sodium hydroxide would give better results. This did not prove to be so.

Advantage was taken of the amphoteric properties of zinc. In strongly alkaline solution only the zincate ion would remain in solution while the interfering ions precipitated as the insoluble hydroxides. This approach gave fairly satisfactory results but the reaction was not sensitive and large amounts of cobalt and copper interfere. It was not possible to detect 50γ Zn(II) in the presence of 5000γ Cobalt. Palladium also interfered since $Pd(OH)_2$ redissolves in alkaline solution.

It was finally decided to resort to complexing with cyanide since all these ions formed stable cyanide complexes. Examination of the pK_c values of the cyanide complexes shows that cyanide will effectively mask copper, cobalt, nickel and palladium whereas the cyanide complexes of cadmium and zinc are not very stable. Therefore addition of cyanide will readily complex all the interfering ions except cadmium. Addition of excess cyanide would completely remove the cadmium but the zinc sensitivity will be very much reduced. This would therefore require addition of the minimum amount of cyanide in order not to complex the zinc.

The possibility of utilizing chloral hydrate to liberate the zinc from the cyanide complex did not prove to be very successful because cadmium is also freed and therefore interferes.

It was however found that Cd(II) would be very readily and conveniently removed by treatment with thiourea in basic solution when a yellow precipitate of cadmium sulfide is slowly formed; zinc is not affected and separation by centrifugation is not necessary.

Welcher[26] reports a method where thiourea is used to precipitate and complex interfering ions in a gravimetric determination of zinc. Further investigation has shown that thiourea not only masked cadmium but also prevented the interfering effects of copper and palladium. Had it not been for cobalt and nickel which require cyanide, thiourea would have been sufficient to mask all the interfering ions.

The method finally adopted consisted therefore in adding sufficient cyanide to complex cobalt, copper, nickel and palladium and removing the cadmium with thiourea.

Procedures adopted for the detection of zinc in the presence of different concentrations of one foreign interfering ion

For the detection of zinc in the presence of one foreign interfering ion solutions containing per drop, 5γ Zn(II) and 500γ , 900γ , 4500γ of the interfering ion were prepared. Although thiourea is not required when cyanide is used to mask copper, cobalt, nickel and palladium, it is however used for the sake of uniformity in the procedure. This normally reduces the sensitivity of the zinc but the results obtained are quite satisfactory.

Procedure: To one drop of the solution containing 5 γ Zn(II) and the foreign ion at different concentrations, the following is added:

1) 2 drops saturated thiourea solution.
2) drops of KCN. For number of drops and concentration of KCN refer to table 5 below.
3) 3 drops of 10% NaOH.

The mixture is shaken and placed in a water bath for two minutes. Four drops of the reagent are added and the mixture again heated in a water bath. Four mls of distilled water and 3-4 drops of chloroform (enough to extract the colour) are then added and the colour of the chloroform layer observed.

<u>Reagents</u>: Saturated thiourea solution.

5% and 1% KCN solutions.

10% NaOH solution.

Saturated solution of the reagent in chloroform .

Chloroform.

The following table summarizes the results observed.

Table 5

Detection of Zinc in the Presence of Interfering Ions

Ions	5γ Zn(II) +500γ cation	5γ Zn(II) +900γ cation	5γ Zn(II) +4500γ cation	Colours obtained
	Drops of CN^- added	Drops of CN^- added	Drops of CN^- added	
Co(II)	3 (5%)	4 (5%)	13 (5%)	yellow
Co(II)+Zn(II)	3 (5%)	4 (5%)	13 (5%)	red-orange
Ni(II)	2 (5%)	4 (5%)	9 (5%)	yellow
Ni(II)+Zn(II)	2 (5%)	4 (5%)	9 (5%)	red-orange
Cu(II)	3 (5%)	4 (5%)	8 (5%)	yellow
Cu(II)+Zn(II)	3 (5%)	4 (5%)	8 (5%)	red-orange
Pd(II)	8 (1%)	8 (1%)	---	---
Pd(II)+Zn(II)	8 (1%)	8 (1%)	---	---
Cd(II)	1 (1%)	1 (1%)	1 (5%)	yellow
Cd(II)+Zn(II)	1 (1%)	1 (1%)	1 (5%)	yellow-orange

N.B.: Figures in brackets refer to concentration of KCN solutions.

Determination of the Limit of Identification of Zn(II) in the presence of 500γ interfering Ions

Solutions containing 500γ of interfering cation and various concentration of zinc down to 1γ per drop were prepared. The procedure for the detection of zinc is the following:

Procedure: To one drop of the solution containing 500γ of interfering ion and zinc at different concentrations the following is added:

1) 2 drops of saturated thiourea solution.
2) drops of KCN. For number of drops and concentration of KCN refer to table below. In general KCN is added dropwise until the precipitate formed just redissolves.
3) 3 drops of 10% NaOH.

The mixture is shaken and placed in a water bath for two minutes. Three drops of the reagent and four mls of distilled water are added. The colours of the bead which is formed at the bottom of the test tube is observed.

Reagents: Saturated thiourea solution.
5% and 10% KCN solutions.
10% NaOH solution.
Saturated solution of the reagent in chloroform.

The following results are obtained for the determination of the limit of zinc in the presence of 500γ of foreign ions.

Table 6

Limits of Identification of Zinc in the Presence of 500 γ of Interfering Ions

Ions	Number of drops & concentration of KCN	Colour obtained
1γZn(II)+500γCo(II)	2 drops 5% KCN	red-orange
500γCo(II)	2 drops 5% KCN	yellow
1γ Zn(II)+500γPd(II)	1 drop 5% KCN	orange
500γPd(II)	1 drop 5% KCN	yellow
1γ Zn(II)+500γCu(II)	1 drop 5% KCN	orange
500γCu(II)	1 drop 5% KCN	yellow
2γ Zn(II)+500γNi(II)	2 drops 5% KCN	orange
500γNi(II)	2 drops 5% KCN	yellow
3γZn(II)+500γCd(II)	1 drop 1% KCN	orange
500γCd(II)	1 drop 1% KCN	yellow

The limit of identification for Zn(II) in the presence of 500γ Co(II), Cu(II), Pd(II), Ni(II) and Cd(II) is therefore 1γ, 1γ, 1γ, 2γ, and 3γ respectively.

A third series of experiments was carried out in which a drop containing a high concentration of interfering ion was added to a drop containing 5γ of zinc.

Procedure: In a test tube, a drop of the interfering ion is added to a drop containing 5γ zinc. The following is then added:

1) 2 drops saturated thiourea (5 drops when cadmium is the interfering ion.)
2) 5% KCN. Number of drops shown in table 7.
3) 3 drops 10% NaOH.

The solution is shaken and heated in a water bath for two minutes. Three drops of reagent are added and the mixture is heated again. Four mls of distilled water and a few drops of chloroform (enough to extract colour) are then added. Colour is observed in the chloroform layer.

