

Analytical properties of 2-acetylthiophene-4-phenyl-3-thiosemicarbazone: Spectrophotometric determination of copper(II) in edible oils and seeds

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The analytical properties of 2-acetylthiophene-4-phenyl-3-thiosemicarbazone (ATPT) are described for the first time. The reagent gives yellowish green coloured complex with copper(II) in sodium acetate - acetic acid buffer (pH 3.0 - 6.0) medium. This colour reaction (molar absorptivity, $2.92 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ at 385 nm) has been investigated for the spectrophotometric determination of copper in edible oils and seeds.

Thiosemicarbazones are interesting analytical reagents¹⁻³. Although thiosemicarbazones used for the spectrophotometric determination of metal ions, heterocyclic thiosemicarbazones are not exploited much. It will be of interest to investigate the analytical properties of thiosemicarbazones derived from sulphur containing heterocyclic carbonyl compounds. In the light of the above and in continuation of our previous work,^{4,5} herein we report the synthesis, characterization and analytical properties of 2-acetylthiophene-4-phenyl-3-thiosemicarbazone (ATPT). The spectrophotometric determination of copper(II) in edible oils and seeds using this reagent is discussed in this note.

Experimental

The reagent (ATPT) was prepared by simple condensation of 1 mol of 2-acetylthiophene with 1 mol of thiosemicarbazide. In a 250-ml Erlenmeyer flask, a hot methanolic (100 ml) solution of 2-thienyl methyl ketone (0.020 mol, 2.6 ml) and 4-phenyl-3-thiosemicarbazide (0.024 mol dissolved in 50 ml of hot distilled water and 20 ml of glacial acetic acid) were mixed and heated under reflux for 3 h. Shiny milky white crystals separated out on cooling the

reaction mixture. The crystals were collected by filtration and washed with hot water and 50 per cent cold methanol. The compound was recrystallised from benzene and dried *in vacuo*, yield 65%; m.p.(D) 181°C.

The compound was characterized by IR, ¹H-NMR and mass spectral data. Infrared spectrum of ATPT shows bands at 3297(m), 3223(m), 1589(s) and 1194(s) cm^{-1} respectively corresponding to ν_{NH} (imino), ν (C=N) and ν (C=S) stretching vibrations. ¹H-NMR spectra δ 2.344(s), 3H, CH₃; 7.059(dd), 1H, thiophene H (3&5); 7.706(d), 2H thiophene H(4); 7.248-7.437(m) 5H phenyl H, 8.701(s) 1H hydrazino proton; 9.308(s), 1H, phenyl imine (-NHPh).

Mass spectrum of the reagent shows signal at 275 (m/z) corresponding to its molecular ion peak. Other peaks at 260, 199, 182, 139 correspond to the loss of methyl radical, C₆H₄ molecule, aniline molecule and $\dot{\text{C}}(\text{S})\text{NH-NHC}_6\text{H}_5$ radical respectively. The fragmentation scheme of ATPT is given in Fig. 1.

The pK_a values were determined by recording the UV-visible spectra of micromolar ($1.6 \times 10^{-5} \text{ M}$) solution of ATPT at various pH values and by taking arithmetic mean of the values obtained from the measurements at four different wavelengths (Phillips and Merritt method). The value of the deprotonation of the reagent is found to be 6.0.

The reagent solution (0.01 M) was prepared by dissolving 275 mg of the compound in 100 ml of dimethylformamide and it is stable for at least 12 h.

Hydrochloric acid (1 M) - sodium acetate (1 M) (pH 0.5 - 3.5); (0.2 M) NaOAc - (0.2 M) AcOH (pH 4-6) and (2 M) NH₄Cl - (2 M) NH₄OH buffer solutions were used in the present study. The standard copper(II) solution ($1 \times 10^{-2} \text{ M}$) was prepared by using analytical reagent grade CuCl₂·2H₂O. The stock solution was standardized titrimetrically⁶.

