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Use of Tetrazolium Salts as Selective and Sensitive Reagents for Iron in Environmental Samples

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Tetrazolium salts have been suggested as analytical reagents for metals and non-metals. The elements Tl(III), Cr(VI), Fe(III) (HASHMI et al. 1965, ALEXANDROV et al. 1976), Sn (NUTESCU & TODOR 1979), Fe(II) (HASHMI et al. 1972), Co(II) (MEHRA & LEBLANC 1979), Au(III), Ce(IV), Pt(II) (HASHMI & RASHID 1966), and Zn(II) (ALEXANDROV & KAMBUROWA 1976) have been determined in aqueous systems in the microgram range through the use of tetrazolium salts. Non-metallic species such as BrO_3^- and IO_3^- (HASHMI et al. 1970) and the insecticide "Gardona" (VASSILEVA & SHISHMANOV 1977) have also been determined through their colored products which were generated with tetrazolium compounds.

The usefulness of these versatile reagents has been extended to the analysis of trace amounts of iron in environmental, industrial, and pharmaceutical samples. The present communication deals with the analytical use of 2,3,5-triphenyl-2H-tetrazolium chloride (TTC), 3,3'-(4-4'biphenylene)-bis-2,5-diphenyl-2H-tetrazolium chloride (NTC - also termed as neotetrazolium), 3-(4,5-dimethylthiazolyl-2,5-diphenyl-2H-tetrazolium bromide (DTB) and 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl-2H-tetrazolium chloride (PTC). The chemical structures of these species are shown in Figure 1. These reagents undergo a reductive reaction with the species Fe(II) in an alkaline medium thereby producing a colored water-insoluble formazyl derivative. In fairly acidic conditions, 0.5 - 3.0 N H_2SO_4, this colored pigment is extractable in non-aqueous solvents such as methyl isobutyl ketone (MIBK), and the absorption of the extract in the visible region varies linearly with Fe(II) concentration. This characteristic has been employed in the quantification of iron in a variety of samples and even speciation of Fe(II) and Fe(III) has been accomplished.

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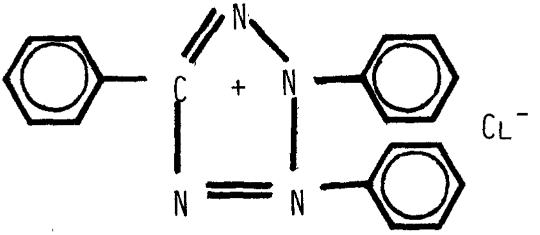
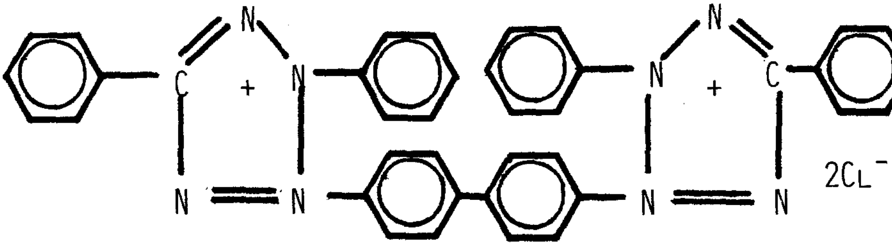


2,3,5-triphenyl-2H-tetrazolium chloride (TTC)



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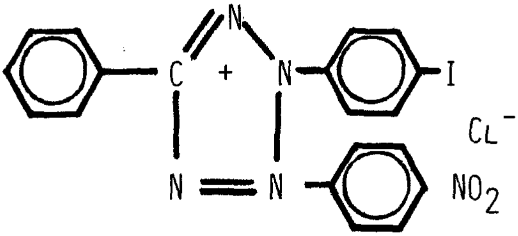
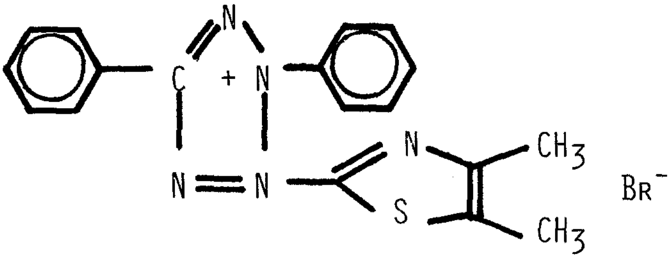


2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl-2H-tetrazolium chloride (PTC)