Reagents: Saturated thiourea solution.
5% NaOH solution
Saturated solution of the reagent in chloroform.
Chloroform.

The following results are obtained:

Table 7

Mixtures of Zinc with Interfering Ions

Mixture of Ions	Number of drops of 5% KCN	Colours obtained
1 dp 5000γCo(II) + 1 dp 5γZn(II)	13 5% KCN	red-orange
1 dp 5000γCo(II)	13 5% KCN	yellow
1 dp 5000γNi(II) + 1 dp 5γZn(II)	11 5% KCN	orange
1 dp 5000γNi(II)	11 5% KCN	yellow
1 dp 5000γCu(II) + 1 dp 5γ Zn(II)	11 5% KCN	orange
1 dp 5000γCu(II)	11 5% KCN	yellow
1 dp 5000γ Cd(II) + 1 dp 5γ Zn(II)	1 5% KCN	orange
1 dp 5000γCd(II)	1 5% KCN	yellow
1 dp 1000γPd(II) + 1 dp 5γ Zn(II)	1 5% KCN	orange
1 dp 1000γPd(II)	1 5% KCN	yellow

<u>Conclusions:</u> The results summarized in Tables 5, 6, and 7 show that the proposed procedure is suitable for detecting zinc in the presence of interfering foreign ions.

Table 5 shows that 5γ Zn(II) can be detected in the presence of 900 times its concentration of cobalt, nickel, copper and cadmium. With palladium it was not possible to prepare solutions which contained more than 1000γ Pd(II) per drop; therefore the proportion limit for zinc to palladium was 1 to 180.

Table 6 also shows that fairly high sensitivities are obtained and that 1γ zinc could be detected in certain cases.

Table 7 confirms the results obtained in Table 5. It shows that in a mixture obtained from a drop of the interfering cation and a drop of zinc, the latter could be detected in the presence of 1000 times its concentration of cobalt, nickel, copper, cadmium and 200 times its concentration of palladium.

The amounts of cyanide which have to be added depend on the nature and concentration of the interfering ion that has to be masked. It is however, found that in most cases the amount of cyanide required is in agreement with expected behaviour. Thus $Pd(CN)_4^=$ which is a very stable complex, will require the least amount of cyanide. $Cu(CN)_4^=$, $Ni(CN)_4^=$, and $Co(CN)_4^=$ with pK_c 27.3, 22.0, 19.1 respectively require more cyanide to mask them. $Cd(CN)_4^=$ and $Zn(CN)_4^=$ with pK_c 18.85 and 16.89 respectively, have stabilities of the same order of magnitude. Therefore any cyanide which is added to complex cadmium is also likely to complex zinc. Hence the danger of adding too much cyanide. The masking of zinc and cadmium would therefore require the largest amount of cyanide. Tables 5, 6, 7 show however that the smallest amounts of cyanide are required to mask cadmium; this is due to the fact that the greatest part of cadmium has already been precipitated by thiourea.

General Method for Removing Interfering Ions

The previous work has shown that zinc can be detected in small amounts in the presence of interfering ions. The procedures described suffer from the disadvantage of not being uniform. Thus the amount of cyanide added is critical; an operator is likely to add excess cyanide thus making the test for zinc less sensitive or even negative.

To overcome this objection an attempt was made to develop a standard procedure that would apply to all cases irrespective of the ions present and their concentrations.

The separation of zinc using its amphoteric property was further investigated. As mentioned previously cobalt and palladium interfered strongly, the first by producing an intense coloration probably through incomplete precipitation, while the latter redissolved in strongly alkaline solution. Copper in high concentrations also interferes.

Cadmium when precipitated as the hydroxide does not interfere, thus 50γ Zn(II) in the presence of 10,000γ Cd(II) gave a positive test while 10,000γ Cd(II) alone failed to do so. 5000γ Cu(II) alone gave a positive response. 5000γ Ni(II) in the presence of 50γ Zn(II) gave a positive response while 5000γ Ni(II) alone failed to do so. Palladium 1000γ gives a positive response. Therefore the cations that do not interfere at relatively high concentrations are cadmium and nickel. The problem consisted then in removing the effect of Co(II), Cu(II) and Pd(II).

Oxidation by persulfate in alkaline solution converts nickel and cobalt to the corresponding very insoluble black peroxides: Ni_2O_3 and Co_2O_3 probably in their hydrated forms. Copper is converted to the insoluble black oxide CuO. Cadmium and palladium do not interfere in the presence of thiourea and cyanide.

A series of experiments were made in which 5000γ of Co(II), Cu(II), Ni(II), Cd(II) and 1000γ Pd(II) were mixed with different concentrations of zinc. The following procedure is adopted and the results are shown below.

Procedure: A drop containing 5000γ interfering ion (1000γ for Pd(II)) is added to a drop of zinc in a test tube. The solution is treated with:

1) 6 drops 10% NaOH.

2) a crystal of persulfate.

The mixture is placed in a water bath for at least two minutes to completely destroy excess persulfate. The black precipitate is centrifuged, the supernatant solution collected and treated with one drop saturated thiourea and one drop 5% KCN. Three drops of reagent are added, the solution is placed in a water bath, water and chloroform finally added. Colour is observed in the chloroform layer.

Reagents: Solid potassium persulfate
10% NaOH solution
Saturated solution of thiourea
5% KCN solution
Saturated solution of the reagent in chloroform
Chloroform

Results:

50γ zinc are detected in the presence of 5000γ of Cd(II), Co(II), Cu(II), Ni(II) and 1000γ Pd(II).

Note: If Pd is known to be absent there is no need to add thiourea or cyanide.

In the following set of experiments the same procedure was applied for the detection of zinc in seven different solutions each containing at least five cations. Standard solutions containing 1 % of the cation were prepared (i.e. 500γ per drop) one drop from each selected solution was taken and mixed in a test tube.

The procedure is identical to the previous one except that excess NaOH i.e. about 10 drops are being used because of the larger volume of the unknown.

Results are shown in Table 8 below.

Table 8

Detection of Zinc in Various Mixtures

Mixture of Ions	Colour obtained
Ag + Sn + Co + Fe + Ni + Zn	red
Ag + Sn + Co + Fe + Ni	yellow
Sr + Hg(I) + K + Ba + Tl + Zn	red
Sr + Hg(I) + K + Ba + Tl	yellow
Cu + Ca + Li + Mn + Na + Pb + Zn	red
Cu + Ca + Li + Mn + Na + Pb	yellow
Hg(II) + Cd + As + U + Sb + Zn	red
Hg(II) + Cd + As + U + Sb	yellow
Bi + Mo + Ce + Ti + Cr + Zn	red
Bi + Mo + Ce + Ti + Cr	yellow

Table 8 - continued

Mixture of Ions	Colour obtained
Be + Zr + Al + Mg + Pt + Pd + Zn	red
Be + Zr + Al + Mg + Pt + Pd	yellow
Co + Cu + Ni + Pd + Cd + Zn	red
Co + Cu + Ni + Pd + Cd	yellow

The procedure described above was finally tested by detecting zinc in a solution containing 33 other ions. This solution was prepared by taking a drop from each of the 33 cations mixed with 5 drops of 1% Zn(II). A solution containing the same cations but without zinc was also prepared. The procedure was applied using 18 drops of 10% NaOH.