The reactions of some important metal ions were tested at different pH values. The samples were prepared in 25-ml volumetric flasks by mixing 10 ml of buffer, metal ion. 2.5 ml of dimethylformamide (DMF) and 1 ml 0.01 M ATPT solution. The reaction mixture was diluted to the mark with distilled water. The absorbance was measured in 250-600 nm range against the reagent blank. Important analytical characteristics viz., pH and λ_{max} for ATPT complexes

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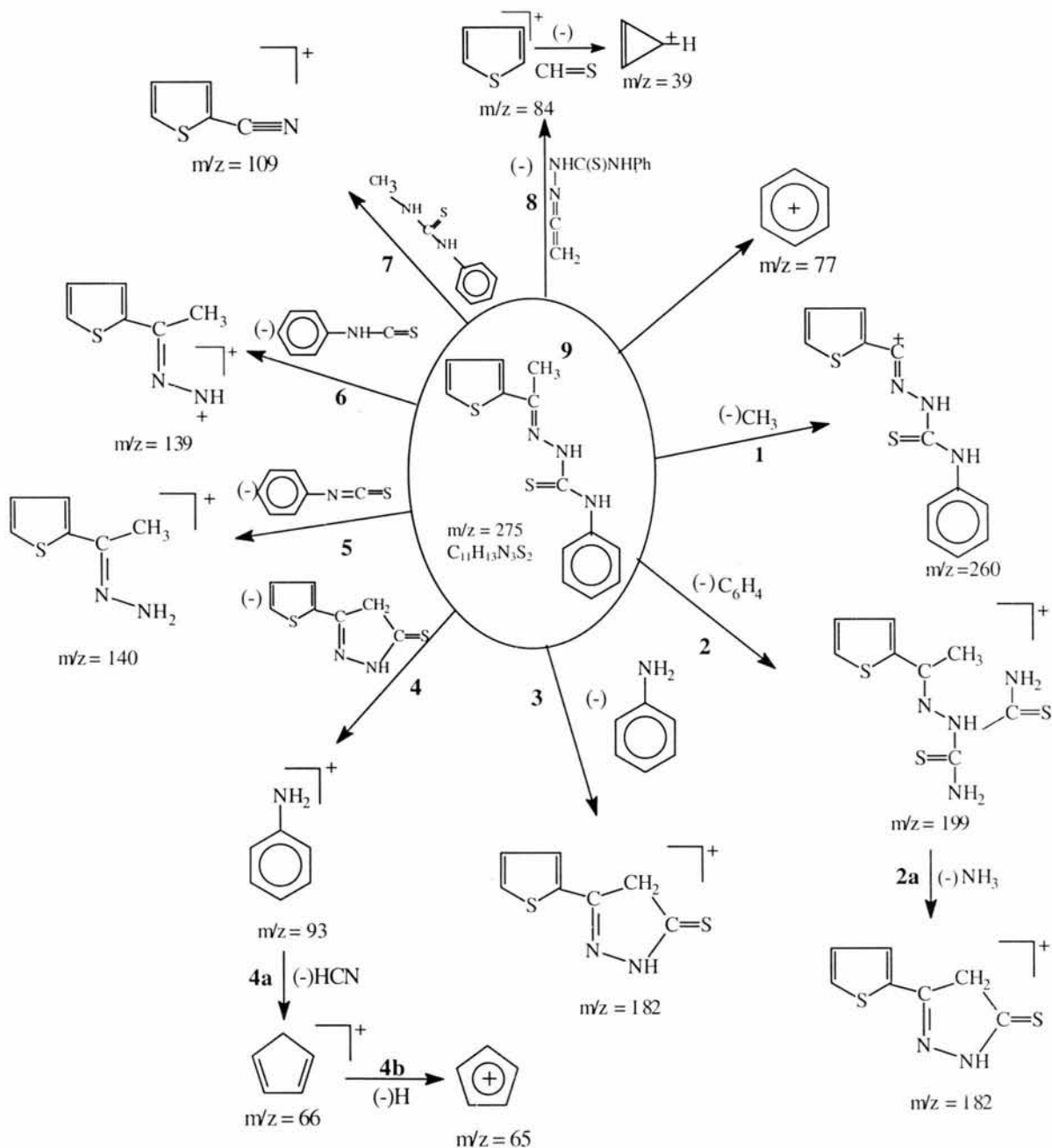


Figure 1—Mass spectral fragmentation pattern of ATPT

of Cu(II), Ni(II) and Co(II) are 3-5, 4-7, 6-7; 385, 370, 385 nm respectively. The results suggest that the reagent reacts with limited number of metal ions. Further, the molar absorptivities of Ni(II) and Co(II) complexes are quite low ($\epsilon \sim 10^3$ L. mol⁻¹ cm⁻¹). Therefore, the reagent is potential for the selective spectrophotometric determination of copper(II).