3-(4,5-dimethylthiazoyl-2)-2,5-diphenyl-2H-tetrazolium bromide (DTB)

Figure 1: Chemical Structures of Tetrazolium Salts

MATERIALS AND METHODS

Reagents: Standard iron(II) solution was prepared by dissolving the required ferrous ammonium sulphate hexahydrate in double-distilled water acidified with dilute sulphuric acid. Standard solutions of TTC (2%, Aldrich), NTC (0.2% in 10% ethanol; Aldrich), DTB (0.2% in 10% ethanol; Eastman) and PTC (0.2% in 10% ethanol; Aldrich) were prepared by direct weighing. All other reagents employed were also of analytical grade purity. The standard solutions of metal cations were prepared by dilution of 1000 ppm atomic absorption standards obtained from Fisher Scientific Co.

Apparatus: A double-beam recording spectrophotometer was used for absorbance measurements. The analytical data were obtained through the use of 1-cm optically-matched cells.

Procedure for Iron Analysis: To an appropriate volume of the samples solution containing up to 180 μg of iron(II) in a 25-mL flask, add 2 mL (0.2 mL in case of TTC) of the tetrazolium reagent solution followed by 0.2 mL (2 mL in case of PTC) of 10% sodium arsenite solution, 0.6 mL of 20% citric acid and 5 mL of dimethylformamide (DMF). Shake the solution and add concentrated sulphuric acid to make it 1 N with respect to the acid. Extract the colored species formed with 10 mL of MIBK and measure the absorbance of the extract at its λ_{max} against distilled water blank as reference.

RESULTS AND DISCUSSION

The absorption characteristics and other pertinent data of the extracted species in MIBK from 1 N H_2SO_4 are shown in Table 1. The analytical response of the Fe-PTC system (ϵ - 1.1 x 10^4L $mol^{-1}cm^{-1}$) evidently is superior to the others investigated. Hence experimental conditions were further established for the PTC system in order to develop a general analytical method for iron analysis in practical samples.

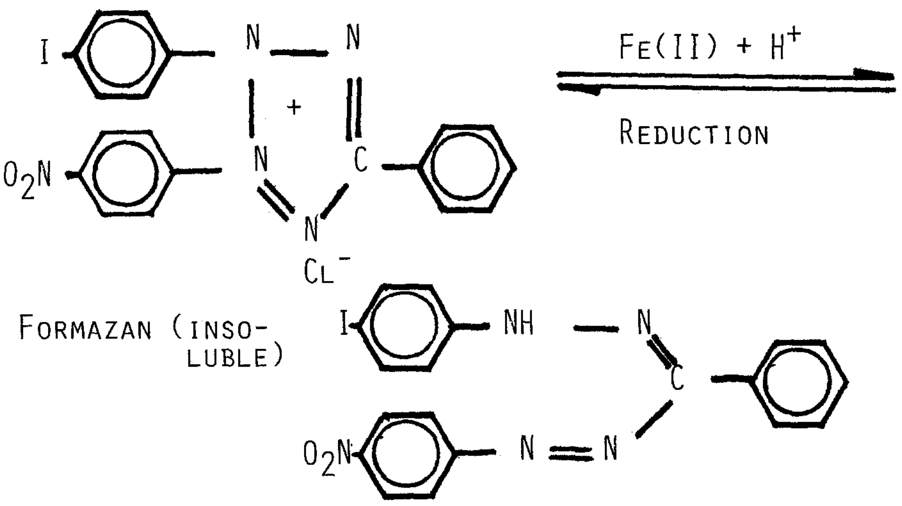
The extracted species in MIBK suffers no deterioration even on prolonged standing (several days). The acidity range 0.5 - 3.0 N H_2SO_4 does not influence the absorbance characteristics, and this in itself is the most useful feature of this procedure. In an earlier study on the Fe(II)-TTC system (HASHMI & RASHID 1966) in alkaline conditions (pH $\simeq$ 8), the absorbance was stabilised with sodium arsenite and citric acid in a dimethylfforamide medium. However, the

Table 1. Characteristics of Iron Complexes with Tetrazolium Salts in Methylisobutyl Ketone

Complex	Color	λ_{max} (nm)	ε ($L \cdot mol^{-1} \cdot cm^{-1}$)	Linear analytical range (ppm)
Fe-PTC	Orange-red	480	1.1×10^4	0 - 13.5
Fe-DTB	violet	555	5.3×10^3	2 - 18
Fe-TTC	Orange-red	480	2.2×10^3	3 - 12
Fe-NTC	Orange-red	500	2.2×10^3	1 - 13

linear analytical range reported in this case lies between 50 and 160 ppm. The present study with PTC in an acidic medium has a linear range between 0 and 13.5 ppm Fe(II), and thus not only offers an increased sensitivity but also an operational simplicity. The samples requiring strong acid treatment (soils, alloys, biological materials) are thus amenable to iron analysis in a straightforward manner.