Solution (1): Ag(I) + Sn(II) + Co(II) + Fe(III) + Ni(II) + Sr(II)
+ Hg (I) + Na(I) + K(I) + Ba(II) + Tl(I) + Pt(II)
+ Pd(II) + Cu(II) + Ca(II) + Mn(II) + Pb(II) +
Li(I) + Hg (II) + Cd(II) + As(V) + U(VI) + Sb(III)
+ Bi(III)+ Mo(VI) + Ce(IV) + Ti(III)+ Be(II) +
Cr(III) + Zr(IV) + Al(III) + V(V) + Mg(II).

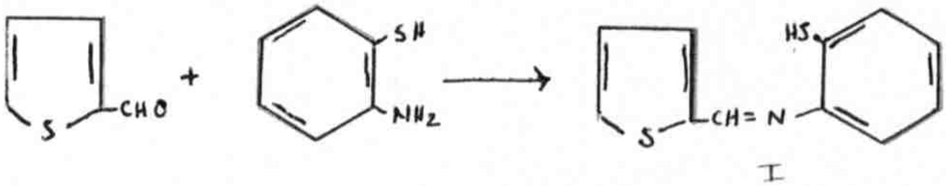
Solution (2): Solution (1) + Zn(II).

Results: Solution (2) produced a red colour while solution (1) gave a yellow colour.

These results are extremely satisfactory and show that the procedure is functional and can be applied for the detection of zinc in the presence of a very large number of foreign ions.

IV. DISCUSSION

The condensation of thiophene 2-aldehyde with o-aminobenzenethiol forms the following Schiff base (I)



I

In the absence of a study of the nature of the complex formed in solution with metal ions and its empirical formula it is difficult at this stage to postulate a definite structure for the complex.

The Schiff base appears to be a tridentate chelating agent having two atoms acting as donors: the nitrogen atom and the thiophene sulfur; the third point of attachment being through the salt forming mercapto sulfur. However, it is doubtful that a flat tridentate chelating agent could satisfy the stereochemical requirements of such a variety of metals as Zn(II), Cu(II), Pd(II), Co(II), Ni(II), Cd(II) all of which form red complexes with the reagent. It is very unlikely that such a tridentate could at the same time satisfy metals like Cu(II) and Pd(II) which are known to form planar complexes, Zn(II) and Cd(II) which form tetrahedral complexes and Co(II) which normally forms octahedral complexes.

On the other hand there is evidence that the Schiff base is acting as a bidentate, the points of attachment being coordination through the nitrogen atom and salt formation with the mercapto sulfur.

The latter point is substantiated by the fact that complex formation takes place in alkaline conditions when one hydrogen ion may be lost from o-aminobenzenethiol. The sulfur atom of the thiophene 2-aldehyde is not necessarily acting as a donor for complex formation because it appeared during the course of the work (see Part II) that equally good chelating agents were obtained when o-aminobenzenethiol was condensed with α-β unsaturated aldehydes which did not have atoms acting as donors. Further, an equally good chelating agent has also been obtained when o-aminobenzenethiol was condensed with furfural. However, when condensation took place with pyrrole aldehyde a Schiff base was obtained which did not give stable complexes. It would therefore seem that although the thiophene sulfur and the furfural oxygen do not act as donors they play an important role through their strong electronegative character. The sulfur of the aminobenzenethiol is however necessary because when o-aminophenol is used instead no chelation takes place.

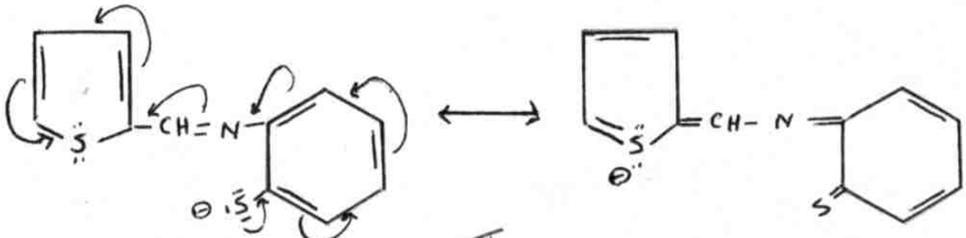
Since condensation of the benzenethiol with α-β unsaturated aldehydes gave a good chelating agent it would therefore seem that the reagent proposed in this work is a bidentate. Such an assumption would then explain the chelation of metals which normally form planar, tetrahedral and octahedral complexes. Thus two molecules of the base would be required for each ion having coordination number 4 irrespective of whether it is planar or tetrahedral, whereas three molecules would be required for octahedral complexes.

The presence of one nitrogen atom acting as donor, salt formation through replacement of an acidic hydrogen and the formation of a

5-membered ring may not however be sufficient to form red coloured complexes with different metals. Thus when o-aminobenzenethiol is condensed with an ordinary aldehyde the above conditions are satisfied and yet no red complexes are obtained. This would suggest that an additional factor is necessary which could be resonance arising through conjugation. This is substantiated by similar results obtained by Goldstein[27] and Hagopian[28]. Such an explanation could account for the behaviour of the reagent in the case of thiophene 2-aldehyde and pyrrole aldehyde but fails to explain the behaviour of furfural.

Thus Schiff bases obtained by condensing o-aminobenzenethiol with thiophene 2-aldehyde and furfural gave stable intensely coloured complexes whereas with pyrrole aldehyde unstable and weakly coloured complexes are obtained.

With thiophene 2-aldehyde the reagent can resonate as in (II),



II

producing a conjugation involving seven double bonds. With pyrrole aldehyde no such resonance could take place because the pyrrole nitrogen cannot expand its octet. Further in alkaline medium the pyrrole hydrogen is ionized leaving a negative charge on the nitrogen; this charge will not favour the inflow of electrons from the mercapto sulfur. This would account for the unstable slightly coloured complexes obtained in this case.

The explanation put forward above fails however in the case of furfural. It could be argued that since oxygen is more electronegative than sulfur, furfural should therefore favour resonance and more stable complexes formed. In fact with furfural more stable and more intensely coloured complexes are formed. However, such an explanation is not valid because it would force the oxygen to expand its octet, which it cannot do.

The mechanism evidently requires further investigation. One possibility to consider would be the formation of a carbanion next to the oxygen and nitrogen in furfural and pyrrole aldehyde respectively, whereas in the case of thiophene 2-aldehyde the negative charge is localized on the sulfur. Since oxygen is more electronegative than nitrogen it would be reasonable to expect that chelates obtained with furfural should be more stable than those obtained with pyrrole aldehyde.

