CHAPTER III

ANALYTICAL APPLICATIONS OF ALLYL-ISOThIOCYANATE (AITc)

The literature on isothiocyanates abounds with references on the analysis of compounds with -NCS group. The majority of these references reveals the detection and determination of isothiocyanates in plant seeds and in mustard oils extracted from them, and most of them deal with allyl-isothiocyanate.

Despite of the fact that a variety of analytical reactions, meant for the analysis of isothiocyanates themselves have been reported in the literature, not a single chemical/analytical reaction has so far been worked out by any one that could describe an isothiocyanate as an analytical reagent for an anion or cation. This trend is quite reasonable because the isothiocyanates are, in general, considerably less reactive than the corresponding isocyanates, with whom they are isoelectronic.¹

¹This chapter also includes analytical work on certain other ligands besides AITc.
As far as the case of AITc is concerned, only two or three reactions of analytical importance are described in the literature.\textsuperscript{1-3} Thus, AITc has been reported to give a colour reaction with acidic fluoroglucinol solution directly,\textsuperscript{1} and with bismuth ion solution via the formation of 1-allyl-2-tetrazoline-5-thione through sodium azide reaction.\textsuperscript{2,3}

Our repeated efforts to explore AITc as an analytical reagent proved futile. It did not give any useful reaction product of analytical importance with any of the anions or cations. However, we did succeed in the case of bismuth(III) with which AITc gave an interesting colour reaction in presence of iodide ion. Thus, when a drop of 20\% AITc solution (in acetone, methanol or ethanol) was added to a slightly acidic bismuth(III) ion solution, and a drop of potassium iodide solution added, an intense red-orange precipitate or colouration developed. Interestingly enough, this reaction of AITc is like many other nitrogenous organic bases.\textsuperscript{4} The organic bases react with bismuth in presence of potassium iodide to form coloured products (double iodides) of general formula BiI\textsubscript{3}.base.HI, which may be regarded as salts of the base with iodo-bismuthic acid, H[BiI\textsubscript{4}]. The literature on AITc does not clearly state that AITc is a "base". The test for bismuth with AITc is, therefore, an unambiguous proof of the basic character of this mustard oil (cf. cinchonine, quinine, quinoline, etc.).\textsuperscript{5}

Similarly, using another method, AITc was converted to some unidentified thiourea compound\textsuperscript{*} on reaction with ethylene-

\textsuperscript{*Studies are in progress to characterize and identify the thiourea formed by the interaction of AITc with ethylenediamine.}
diamine. The resultant thiourea, like other thioureas, gave a yellow complex with Bi(III), which on further treatment with potassium iodide gave a deep orange product.

The above colour reactions involving the use of AITc, ethylenediamine, Bi(III), and iodide, have been used as sensitive and selective spot test methods for the detection of bismuth and iodide ions.

**EXPERIMENTAL**

**Reagents**

All the reagents used were of chemically pure grade.

**Allyl isothiocyanate:** 20% v/v solution in acetone or methanol.

**Ethylenediamine:** 20% v/v solution in water.

**Bismuth(III) nitrate pentahydrate:** 1% w/v solution in dilute nitric acid.

**Potassium iodide:** 1% w/v solution in water.

**Procedures**

**Micro Detection of Bismuth(III):**

(1) To one drop of the slightly acidic test solution add successively one drop of AITc solution and one drop of potassium iodide solution. A positive response is indicated by the formation of a deep red orange fleck (if test is conducted on a spot
paper or white filter paper) or precipitate (if the test is performed on a spot plate).

**Limit of detection:** 10 µg Bi(III)  
**Limit of dilution:** 1:5,000

(2) Two drops each of AITc and ethylenediamine solution are mixed in the groove of a spot plate and a drop of test solution is added. Immediate appearance of a canary yellow precipitate or colouration indicates the presence of Bi(III).

**Limit of detection:** 25 µg Bi(III)  
**Limit of dilution:** 1:2,000

**Micro Detection of Iodide:**  
When a drop of AITc solution is mixed on a spot plate with a drop of bismuth(III) nitrate solution, followed by the addition of a drop of test solution, a deep-orange precipitate or colouration appears immediately, indicating the presence of iodide.