The tetrazolium salts are known to undergo reduction with reducing agents and the red species in all probability stems from the ring clevage leading to the formation of a formazan. In this case where Fe(II) acts as a reductor, the Fe-PTC reaction may be described as



Since very few inorganic cations exercise such a reductive influence on tetrazolium compounds, this reaction thus is selective toward iron analysis. The ready extraction of formazan produced in a non-aqueous phase further augments the sensitivity. In fact the common cations and anions present in low concentrations do not interfere with iron analysis by this method. Furthermore Fe(III) up to 5 fold excess over Fe(II) does not cause any ill effects. This in turn has been made use of in the speciation of iron in a sample by successive determination of Fe(II) and Fe(III). Initially Fe(II) is determined in an aliquot in the usual manner and then the total iron is measured through the use of a suitable reductor (hydroxylamine) to render the entire system into the Fe(II) state. some results of the synthetic mixtures of Fe(II) and Fe(III) are shown in Table 2.

Table 2. Successive Analysis of Fe(II) and Fe(III) with 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazolium (PTC)

Iron added (ppm)		Iron found (ppm)
Fe(II)	Fe(III)	total
2.24	-	2.24
2.24	8.0	2.24
2.24	2.0*	4.24
-	2.0*	2.00

* 1% Hydroxylamine hydrochloride employed as reductor to reduce Fe(III) to Fe(II).

Iron has also been determined in a variety of environmental and industrial samples. The iron contents of pharmaceutical products, alloys, and soil samples have been analyzed employing this simple photometric procedure and results compared with another sensitive spectrophotometric reaction of iron with a newly reported reagent ferene (ε - 3.5 x 10^4L mol^{-1} cm^{-1})(ARTISS et al. 1981).

The general procedure for the pharmaceutical products analysis entailed dissolution of a weighed quantity of the sample in a minimum quantity of HCl and dilution to an appropriate volume to render the final HCl concentration to the order of 0.03 M. A suitable aliquot was then analyzed for the iron content by the described procedure. The sample was reduced with 1% $NH_2OH{\cdot}HCl$ if Fe(III) was known to be present in a sample.

The soil or an alloy sample (0.5g) on the other hand was treated with 10 mL of 6 N HCl and heated for 30 min and further treated with 2 mL of H_2O_2, filtered, and made up to 1-L volume. A suitable aliquot in either case was further treated with 2.5 mL of 10% $NH_2OH \cdot HCl$ and the iron contents determined in the usual manner.

The comparative data for some analyses are shown in Table 3. The results obtained compare favorably in either case. The analytical precision is good, since for repetitive determination (at least 6 determinations), coefficient of variation did not exceed 2% at the most. The data show that relatively inexpensive tetrazolium salts in an acidic medium can be successfully employed for the trace analysis of iron in samples of interest.

Table 3. Comparative Data on the Spectrophotometric Analysis of Iron in Pharmaceuticals, Alloys, and Soils with 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazolium chloride (PTC)

	Commercial name	Form	PTC Method	Ferene Method
1.	Pharmaceuticals			
	Palafer Capsule (Beecham)	Solid	27.1 mg/ 100 mg	27.0 mg/100 mg
	Ferograd (Abbott)	Solid	24.28 mg/ 100 mg	25.02 mg/100 mg
	Fergon (Winthrop)	Solid	6.14 mg/ 100 mg	6.13 mg/100 mg
	Palafer liquid (Beecham)	Liquid	14.25 mg/ g syrup	-
	Incremin Syrup (Lederle)	Liquid	2.03 mg/ 5 mL syrup	-
2.	Soils and Alloy Samples			
	Elmwood Drive		16.90 mg/g	17.25 mg/g
	Steadman Street		21.66 mg/g	22.25 mg/g
	Botsford Street		22.20 mg/g	23.25 mg/g
	K-041 (Al-alloy)		11.60 mg/g	11.86 mg/g

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