PART TWO: NEW SPOT TESTS FOR CERTAIN ORGANIC COMPOUNDS

I. INTRODUCTION

In the course of the work performed in the first part of this thesis it appeared that o-aminobenzenethiol formed condensation products with a large number of aldehydes in acid solution. A total of 42 different aldehydes were investigated. It quickly became apparent that only α-β unsaturated and heterocyclic aldehydes such as thiophene 2-aldehyde, pyrrole aldehyde and furfural gave intense coloration when condensed with o-aminobenzenethiol.

A specific spot test for α-β unsaturated aldehydes was therefore developed.

While investigating the reactions of various aldehydes with o-aminobenzenethiol it was noticed that glyoxal behaved in a very remarkable manner, producing an intense violet coloration even at very low concentrations. A very sensitive test was therefore developed using this property.

It was also observed that the Schiff base obtained from the condensation of o-aminobenzenethiol and cinnamaldehyde proved to be an excellent chelating agent for zinc. This property was used to design a new sensitive and specific spot test for cinnamaldehyde.

Since cinnamaldehyde is usually obtained by condensing acetaldehyde with benzaldehyde, a very sensitive and selective test for acetaldehyde was also developed; it is based on the fact that cinnamaldehyde will be formed if benzaldehyde is added to an unknown which contains acetaldehyde. The cinnamaldehyde in its turn is detected through its own reaction.

Evidently the next obvious test to develop would be for ethyl alcohol since controlled oxidation of the alcohol would yield acetaldehyde which in turn can be detected. Satisfactory results are obtained when the ethyl alcohol is oxidized with a heated copper wire.

II. HISTORICAL

In the following sections some of the tests reported for the detection of the organic compounds that were investigated in the course of this work are summarized.

1.- α-β Unsaturated aldehydes

A literature survey of the methods reported for detecting α-β unsaturated aldehydes shows that all the spot tests recorded are applicable to α-β unsaturated as well as to aromatic aldehydes. Thus when an aqueous yellow solution of sodium pentacyanoammine ferroate, $Na_3[Fe(CN)_5NH_3]$ is treated with aromatic and α-β unsaturated aldehydes in the presence of hydrogen sulfide a deep blue colour is produced.[29]

A most recent method for detecting aromatic and α-β unsaturated aldehydes is based on the condensation of the aldehyde with thiobarbituric acid to yield orange products. Positive response is given also with polysaccharides, ethers, esters of cellulose and N-acrylic compounds. The test has microanalytical detection limits.[30]

2.- Glyoxal

A search of the literature has shown that the only valid test reported for glyoxal is the one based on the condensation of o-aminophenol with glyoxal giving a colorless Schiff base which, when treated with calcium ions forms a water soluble red inner complex. The limit of identification reported is 3γ.[31]

3.- Cinnamaldehyde

Methods for detecting cinnamaldehyde are numerous, however, most of them are tests which are common to all aliphatic and aromatic aldehydes and ketones.

An aqueous solution of azobenzene sulfonic acid when treated with aldehydes gives a blue or red colour. For cinnamaldehyde the limit of identification is 0.11γ.[32] Likewise the test mentioned in [30] above applies to cinnamaldehyde since it is both an aromatic and α-β unsaturated aldehyde. However, in this test all aromatic and α-β unsaturated aldehydes interfere.

Aldehydes may also be detected by the action of Ag_2CrO_4 in NH_4OH on the reaction product of dimedone and aldehyde. An intense red brown colour is obtained. The aldehydes which responded to this test are formaldehyde 1γ, citral 4γ, β-hydroxy naphthoic aldehyde 25γ, valeraldehyde 50γ, m-hydroxybenzaldehyde 50γ, cinnamaldehyde 60γ, and furfural 200γ.[33]

When a reagent composed of 100 ml of H_2SO_4 and 33.3 ml of H_2O and 0.7 gr. of 1-amino 8-naphtol 3,6 disulfonic acid is added to dilute aqueous solutions of an aldehyde a yellow colour is obtained. Sensitivities are as follows: formaldehyde 0.01%, benzaldehyde 0.01%, cinnamaldehyde 0.002%, furfural 0.02%. Ketones also give the same reaction but are much less sensitive.[34]

Methylene ketones with sodium nitroprusside and NaOH give a red-yellow colour which when acidified with acetic acid, changes to pink-violet. The only aldehyde which gives this colour reaction is cinnamic aldehyde. The limit of identification is not reported.[35] Rosenthaler has reported a similar method of identification for cinnamaldehyde: sodium nitroprusside and piperazine added to cinnamaldehyde gives a blue colour, ethyl alcohol in large amounts inhibits the reaction. The test is sensitive to 1 mg. of cinnamaldehyde.[36]

4.- Acetaldehyde

Acetaldehyde may be detected as an aliphatic aldehyde. Thus when an aldehyde is treated with $\phi NHNH_2.HCl$, diazobenzene sulfonic acid and NaOH, a colour is developed. Upon addition of $MgSO_4$ a precipitate is formed with a new colour. In this procedure the colours obtained are observed at three stages: in acid, in base and after the precipitate of magnesium is formed. For acetaldehyde, pink, carmine and red-violet colours were obtained. Colours were also formed with formaldehyde, propionaldehyde, butyraldehyde, isovaleraldehyde, salicylaldehyde, vanillin and p-dimethyl aminobenzaldehyde.[37]

Ethanol, methyl ketones and acetaldehyde when treated with o-nitrobenzaldehyde and KOH pellets produce indigoid colours which are extractable in chloroform.[38]

A selective and sensitive test for acetaldehyde employs sodium nitroprusside. When acetaldehyde or its vapors come in contact with a solution of the nitroprusside containing morpholine or piperidine, a blue colour is formed. Limit of identification: 1γ acetaldehyde.[39] However, acrolein, crotonaldehyde and tiglaldehyde interfere even at small concentrations. Propionaldehyde interferes at high concentrations.[40]

5.- Ethyl alcohol

Ethyl alcohol can be detected as an alcohol or as acetaldehyde through oxidation of the alcohol.

Small concentrations of ethyl alcohol are detected with a flame test using boric acid. Vapors of ethyl alcohol treated with borax in the presence of concentrated H_2SO_4 are converted to the ethyl borate ester characterized by a green flame. Methyl alcohol interferes.[41]

Alcohols are detected with 8-quinolinol complex of vanadium. If an aqueous solution of NH_4VO_3 is heated with oxime and then the alcohol, a red colour appears. Seventy three compounds were tested, of which only 25 alcohols, keto alcohols, and ether alcohols, gave positive response. All the rest, including phenols and enols gave negative results.[42]

To detect micro quantities of ethyl alcohol, a solution is treated with $K_2Cr_2O_7$, Na_2CO_3, phenylmethylaminopropane-HCl and 1% $Na_2Fe[NO(CN)_5]$ and boiled. A blue colour indicates ethyl alcohol. Limit of identification 50 γ . Ethyl acetate interferes.[43]

Finally ethyl alcohol may be detected best by oxidizing it to acetaldehyde by permanganate in the presence of sulfuric acid.[44] Once converted to acetaldehyde it is detected with nitroprusside containing piperidine or morpholine. Limit of identification:150γ .[44]

III. EXPERIMENTAL

In the following section, experiments carried out on various aldehydes are reported. In all cases the reagent used is a 25% solution of o-aminobenzenethiol in chloroform. All droppers were calibrated to deliver 0.05 ml drops.