**Limit of detection:** 15 µg I⁻  
**Limit of dilution:** 1:3,300

**RESULTS AND DISCUSSION**

**Interferences**

The study of interferences in the various tests were performed with the following ionic species:
Na(I), K(I), Li(I), NH₄(I), Ag(I), Hg(I), Hg(II), Ni(II), Co(II), Mn(II), Mg(II), Cu(II), Ca(II), Sr(II), Ba(II), Sn(II), Pb(II), VO(II), UO₂(II), Pd(II), Fe(II), Fe(III), Al(III), Cr(III), As(III), Sb(III), La(III), Rh(III), Ru(III), Eu(III), Pt(IV), Th(IV), Zr(IV), V(V), Mo(VI), W(VI), Cl⁻, Br⁻, F⁻, I⁻, NO₂⁻, CN⁻, N₃⁻, NO₃⁻, HCOO⁻, CH₃COO⁻, C₆H₅COO⁻, C₆H₄(o-OH)COO⁻, SO₃²⁻, SO₄²⁻, C₂O₄²⁻, CrO₄²⁻, Cr₂O₇²⁻, PO₄³⁻, IO₃⁻, IO₄⁻, MnO₄⁻, [Fe(CN)₆]³⁻, [Fe(CN)₆]⁴⁻, and [Fe(CN)₅NO]²⁻.

In the detection of iodide, the interfering ions are IO₃⁻, IO₄⁻, and S₂O₃²⁻. Small quantities of NO₂⁻ do not interfere, whereas comparatively larger amounts do.

In the test for bismuth, only Cu(II), Fe(III), S₂O₃²⁻, IO₃⁻, IO₄⁻ and larger amounts of NO₂⁻ interfere. No interference is caused by Pb(II), Hg(II), Cd(II), Sb(III), As(III) etc.

The above mentioned analytical work, though quite meager, clearly indicates the analytical potentiality of AITc, especially in the field of qualitative microanalysis. It is for the first time, therefore, that a compound containing -NCS group has been worked out as an analytical reagent. This work therefore, also opens the way to explore further analytical applications of AITc and other compounds containing -NCS group.
MISCELLANEOUS ANALYTICAL WORK

Analytical Applications of Phenothiazine

Very little work has been reported in the literature on the analytical applications of (I)\textsuperscript{6-9} which finds use as a major veterinary medicine to combat intestinal parasites in the cattle. The reaction of (I) with Pd(II) described initially by YOE & OLVERSER\textsuperscript{9} has now been found to possess special merit in qualitative analysis. A dilute acidic solution of PdCl\textsubscript{2} forms a very deep blue product with an acetone solution of (I). The test is highly sensitive and selective for detecting Pd and Pt when present in submicrogram amounts. The results of the reaction of (I) with Pd(II) is observed on spot plate and on the other hand with Pt(IV) in micro test tube.

EXPERIMENTAL

Reagents

Phenothiazine: 2% solution in acetone.

Palladium(I) Chloride: 0.5 g of PdCl\textsubscript{2} was taken in 100 ml of water, 5 ml of concd. HCl added, and then diluted to 250 ml. This solution was standardized by the oxine method, and further
diluted in parts to obtain solutions containing $\leq 50 \mu g$ of Pd$^{2+}$/ml.

PtCl$_4$ : 0.5% solution in water.

Ammonia solution: A 1:1 solution of concd. ammonia (sp. gr. 0.8 - 0.9) in water.

Recommended Procedures

Detection of Pd(II):
To a drop of slightly acidic test solution on the spot plate add a drop of 2% acetone solution of I. A positive response is indicated by the appearance of a deep blue colour or precipitate depending upon the quantity of palladium present.

Limit of detection: 1 $\mu g$ Pd$^{2+}$

Limit of dilution: 1:50,000

Detection of Pt(IV):
To 10 drops (0.5 ml) of the test solution in a micro test tube add 2-4 drops of 2% I solution in acetone. After shaking for about 2 min. green precipitate is formed indicating the presence of Pt(IV). On adding 1 or 2 drops of ammonia solution the colour of the precipitate appears more clear greenish.