An aliquot of the solution, 0.10 - 0.51 $\mu\text{g ml}^{-1}$ (or ppm) of copper(II), 10 ml of NaOAc - AcOH buffer

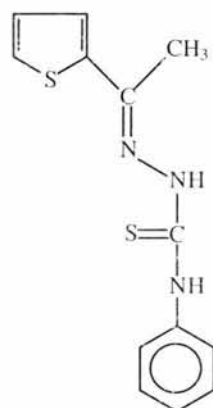
solution (pH 4.0), 2.5 ml of DMF and 2 ml of 1×10^{-3} M ATPT solution were combined in a 25-ml standard flask and the mixture was diluted to the mark with distilled water. The absorbance of the solution was measured at 385 nm against reagent blank. The measured absorbance is used to compute the amount of copper from pre-determined calibration curve.

Schimidzu 160A UV visible spectrophotometer equipped with 1.0 cm quartz cell and an ELICO

model LI-120 pH meter were used in the present study.

Results and discussion

The reagent, 2-acetylthiophene-4-phenyl-3-thio-semi-carbazone (**I**) is easily obtained under reflux conditions. A 0.001 M solution of ATPT is stable for 12 h. The bathochromic shift of absorption band from 330 to 340 nm indicates that in solution on increasing the pH, the acid is neutralized and the >C=S group is enolized or dissociated⁷.



(I)

In basic medium (above pH 6), the ligand presumably exists in enolic form and coordinates the divalent metal ion as mono anion to give (1:2) neutral complex.

Determination of copper(II)

Copper(II) reacts with ATPT in acidic pHs to give pale yellow coloured complex. The colour reaction is instantaneous even at room temperature. The order of addition of reagent, metal ion, buffer has no effect on the absorbance of the complexes, provided DMF is added prior to the addition of reagent solution. The absorbance of the yellow coloured complex (λ_{\max} 385 nm) remains constant for 5 h. The maximum colour intensity is observed in the pH range 3.0-6.0. A 5-fold molar excess of the reagent is adequate for full colour development. Addition of excess reagent has no adverse effect on the absorbance of the complex.

The system obeys Beer's law over the concentration range 0.10 to 0.51 $\mu\text{g ml}^{-1}$. The optimum range for the determination of copper(II) from Ringbom's plot is found to be 0.13-0.484 $\mu\text{g ml}^{-1}$. The molar absorptivity and Sandell's sensitivity of the method are found to be $2.92 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ and 0.0218 $\mu\text{g cm}^{-2}$ respectively. The specific absorptivity of the system is 0.460 $\text{ml g}^{-1} \text{ cm}^{-1}$. The

relative standard deviation for ten replicate analyses of solution containing 0.41 $\mu\text{g ml}^{-1}$ copper is 2.86%.

Job's and molar ratio methods gave the composition of the complex as 1:1 (M:L). The stability constant of the complex as determined by Job's method is found to be 2.1×10^6 .

Interferences

The effect of some of the ions which often accompany copper has been studied by adding different amounts of anions and cations to 0.41 $\mu\text{g ml}^{-1}$ of copper in solution. The colour is developed as described in the recommended procedure. An error of $\pm 2\%$ in the absorbance reading is considered tolerable. The results are given in Table 1. Higher amounts of iron(III) could be tolerated in the presence of fluoride.