1.- α-β Unsaturated aldehydes

Test with o-aminobenzenethiol.

Procedure: A drop of the test solution is treated in a test tube with four drops of concentrated HCl, two drops of the reagent and 10 - 15 drops of ethyl alcohol. The mixture is shaken and put in a water bath for one minute. One ml of petroleum ether is added and the colour of the lower layer observed.

A dark red colour indicates α-β unsaturated aldehydes.

Reagents: 25% of o-aminobenzenethiol in chloroform.
Ethyl alcohol.
Petroleum ether.
Concentrated HCl.

The above procedure was applied to a series of aldehydes representing α-β unsaturated, aromatic and aliphatic, unsaturated, heterocyclic aldehydes and dialdehydes. The following results were obtained:

Table 9

Colours obtained with various Aldehydes

Aldehyde	Colour obtained
Acrolein	dark red
Crotonaldehyde	dark red
Tiglaldehyde	dark red
2-Hexene-1-al	dark red
Citral	dark red
Cinnamaldehyde	dark red
All-trans Vitamin A aldehyde	dark red
Furfural	dark red
Thiophene 2 -aldehyde	dark red
Pyrrole aldehyde	dark red
Pyridine 2-aldehyde	orange
Pyridine 2,6 dialdehyde	orange
Salicyl aldehyde	orange
o-nitro salicyl aldehyde	yellow
Anisaldehyde	yellow
Benzaldehyde	yellow
o-chlorobenzaldehyde	yellow
Dimethylaminobenzaldehyde	orange
Vanillin	Yellow
Piperonal	yellow
Protocatechualdehyde	yellow
Veratraldehyde	yellow
Phtaldialdehyde	pale brown
Glutar dialdehyde	yellow
Glyoxal	black-violet
Formaldehyde	yellow
Acetaldehyde	dark orange
Propionaldehyde	yellow-orange
Butyraldehyde	yellow

Table 9, continued

Aldehyde	Colour obtained
Isobutyraldehyde	yellow
Isovaleraldehyde	yellow
n-Heptaldehyde	yellow
Caprylaldehyde	yellow
Chloral	colorless
Paraldehyde	orange
Paraformaldehyde	colorless
Citronellal	yellow
10-Undecen 1-al	yellow
Galactose	yellow
Lactose	yellow
Glucose	yellow
Maltose	yellow

Observations and Comments:

It is clear from table 9 that only the seven $\alpha-\beta$ unsaturated, furfural, thiophene and pyrrole aldehydes give an intense dark red colour.

Glyoxal can be differentiated from $\alpha-\beta$ unsaturated aldehydes by the colours produced. It is the only aldehyde amongst those tried that turns dark green upon addition of the reagent and black-violet when ethyl alcohol is added.

All-trans Vitamin A aldehyde develops a dark permanganate colour with the reagent but upon addition of petroleum ether the colour turns to a very intense red.

Acetaldehyde gives a colour which is similar to that produced by the α-β unsaturated aldehydes: if heating is omitted acetaldehyde will yield a yellow colour while the α-β unsaturated aldehydes will form an orange to light red colour.

Paraldehyde forms an orange colour; this is to be expected since in acid solution paraldehyde gives acetaldehyde.

Furfural when acidified gives a violet red colour prior to the addition of any reagent.

Pyrrole aldehyde gives with the reagent a red colour which upon heating and addition of petroleum ether turns brown.

The above procedure was also applied to the following possible interfering compounds. All solids were dissolved in chloroform. The following results are obtained.

Table 10

Colours obtained with possible interfering ions

Compounds	Colour obtained
Acrylic acid	pale brown
Oleic acid	yellow
Fumaric acid	yellow
Crotonic acid	yellow
Acetophenone	yellow
Benzophenone	yellow
Hydroquinone	yellow
Methyl 3-keto-Δ^1-cholenate	yellow
Δ^4- cholestene-3-one	yellow
Chloroform	yellow
Ethyl alcohol	yellow
Water	yellow

Determination of the Limits of Identification of the Various α-β Unsaturated Aldehydes

In order to obtain the highest sensitivities the procedure described above had to be slightly modified. The following was finally adopted.

Procedure: To one drop of the pure aldehyde or in chloroform add one drop of the reagent, one drop of concentrated HCl and heat to dryness. Compare with a blank.

The results obtained are summarized in Table 11:

Table 11

Limits of Identification of α-β Unsaturated Aldehydes

Aldehydes	pure or practical	500γ		250γ	50γ	5γ
		hot	cold			
Acrolein	dark red	orange	orange	pale yellow		
Crotonaldehyde	dark red	orange	orange	pale yellow		
Tiglaldehyde	dark red	dark yellow	pale yellow	white		
2-Hexen-1-al	dark red	orange	yellow	yellow		
Citral	dark red	orange	yellow	dark yellow		
Cinnamaldehyde	dark red	dark red	dark red	dark red	red-ora.	yellow
All-trans Vitamin A ald.	dark red	intense violet	intense violet	dark red	permanganate color	amethyst
Chloroform	yellow	white	white	white	white	white

Note: 0.5γ All-trans Vitamin A aldehyde still responds and gives a pale violet colour.

Observations and comments

In the above table the terms hot and cold refer to the observations made when the solution is heated to dryness and the colours noted when the test tube is still hot and cold respectively.

With All-trans Vitamin A aldehyde the following observations were made:

With 500γ the solution upon addition of petroleum ether goes blue by transmitted light and violet by reflected light.

With 50γ the solution goes pink-violet in both transmitted and reflected lights.

No great significance can be attached to the limits of identification of tiglaldehyde and citral since these were only available in "practical" form.

2. Glyoxal

Test with o-aminobenzenethiol.

Procedure: In a test tube one drop of the glyoxal test solution is treated with four drops of concentrated HCl and one drop of the reagent. The mixture is shaken for a minute and the colour is observed at this stage. (stage$_1$). Ten drops of ethyl alcohol are added, the test tube again shaken for a minute and the colour observed. (stage$_2$).

A very dark green colour at stage$_1$ and a dark permanganate colour at stage$_2$ indicate glyoxal.

Reagents: 25% of o-aminobenzenethiol in chloroform.
Concentrated HCl.
Ethyl alcohol 95%.