Limit of detection: 50 $\mu g$ Pt$^{4+}$

Limit of dilution: 1:5,000
RESULTS AND DISCUSSION

Interferences

In the test for Pd: Following ions interfere: I\(^-\), NO\(_2\)^-, CN\(^-\), SCN\(^-\), SO\(_3\)^2-, S\(_2\)O\(_3\)^2-, CrO\(_4\)^2-, Cr\(_2\)O\(_7\)^2-, [Fe(CN)]\(_6\)^3-, [Fe(CN)]\(_6\)^4-, Au(III), and Fe(III), EDTA, thiourea, and dimethylglyoxime also interfere, Br\(_2\)^-, IO\(_3\)^- and IO\(_4\)^- do not interfere at pH 7, but they do below this pH level. In presence of [Fe(CN)]\(_5\)NO\(_2\)^-, Pd is identified by the development of deep violet precipitate.

In the test for Pt: The following ions cause interference: Fe(II), Fe(III), Au(III), NO\(_2\)^-, VO\(_3\)^-, MnO\(_4\)^-, [Fe(CN)]\(_5\)NO\(_2\)^-, and [Fe(CN)]\(_6\)^4-. In acid medium, Br\(_2\)^-, IO\(_3\)^-, IO\(_4\)^- and Cr\(_2\)O\(_7\)^2- seriously interfere, but exert no effect in neutral medium. The behaviour of CN\(^-\), SCN\(^-\), F\(^-\), N\(_3\)^-, SO\(_3\)^2- and CrO\(_4\)^2- is just reverse.

Nature of product in the phenothiazine test for palladium(II)

The elemental analysis of the PdCl\(_2\)/phenothiazine reaction product revealed a 1:2 (metal:ligand) ratio, i.e., Pd(\(_2\))^2Cl\(_2\):

Found : N, 5.2; S, 11.3; Pd, 18.2%.

Reqd : N, 5.0; S, 11.1; Pd, 18.4%.

The characteristic features of the I.R. spectrum of the product are the following: The C-S stretching frequency of the "Thia" group of I (at 690 cm\(^{-1}\)) has shifted to lower wave number by 30 cm\(^{-1}\), and the intensity of the band has also been reduced. This indicates coordination of Pd through sulphur atom of I.
This is corroborated by a new band at 350 cm\(^{-1}\) which is assigned to \(v(Pd-S)\). There is a single\(^{10}\) \(v(N-H)\) band at 3330 cm\(^{-1}\) in I, but in the complex, this band has splitted into two; one at 3380 cm\(^{-1}\) and the other at 3320 cm\(^{-1}\). The latter band is possibly due to the associated N-H group\(^{10}\) as a consequence of hydrogen bonding. This is believed to be a case of intermolecular hydrogen bonding between the hydrogen of the N-H group and the halogen of the metal halide. Such types of hydrogen bondings have been observed earlier by several workers.\(^{11,12}\) That N-H group is not involved in coordination is shown by the unchanged position and intensity of the \(\delta(N-H)\) group in I as well as in the complex, at 1620 cm\(^{-1}\). The C-N stretching vibration at 1320 cm\(^{-1}\) is also almost unchanged in both the spectra. The band at 260 cm\(^{-1}\) is associated to \(v(Pd-Cl)\).

The electronic spectrum of the complex shows an intense band at 40,000 cm\(^{-1}\) and a medium band at 20,833 cm\(^{-1}\). The latter band appears to be due to d-d transition,\(^{13}\) while the former may be an electron transfer band.\(^{13,14}\) These bands are therefore, assigned to \(^1A_{1g} \rightarrow ^1A_{2g}\) and \(^1A_g \rightarrow ^1B_{1u}\) transitions. The diamagnetism of the complex further confirms that the metal ion is in the singlet ground state. The singlet ground state of four coordinate Pd(II), and inferences from the I.R. and electronic spectral data suggest a square-planar geometry of the field around the metal ion. The structure II proposed for the complex is also confirmed by the conductance
experiment in THF, showing it to be non-ionic complex.

\[
\text{\textcolor{red}{S}} \quad \text{Cl} \quad \text{Pd} \quad \text{Cl} \quad \cdots \quad \text{H} \quad \text{N} \quad \text{S} \quad \text{Pd} \quad \text{S} \quad \text{N} \quad \cdots \quad \text{Cl} \quad \text{Pd} \quad \text{Cl}
\]

(Structure II)

**Analytical Applications of Acriflavine**

Various micro- and spot-test procedures are available in literature\textsuperscript{15,16} for the detection of palladium(II). A new simple and sensitive spot test for palladium(II) is described using the common antiseptic dye acriflavine (3,5-diamino-10-methylacridinum chloride monohydrochloride) (I) as the reagent.