Analysis of edible oils and seeds

Edible oil and oil seed sample solutions were prepared by following the procedure given in the literature⁸. A 50 g of the sample (in the case of seeds it was finely grinded) was heated in a 500 ml conical flask with 40 ml of concentrated HNO_3 on a steam bath and shaken vigorously until a fine emulsion was formed. The heating was continued with the gradual addition of 40 ml of 6% H_2O_2 . The aqueous phase was then transferred to the beaker. The extraction was

Table 1—Tolerance limit of foreign ions in the determination of 0.41 $\mu\text{g/ml}$ of copper

Ion added	Tolerance limit ($\mu\text{g/ml}$)	Ion added	Tolerance limit ($\mu\text{g/ml}$)
Iodate	1525	W(VI)	2205
Tartarate	1185	Ba(II)	1650
Bromide	960	Sr(II)	1050
Sulphate	770	Ca(II)	480
Phosphate	760	Al(III)	320
Nitrate	495	Mg(II)	290
Bicarbonate	490	Pb(II)	250
Urea, carbonate	480	Cd(II)	135
Acetate	470	Zr(IV)	110
Thiocyanate	465	Zn(II)	80
Chloride	425	Co(II), Fe(III) ^a	70
Citrate	380	Ni(II)	70
Ascorbate	350	Mn(II)	65
Cyanate	335	Sn(II)	50
Fluoride	230	Mo(VI)	40
Iodate	75	Fe(II)	20
Thiosulphate	45	Ag(I)	1.3
Thiourea	30	V(V)	0.76 ^a
Oxalate	7	Hg(II)	0.25 ^b
EDTA	0.06		

^a Masked with 200 $\mu\text{g/ml}$ of fluoride

^b Masked with 400 $\mu\text{g/ml}$ of chloride

Table 2—Determination and recovery of copper in edible oils and oil seeds

Sample	Copper found* ($\mu\text{g/g}$)		Copper added ($\mu\text{g/ml}$)	Copper (found)*		Recovery (%)	
	ATPT method	AAS method		ATPT method	AAS method	ATPT method	AAS method
Groundnut seed	4.373	4.440	1.0	0.987	1.026	98.7	102.6
Sesame seed	11.510	11.748	1.0	1.015	0.892	101.5	89.2
Groundnut oil	0.579	0.596	1.0	0.979	0.995	97.9	99.5
Sesame oil	1.270	1.289	1.0	0.993	0.988	99.3	98.8
Palmolen	N.D	0.256	1.0	1.021	0.981	-	98.1
Coconut oil	N.D	0.099	1.0	1.018	0.983	-	98.3
Mustard oil	1.215	1.236	1.0	1.027	1.015	102.7	101.5
Sunflower oil	0.641	0.648	1.0	0.964	1.016	96.4	101.6
Hydrogenated Vegetable oil	N.D	0.270	1.0	1.024	0.988	102.4	98.8
Chocolates (Eclairs)	0.975	0.989	1.0	1.015	1.018	101.5	101.8

*Average of three determinations.

N.D. Not determined.

repeated twice with further addition of 20 ml of concentrated nitric acid and 20 ml of 6% H_2O_2 . The combined extracts were evaporated to dryness. The residue was dissolved in minimum amount of dil. HCl and transferred into a 50 ml standard flask quantitatively. The contents were diluted to the mark with distilled water.

To 10 ml of NaOAc-AcOH buffer solution (pH 4.0), 1 ml of 0.1 M sodium fluoride, oil sample solution (in the optimum concentration range), 2.5 ml of DMF and 1 ml of 0.001 M reagent solution were added. The solution was diluted to volume (25 ml) with distilled water and the absorbance was measured at 385 nm against reagent blank. The results are given in Table 2.

A known quantity (1 $\mu\text{g/ml}$) of copper(II) were added to the sample and the amount of metal ion was estimated as described above. The recovery data are incorporated in Table 2.

The reagent, 2-acetylthiophene-4-phenyl-3-thiosemicarbazone may be considered as heterofunctional ligand which is found to be potential for the spectrophotometric determination of copper(II) in aqueous medium. The method compares favourably with several spectrophotometric methods⁹⁻¹⁸ using phenyl thiosemicarbazones. These methods involve extraction procedures which are expensive, laborious and time consuming while in the present method copper is estimated in aqueous medium. The reagent is easy to synthesize using commercially available precursors. Further, the present method is applied for the determination of copper in edible oils. Moreover, the method is simple, rapid, and selective without the need for heating or extraction.

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