The above procedure was applied to a series of aldehydes and the results are shown in the following table:

Table 12

Colours obtained by aldehydes in the glyoxal test

Aldehyde	Colour at stage$_1$	Colour at stage$_2$
Acrolein	Pale brown	Pale brown
Crotonaldehyde	orange-red	orange-red
Tiglaldehyde	orange-red	orange-red
2-Hexen 1-al	red	red
Citral	brown-red	red
Cinnamaldehyde	red	red
All-trans vitamin A ald.	dark brown	dark permanganate colour
Furfural	brown	brown
Thiophene 2-aldehyde	scarlet-red	orange
Pyrrole aldehyde	brown-red	brown-red
Pyridine 2-aldehyde	yellow	yellow
Pyridine 2,6 dialdehyde	yellow	yellow
Salicylaldehyde	pale orange	yellow
o-nitrosalicylaldehyde	yellow	yellow
Anisaldehyde	orange	yellow
Benzaldehyde	orange	yellow
o-chlorobenzaldehyde	orange	yellow
Dimethylaminobenzaldehyde	orange	yellow
Vanillin	yellow	yellow
Piperonal	orange-red	yellow
Protocatechualdehyde	yellow	yellow
n-Veratraldehyde	orange	yellow
Phthaldialdehyde	turbid brown	brown
Glutardialdehyde	yellow	yellow
Glyoxal	dark green	very intense violet

Table 12 - continued

Aldehyde	Colour at stage$_1$	Colour at stage$_2$
Formaldehyde	yellow	yellow
Acetaldehyde	yellow	yellow
Propionaldehyde	yellow	yellow
Butyraldehyde	yellow	yellow
Isobutyraldehyde	yellow	yellow
Isovaleraldehyde	yellow	yellow
n-Heptaldehyde	yellow	yellow
Caprylaldehyde	yellow	yellow
Chloral	colorless	colorless
Paraldehyde	yellow	yellow
Paraformaldehyde	colorless	colorless
Citronellal	colorless	colorless
10-undecen 1-al	yellow	yellow
Galactose	colorless	colorless
Lactose	colorless	colorless
Glucose	colorless	colorless
Maltose	colorless	colorless

In order to ascertain that the colours obtained with glyoxal were not due to the reagents used the following experiments were carried out and the colours observed.

4 drops concentrated HCl + 2 drops reagent = colorless.
1 drop glyoxal 40% + 4 drops conc. HCl .. shake, heat = colorless.
1 drop glyoxal 40% + 4-5 drops EtOH .. shake, heat = colorless.
1 drop glyoxal 40% + 2 drops of reagent ..shake, heat = yellow.
1 drop glyoxal 40% + 2 drops of reagent + 2 drops NaOH .. heat = yellow.

Observations and comments

It appears from Table 12 that it is possible to differentiate between α-β unsaturated aldehydes and glyoxal at the first stage of the procedure. At stage$_2$ all α-β unsaturated aldehydes with the exception of cinnamaldehyde and all-trans Vitamin A aldehyde fail to respond at concentrations of 500γ, while glyoxal still gives a very intense colour.

Interesting colours changes are observed for different concentrations of glyoxal and at different stages of the procedures. These are reported below.

40% glyoxal: 1 drop of 40% glyoxal (in H_2O) + 4 drops of concentrated HCl, water bath for one minute, add 1 drop of the reagent, a blue-black colour forms. Add 10 drops of ethyl alcohol, a very dark violet colour is formed both in transmitted and reflected lights. Add 2 more mls of ethyl alcohol, a very dark red-violet colour is produced, add 8 more mls of ethyl alcohol the colour is still dark red-violet. Upon heating in water bath, colour remains the same. Addition of 20 mls of ethyl alcohol will not change the colour.

500γ Glyoxal: 1 drop of 500γ glyoxal (in H_2O) + 4 drops of concentrated HCl, water bath for one minute, add 1 drop of the reagent, a permanent deep green colour is formed. Add 10 drops of ethyl alcohol a violet colour is obtained in reflected light and dark Bordeaux in transmitted light. When heated in water bath for a minute colour goes darker. Further dilution with 1-2 mls of ethyl alcohol changes the colour to red-violet.

50 γ Glyoxal: 1 drop of 50 γ glyoxal (in H_2O) + 4 drops concentrated HCl, water bath for one mimute, add one drop of the reagent, a brilliant green colour is obtained both in reflected and transmitted lights. Add 10 drops of ethyl alcohol and shake a light amethyst colour both in reflected and transmitted light is formed. Heat in water bath, colour changes to violet. Further dilution with ethyl alcohol (1-2 mls) colour turns a shade lighter.

5 γ Glyoxal: 1 drop of 5 γ glyoxal + 4 drops concentrated HCl, water bath for one to two minutes, add 1 drop of the reagent, a light yellow colour both in reflected and transmitted light is formed. Add 10 drops of ethyl alcohol and heat in water bath, a very pale amethyst colour is produced in both transmitted and reflected lights. Further dilution will discharge the colour.

Determination of the Limit of Identification of Glyoxal

The following procedure is adopted for the determination of the limit of identification of glyoxal.

Procedure: To one drop of glyoxal test solution in water add 4 drops of concentrated HCl and one drop of reagent. Heat in a water bath for two minutes and then add 1-2 mls of ethyl alcohol and water bath for another two minutes. A pale violet to amethyst colour is formed. Compare with a blank which should give a colourless solution.

Limit of identification: 1 γ glyoxal

Limit of dilution: 1:50,000

3. Cinnamaldehyde

It was found that condensation of o-aminobenzenethiol with cinnamaldehyde gave a Schiff base which proved to be as good a chelating agent as o-mercaptothenalaniline. (see Part I). This property was used to develop a new sensitive test for cinnamaldehyde.

Test with o-aminobenzenethiol.

Procedure: In a test tube one drop of cinnamaldehyde test solution, two drops of reagent, one drop 500γ Zn(II) and one drop of 10% NaOH are added. The mixture is shaken, chloroform added and the colour in the lower layer observed.

A very intense bright red indicates cinnamaldehyde.

Reagents: 25% of o-aminobenzenethiol in chloroform.
10% NaOH.
500γ Zinc solution
Chloroform.

The above procedure was applied to a series of aldehydes. Results are shown in Table 13.

In order to ascertain that the colour obtained with cinnamaldehyde is not due to the reagents used, the following experiments were carried out.

1 drop pure cinnamaldehyde + 2 drops reagent.. shake.. = dark yellow.
1 drop pure cinnamaldehyde + 1 drop 500γ zinc..shake.. = colorless.
1 drop pure cinnamaldehyde + 1 drop 10% NaOH...shake.. = colorless.
1 drop pure cinnamaldehyde + 1 drop 10% NaOH..shake,heat= orange.
1 drop pure cinnamaldehyde + 2 drops reagent + 1 drop 10% NaOH..shake,heat= dark yellow.