The test for palladium involves the reaction of an aqueous acriflavine solution with an acidic solution of Pd(II) ion when a deep orange precipitate is formed. On adding ammonia solution the colour of the precipitate changes to deep red. However, on adding sodium hydroxide solution in place of ammonia, the orange precipitate assumes an intense purple colouration, but is not dissolved. The results of the reaction (with ammonia) are more satisfactorily and interestingly observed on the filter paper. On the other hand, the reaction with sodium hydroxide is best observed in a micro test tube.
**EXPERIMENTAL**

**Reagents**

*Acrididine solution*: 0.1 per cent w/v solution in water.

*Palladium(II) chloride solution*: 0.5 per cent w/v solution in water acidified with sufficient hydrochloric acid.

*Concentrated ammonia*: Sp. gr. 0.8 or 0.9.

*Sodium hydroxide solution*: 5 per cent w/v solution in water.

**Recommended Procedures**

**On filter paper:** Place a drop of 0.1 per cent aqueous acrididine solution on filter paper and mix 1 drop of slightly acidic test solution. Presence of palladium is indicated by the appearance of deep orange-coloured spot or ring surrounded by fluorescent yellow background. Now bring the spotted part of the paper over the mouth of ammonia bottle. The orange spot turns deep red or intense red-orange.

**Limit of detection:** 2 µg Pd(II)

**Limit of dilution:** 1:25,000

**In a micro test tube:** To 5 drops (0.25 ml) of the slightly acidic test solution add 1 or 2 drops of acrididine solution. A positive response is indicated by the formation of an orange precipitate or turbidity depending on the quantity of palladium present. Upon the addition of a drop of 5 per cent sodium
hydroxide solution, the orange compound changes to intense red, purple, or red-brown.

**Limit of detection**: 10 µg Pd(II)

**Limit of dilution**: 1:25,000

RESULTS AND DISCUSSION

Interferences

The following ions do not interfere in the tests for palladium involving acriflavine and ammonia on the filter paper or acriflavine and sodium hydroxide in micro test tube: Tl(I), NH₄(I), Ag(I), Zn(II), Cd(II), Sn(II), Cu(II), Ni(II), Co(II), Mn(II), Ca(II), Sr(II), Ba(II), Mg(II), Fe(II), Fe(III), As(III), Sb(III), Bi(III), In(III), Ru(III), La(III), Eu(III), Al(III), Cr(III), Pt(IV), Zr(IV), Ce(IV), NO₃⁻, VO₃⁻, IO₃⁻, IO₄⁻, BrO₃⁻, F⁻, Cl⁻, Br⁻, HCOO⁻, CH₃COO⁻, SO₄²⁻, CrO₄²⁻, Cr₂O₇²⁻, MoO₄²⁻, WO₄²⁻, S₂O₃²⁻, SiO₃²⁻, [Fe(CN)₅NO]²⁻, [Fe(CN)₆]³⁻, [Fe(CN)₆]⁴⁻, and salicylate. Hydrogen peroxide and formaldehyde also do not interfere.

The following ionic and other species constitute interferences in these tests: Hg(I), Hg(II), Au(III), I⁻, NO₂⁻, N₃⁻, MnO₄⁻, SO₃²⁻, C₂O₄²⁻, pyridine, α,α'-dipyridyl, hydrazine sulphate, and dimethylglyoxime. Whereas EDTA completely inhibits the test on filter paper, its presence gives an entirely different coloured but specific product in the test.
in micro test tube. PO₄³⁻ interferes in the 'filter paper' test, but very slight interference is caused in 'micro test tube' test.

