CHAPTER – II

SECTION – A
DETERMINATION OF SOME THIOUREA DERIVATIVES IN THEIR FORMULATIONS AND RESIDUES
Thiourea, the thio analogue of urea, is the thioamide of carbonic acid, and therefore, often referred to in literature as thiocarbamide. The importance of thiourea is well known. Special chapters in specialized treatise deal with its chemistry, properties, derivatives and applications. Thiourea and its organic derivatives find various synthetic and industrial applications. The most important of the latter are in rubber vulcanization, in electrodeposition of metals, in corrosion inhibition for steel and in polymer, textile, agriculture and pharmaceutical industry.

Ammonia and amines react with organoisothiocyanates yielding mono-, di- and trisubstituted thioureas. Bisthioureas are similarly prepared by reacting diamines with organoisothiocyanates. Though most substituted thioureas have lower melting points than thiourea, they are all nicely crystalline. For this reason, they serve as excellent derivatives of amines for their characterization purposes. Reactions of thiourea and substituted thioureas with alkyl halides to form isothiouronium salts are useful in synthesis and in characterizing the halides.

As the reaction suggests, since both nitrogen and sulphur have free electron pairs, thiourea has two possible reactive sites. Dipole moment measurements have been interpreted as indicating that the thiourea is a resonance hybrid with an almost equal contribution from the following three canonical forms:

Thione form

Isothiouonium forms

Thiourea and its substituted analogues are remarkable for the number and variety of complexes that they form with the metal salts. The specific properties i.e. colour, crystallinity, solubility etc. of many of these complexes are used for analytical purposes. The applications of thiourea and its derivatives in the extraction, separation and spectrophotometric, gravimetric, potentiometric, polarographic or amperometry determination of a variety of metals have been reviewed. With a view of obtaining high degree of specificity and sensitivity in the spectrophotometric determination of metal
ions, many new thiourea derivatives have also been synthesized by various workers\textsuperscript{10-14}. Thiourea derivatives have recently been reported as corrosion inhibitors for mild steel in formic acid\textsuperscript{15}. The role of thioureas in improving the analytical performance of atomic absorption spectroscopic determination of arsenic, antimony, bismuth, selenium, tellurium, tin and platinum has also been reported in literature\textsuperscript{16,17}.

Because of their reactive nature and commercial importance, the determination of thiourea and its organic derivatives has been a subject of extensive analytical researches. The compounds have been determined by numerous methods involving a variety of procedures and techniques. The various methods are based either on their tendency to react with metals to undergo desulphurisation or their oxidation using a variety of oxidants. Various acidimetric, colorimetric and chromatographic methods have also been used. The work has been reviewed in several books/monographs \textsuperscript{1,2,18-20}.

**Desulphurisation methods:**

The determination of thioureas based on their desulphurisation in the presence of silver ion has been the most useful, but zinc, cadmium and lead salts have also been used\textsuperscript{21}. Among the numerous variations involving silver ion, the method of Cuthill and Atkins\textsuperscript{22} is perhaps the most convenient procedure. The authors treated thiourea with an excess of ammonical silver nitrate solution and titrated the excess reagent, after acidification of the solution with nitric acid and removal of the precipitated silver sulphide, against standard thiocyanate using ferric alum as an indicator.

\[
2\text{AgNO}_3 + 2 \text{NH}_4\text{OH} + \text{CS(NH}_2\text{)}_2 \rightarrow \text{Ag}_2\text{S} + \text{CO(NH}_2\text{)}_2 + 2\text{NH}_4\text{NO}_3 + \text{H}_2\text{O}
\]

The method suffers from the disadvantage that thiocyanate and halogens interfere. Such interferences can, however, be removed by using the soluble Na[Ag(CN)\textsubscript{2}] complex to desulphurise thiourea. The amount of silver removed as silver sulphide was determined by the readdition of silver(I) until the Na[Ag(CN)\textsubscript{2}] solution is again in balance. The determination of thioureas by direct potentiometric titrations with silver nitrate using ion sensitive electrodes, has also been reported by some workers\textsuperscript{23,24}.

\[
2 \text{Na[Ag(CN)}_2]\text{]+CS(NH}_2\text{)}_2 + 2 \text{NaOH} \rightarrow \text{CN.NH}_2 + \text{Ag}_2\text{S} + 4 \text{NaCN} + 2 \text{H}_2\text{O}
\]

\[
4 \text{NaCN} + 2 \text{AgNO}_3 \rightarrow 2 \text{Na[Ag(CN)}_2]\text{] + 2 NaNO}_3
\]
Oxidimetric methods:

Based on the reducing behaviour of thioureas, a variety of oxidimetric procedures have been described in literature for the determination in acidic, basic, neutral or buffered solutions.