Observations and comments

An examination of Table 13 shows that only pyridine 2-aldehyde and pyridine 2,6 dialdehyde give a wine red colour while furfural gives an orange colour. Furfural can however be differentiated from cinnamaldehyde by the addition of excess chloroform, when the colour becomes lighter while the cinnamaldehyde colour remains intense. Even the pyridine aldehydes give colours which are different from cinnamaldehyde.

Determination of the Limit of Identification of Cinnamaldehyde

The limit of identification of cinnamaldehyde was determined using exactly the above procedure with the exception that heating in a water bath is required before the addition of chloroform. A blank using chloroform is carried out and compared with the cinnamaldehyde. The colours are viewed from the top of the test tubes for maximum sensitivity.

Limit of identification: 0.5 γ cinnamaldehyde.

Limit of dilution: 1:100,000

A comparison of the two reactions of cinnamaldehyde with the reagent i.e.

a) condensation of the reagent in acid

b) condensation with the reagent and chelation with zinc in alkaline solution.

shows that the second procedure gives darker colours.

4. Acetaldehyde

Since the test for cinnamaldehyde proved to be so selective and sensitive it was decided to apply it to acetaldehyde. The argument being that condensation of acetaldehyde with benzaldehyde in alkaline solution yields cinnamaldehyde.

Test with o-aminobenzenethiol.

Procedure: To one drop of an acetaldehyde test solution in a test tube, one drop of pure benzaldehyde and one drop of 10% NaOH are added. The mixture is shaken, placed in a water bath for half a minute and cooled to room temperature. Two drops of reagent and one drop of 500γ zinc are added, the mixture is shaken, 20 - 30 drops of chloroform added and the colour of the chloroform layer observed.

A bright red colour indicates acetaldehyde.

Limit of identification: 5γ acetaldehyde.

Limit of dilute: 1:10,000.

Reagents: 25% of o-aminobenzenethiol in chloroform.
10% NaOH solution.
pure benzaldehyde.
500γ Zn(II) solution.
Chloroform.

The above procedure was applied to a series of aldehydes. Results are shown in Table 13.

In order to ascertain the effect of the various reagents used in this procedure, the following experiments were carried out.

1 drop pure acetaldehyde + 2 dp.of reagent = yellow..heat..yellow

1 drop pure acetaldehyde + 1 dp.10%NaOH = colorless..heat..brown-yellow

1 drop pure acetaldehyde + 1 dp.pure benzaldehyde + 1 dp.10% NaOH...yellow..heat = orange.

1 drop pure acetaldehyde + 1 dp. 10% NaOH + 2 dp. reagent....
yellow ... heat = orange

1 drop pure acetaldehyde ..acetaldehyde procedure with no
benzaldehyde = dark orange

1 drop benzaldehyde ... acetaldehyde procedure with no
acetaldehyde = yellow

As shown above acetaldehyde in the absence of benzaldehyde gives a dark orange colour.

Observations and comments

An examination of Table 13 shows that only furfural and cinnamaldehyde interfere.

Strangely, pyridine aldehydes which interfere in the cinnamaldehyde test, no longer interfere in the test for acetaldehyde.

Determination of the Limit of Identification of Acetaldehyde

In order to obtain the lowest limit of identification the procedure described above had to be slightly modified.

Procedure: In a test tube to one drop of acetaldehyde (in water or ethyl alcohol) one drop of pure benzaldehyde, one drop of 10% NaOH are added and the mixture is heated in a water bath for a minute. 8 to 10drops of concentrated HCl and two drops of the reagent followed by 10 - 15 drops of chloroform are then added and the colour of the chloroform layer observed. Comparison is made with a blank.

Limit of identification: 0.5 γ acetaldehyde.

Limit of dilution: 1:100,000.

Reagents: 25% of o-aminobenzenethiol in chloroform.

pure benzaldehyde

10% NaOH.

Concentrated HCl.

Chloroform.

5. Ethyl alcohol

Since controlled oxidation of ethyl alcohol yields acetaldehyde, a new test for ethanol is proposed whereby oxidation to acetaldehyde and condensation with benzaldehyde would give cinnamaldehyde. The latter is then identified as previously described.

It was found that satisfactory results are obtained when oxidation is achieved with a heated copper wire which does not oxidize ethyl alcohol to acetic acid.

Test with o-aminobenzenethiol.

Procedure: In a test tube one drop of the ethyl alcohol test solution is taken. A red hot copper wire is dipped and maintained for a few seconds until sizzling takes place. A drop of pure benzaldehyde, one drop of 10% NaOH are added, the mixture is shaken, placed in a water bath for one minute and then cooled to room temperature. Two drops of the reagent and one drop of 500 γ Zn(II) are added, the whole mixture shaken, 10 - 20 drops of chloroform added and the colour of the chloroform layer observed.

An orange to red colour indicates ethyl alcohol.

Limit of identification: 5γ ethyl alcohol.

Limit of dilution: 1:10,000.

Reagents: 25% of o-aminobenzenethiol in chloroform.

pure benzaldehyde.

10% NaOH.

500 γ Zn(II) solution.

Chloroform.

The above procedure was applied to a series of aldehydes. Results are shown in Table 13.

In addition the procedure was applied to the following alcohols and ethers and the results obtained are shown below:

Alcohol or ether used	Colour obtained
Methyl alcohol	yellow-white
Propyl alcohol	yellow-white
Isopropyl alcohol	yellow-white
Butyl alcohol	yellow-white
Ethyl ether	yellow-white
Butyl ether	yellow-white

Observations and comments

Examination of Table 13 shows that furfural, acetaldehyde and cinnamaldehyde interfere.

Determination of the Limit of Identification of Ethyl Alcohol

As with acetaldehyde the sensitivity of ethyl alcohol proved to be higher with a modified procedure.

Procedure: A red hot copper wire is dipped in a drop of the ethyl alcohol solution in a test tube, and maintained for a few seconds until sizzling takes place. One drop of pure benzaldehyde, one drop of 10% NaOH are added and the mixture is left in a water bath for one minute. 8 - 10 drops of concentrated HCl, one drop of reagent are then added and the colour of the bead which forms at the bottom of the test tube observed. Comparison is made with a blank.

Limit of identification: 1 γ ethyl alcohol.

Limit of dilution: 1:50,000.

Reagents: 25% of o-aminobenzenethiol in chloroform.

pure benzaldehyde.

10% NaOH.

Concentrated HCl.

Chloroform.