**Nature of the product in palladium test with acriflavine**

Acriflavine, \([\text{C}_{14}\text{H}_{15}\text{N}_{3}\text{Cl}]^+\text{Cl}^-\), when treated in aqueous solution with complex ions, reveals its tendency to get precipitated in the form of salts. These salts are formed by the replacement of chloride ion attached to the heterocyclic nitrogen. Thus, such salts as acriflavine chromate, \([\text{C}_{14}\text{H}_{15}\text{N}_{3}\text{Cl}]_2\text{CrO}_4\), acriflavine antimonyl tartrate, etc. are described in literature. It has also been observed that acriflavine is also precipitated out from its aqueous solution when treated with such complex ions as ferricyanide, ferrocyanide, nitroprusside, dichromate, thiocyanate, and vanadate etc. In all these cases, the formation of corresponding salts of acriflavine are expected. Certain allied-natured salts, viz., those of acridine, 3,6-diamino-10-iodomethylacridine are also known. Similarly, when anhydrous palladium chloride (PdCl₂) is dissolved in water containing sufficient hydrochloric acid, there is formed a dark red-brown solution which seems to contain unstable chloropalladoic acid \(\text{H}_2[\text{PdCl}_4]\); the latter yielding tetra-chloropalladate ions \([\text{PdCl}_4]^2-\) in solution. In the course of present investigation, it was though that the orange product (precipitate) formed by the interaction of palladium(II) solution and acriflavine, probably involved the reaction of \([\text{PdCl}_4]^2-\)
anion with the dye cation \[C_{14}H_{15}N_3Cl]^+,\] resulting in the formation of the corresponding salt 'acriflavine tetrachloropalladate', i.e., \([C_{14}H_{15}N_3Cl]_2[PdCl_4].\) The analytical, magnetic, infrared and electronic spectral data, however, have ruled out the formation of such type of salt. These finding have given a strong indication to the formation of 1:1 (metal:ligand) chlorine-bridged complex having the structure II, in which the metal is linked to one of the primary amino groups of the dye through nitrogen. The II is practically insoluble or very feebly soluble in cold water and organic solvents, but considerably (though not completely) soluble in hot or boiling water, giving a yellow solution with intense green fluorescence. The elemental analysis of the complex \((C_{14}H_{15}N_3Cl)^+Cl^-\cdot PdCl_2\cdot2H_2O\) gave the following results:

**Found:** C, 32.8; H, 4.4; N, 8.3; Pd, 21.5%.

**Reqd:** C, 33.0; H, 3.7; N, 8.2; Pd, 20.8%.

The I.R. study of the complex points to a direct coordination of palladium with the amino group of acriflavine. The presence of a new band below 300 cm\(^{-1}\) at the same time indicates bridging Pd-Cl grouping i.e. (Pd-Cl-Pd).

In square-planar complexes, one should expect the following spin allowed transitions: (i) \(1\A_1g \rightarrow 1\A_2g\), (ii) \(1\A_1g \rightarrow 1\B_1g\) and (iii) \(1\A_1g \rightarrow 1\E_g\). Since the spectra of square-planar Pd(II) complexes show strong absorption in the range of 200 m\(\mu\)-750 m\(\mu\), it is very difficult to find bands corresponding to
these transitions. However, a single band at 18,518 cm\(^{-1}\) (540 m\(\mu\)) observed in the case of acriflavine/PdCl\(_2\) complex is assigned to \(^1\text{A}_{2g} \rightarrow ^1\text{B}_{1g}\) a transition which usually absorbs in this region.\(^{23}\) A second band observed at 14,705 cm\(^{-1}\) (680 m\(\mu\)) may be due to d-d transition.\(^5\) On the basis of preceding discussion, therefore, the following structure (II) is proposed for the acriflavine/PdCl\(_2\) complex:

![Structure (II)](image)

The structure II is further supported by the following experimental evidences: (i) it is practically insoluble or very slightly soluble in cold water and organic solvents, but considerably (though not completely) soluble in hot or boiling water, giving a yellow solution with intense green fluorescence. With AgNO\(_3\) and Hg\(_2\)(NO\(_3\))\(_2\), this solution gives a positive test for Cl\(^-\) ions. This shows that the chloride ion attached to the heterocyclic nitrogen is intact after complex formation. (ii) The solution of II in hot water is fluorescent yellow (like solution of the free dye) which gives a violet colour with nitrite + acid, a characteristic reaction of acriflavine due to its amino groups. This shows that in II, at least one of
the two amino groups of I is free to undergo diazo reaction. Therefore, the only possibility that remains for the attachment of the inorganic moiety (PdCl₂) to the dye molecule is through the other amino group, as in structure I.

The nature of red-coloured products formed by the action of ammonia or sodium hydroxide on II is not well understood as yet. These may be ammine or hydroxo complexes of II.
REFERENCES


17. G.S. JOHAR et al., New methods for detection of carboxylic acid groups in organic compounds with acriflavin, Talanta, 18, 1051 (1971).