Table 13

Colours obtained by various aldehydes with cinnamaldehyde, acetaldehyde and ethyl alcohol procedures

Aldehydes	Colours obtained		
	Cinnamaldehyde procedure	Acetaldehyde procedure	Ethyl alcohol procedure
Acrolein	yellow	yellow	yellow
Crotonaldehyde	yellow	yellow	yellow
Tiglaldehyde	yellow	yellow	yellow
2-Hexen 1-al	yellow	yellow	yellow
Citral	dark yellow	dark yellow	yellow
Cinnamaldehyde	bright red	bright red	red orange
All-trans Vitamin A Aldehyde	yellow	yellow	yellow
Furfural	orange	orange	orange
Thiophene 2-aldehyde	yellow	yellow	yellow
Pyrrole aldehyde	yellow	yellow	yellow
Pyridine 2-aldehyde	wine red	yellow	yellow
Pyridine 2,6 dialdehyde	wine red	yellow	yellow
Salicyl aldehyde	yellow	yellow	yellow
o-nitrosalicyl aldehyde	yellow	yellow	yellow

Table 13 -continued

Anisaldehyde	yellow	yellow	yellow
Benzaldehyde	yellow	yellow	yellow
o-chlorobenzaldehyde	yellow	yellow	yellow
Dimethyl aminobenzaldehyde	yellow	yellow	yellow
Vanillin	yellow	yellow	yellow
Piperonal	yellow	yellow	yellow
Protocatechualdehyde	yellow	yellow	yellow
n-Veratraldehyde	yellow	yellow	yellow
Phtaldialdehyde	yellow	yellow	yellow
Glutar dialdehyde	yellow	yellow	yellow
Glyoxal	yellow	yellow	yellow
Formaldehyde	yellow	yellow	yellow
Acetaldehyde	yellow	light red	permanent orange
Propionaldehyde	yellow	yellow	yellow
Butyraldehyde	yellow	yellow	yellow
Isobutyraldehyde	yellow	yellow	yellow
Isovaleraldehyde	yellow	yellow	yellow
n-Heptaldehyde	yellow	yellow	yellow
Caprylaldehyde	yellow	yellow	yellow
Chloral	yellow	yellow	yellow
Paraldehyde	yellow	yellow	yellow
Paraformaldehyde	yellow	yellow	yellow
Citronellal	yellow	yellow	yellow
10-undecen 1-al	yellow	yellow	yellow
Galactose	yellow	yellow	yellow
Lactose	yellow	yellow	yellow
Glucose	yellow	yellow	yellow
Maltose	yellow	yellow	yellow

IV. DISCUSSION

The second part of this work has shown that o-aminobenzenethiol readily condenses with aldehydes in acidic solution and in particular with α-β unsaturated aldehydes to form dark red Schiff bases. Feigl[30] reports a similar condensation between aromatic, α-β unsaturated aldehydes and thiobarbituric acid. He does not propose a mechanism for the reaction. It seems however that o-aminobenzenethiol is a better reagent than thiobarbituric acid since it can differentiate between aromatic aldehydes and α-β unsaturated aldehydes.

The method proposed in this work is applicable to aliphatic, aromatic α-β unsaturated aldehydes, and to 5-membered heterocyclic aldehydes.

Colours obtained with pure α-β unsaturated aldehydes are extremely intense, even 1% solutions give dark red to orange colorations. In its present state however the method does not have the sensitivity which characterizes so many spot tests. Comparison of the limits of identification in Table 11 shows that these limits have widely different values, thus the test is capable of detecting 0.5γ All-trans Vitamin A aldehyde, 5γ cinnamaldehyde on the one hand and 500γ acrolein and crotonaldehyde on the other. This would suggest that the presence of an aromatic ring in the aldehyde renders the test more sensitive.

Another important factor that seems to determine the sensitivity of the test is the presence of conjugation. It would appear that the extent of the conjugation plays an important role in the intensity of the colours obtained.

Thus the Schiff bases obtained with acrolein, crotonaldehyde, tiglaldehyde, 2 hexen 1-al and citral, all of them contain in their system five double bonds and the limit of clear identification is 500γ. In citral a sixth double bond exists but is too far to contribute to the conjugation.

With cinnamaldehyde a system including eight double bonds is obtained and the limit of identification is 0.5γ.

With All-trans Vitamin A aldehyde the Schiff base obtained has nine double bonds and the limit is 0.5γ.

Glyoxal which condenses with two molecules of o-aminobenzenethiol will form a system with eight double bonds and the limit of the test is 1γ.

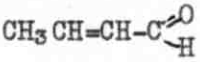
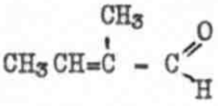
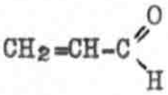
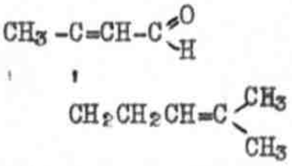
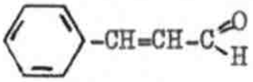
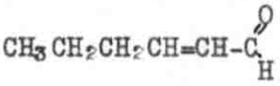
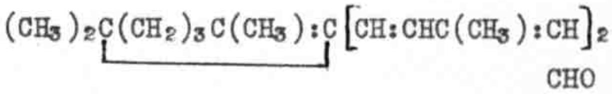
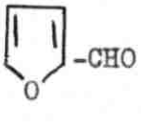
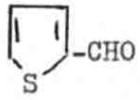
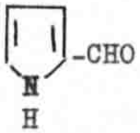
The correlation seems therefore good between sensitivity and number of double bonds in the system.

Another factor can still be considered in the formation of colour and that is resonance. The fact that o-aminophenol does not form coloured Schiff bases suggests that the sulfur atom in o-aminobenzenethiol is playing an important role in the initiation of resonance, probably through its smaller electronegativity and larger radius.

Evidently more work will be required to elucidate the mechanisms which are responsible for the formation of these intense colours.

The sensitive tests for cinnamaldehyde, acetaldehyde and ethyl alcohol which are far more selective than any reported in the literature, are all based on the fact that cinnamaldehyde forms with o-aminobenzenethiol a Schiff base which gives with zinc a red chelate in which conjugation, resonance, and chelation all probably contribute towards increasing its stability.

APPENDIX

Aldehydes	Source	Structure
Crotonaldehyde	Eastman Kodak	 $CH_3CH=CH-C\begin{matrix}O\\H\end{matrix}$
Tiglaldehyde	Eastman Kodak	 $CH_3CH=\overset{CH_3}{C}-C\begin{matrix}O\\H\end{matrix}$
Acrolein	Eastman Kodak	 $CH_2=CH-C\begin{matrix}O\\H\end{matrix}$
Citral	Eastman Kodak	 $\begin{matrix}CH_3-C=CH-C\begin{matrix}O\\H\end{matrix}\\ CH_2CH_2CH=C\begin{matrix}CH_3\\CH_3\end{matrix}\end{matrix}$
Cinnamaldehyde	Merck	
2-Hexen 1-al	Fluka	 $CH_3CH_2CH_2CH=CH-C\begin{matrix}O\\H\end{matrix}$
All-trans Vitamin A aldehyde	Eastman Kodak	 $(CH_3)_2C(CH_2)_3C(CH_3):C[CH:CHC(CH_3):CH]_2CHO$
Furfural	Merck	
Thiophene 2-aldehyde	Fluka	
Pyrrole aldehyde	A.U.B.	
Glyoxal	Fluka	$OHC - CHO$

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