Singh and Verma have found that in acid medium potassium iodate, iodine halides (monochloride, monobromide and trichloride), potassium dichromate, diethylene tetra-ammonium sulphocerate (DTS), potassium bromate, chloramine-T, potassium ferricyanide and cerium(IV) oxidise thiourea and its N-substituted derivatives to the corresponding substituted formamidine disulphides and used these oxidants for the oxidimetric determination of thioureas. Chauhan and Afshan described the use of ammonium hexanitratocerate(IV) in nitric acid medium for the micro scale determination of thiourea and its derivatives. The unreacted reagent was determined by titrating against iron(II) using ferroin indicator. The same authors also used potassium dipertelluratocuprate(II) as an oxidant for the indirect determination of thiourea and its derivatives. The unreacted copper(II) was determined by arsenite method. Kiryushov et al. described the use of potassium iodate for the determination of thiourea in process solutions from gold extraction plants. The use of bromine in acid solutions for the oxidimetric determination of thioureas has also been reported, but oxidation proceeds beyond formamidine disulphide stage and more or less the corresponding urea plus sulphate ion are formed.

Under basic conditions, methods using hypoiodite have received considerable attentions. Some workers have, however, used hypobromite which has been found a satisfactory oxidant in the determination of thiourea and tetramethyl thiourea. Thiourea like other carbonyl compounds has also been determined by its digestion with alkaline hydrogen peroxide followed by the measurement of alkali consumed. Alternatively, sulphate formed can be determined gravimetrically. Thiourea and its derivatives are also oxidized by iodine in bicarbonate-buffered solution; the reaction stoichiometry is comparable to that observed in most strongly basic media. The method gives better results and has the added advantage that if an excess of iodine is used, the excess oxidant can be back-titrated against arseneous oxide in the same medium. The use of copper(II) and cerium(IV) in acetonitrile has been described by Verma et al. for the oxidimetric...
determination of substituted thioureas. The authors extended these methods to the
determination of isothiocyanates after the derivatisation of the latter with secondary
amines, to the corresponding thiourea derivatives.

Voltammetric methods based on the reducing behaviour of thioureas have also
been reported in literature\textsuperscript{45}. The polarographic behaviour of thioureas at dropping
mercury electrode (DME) is generally believed to be due to their oxidation to
formamidine disulphides\textsuperscript{45,46}. Thiourea electro-oxidation ($E_{1/2} < 0.7V$ (vs SCE)) indicate
formamidine disulphide as the main product. Honggnang et al.\textsuperscript{47} supported the above
\begin{equation}
2 \text{RNH}_2 \text{C} \equiv \text{S} \quad \leftrightarrow \quad 2 \text{RNH}_2 \text{C} \equiv \text{SH} \quad \rightarrow \quad \text{RNH}_2 \text{C} \equiv \text{S} \equiv \text{S} \equiv \text{C} \equiv \text{NHR} + 2 \text{H}^+ + 2 \text{e}^{-}
\end{equation}
oxidation of thiourea in acidic solution using voltammetric methods. The cyclic
voltammograms on platinum, gold and glassy-carbon electrodes showed a single pair of
peaks for the oxidation of thiourea to formamidine disulphide and its subsequent
reduction. The electrochemical response of thioureas and formamidine disulphides on
polycrystalline platinum in aqueous sulphuric acid using voltammetry and rotating disc
electrode techniques has also been studied by other workers\textsuperscript{48-51} and subsequently used
the data to develop voltammetric methods for the determination of thioureas. The
polarographic behaviour of thiourea derivatives at DME in solutions of various pH has
been reported in literature \textsuperscript{2,45}. The anodic half-wave potentials for a series of N-
substituted thioureas were found to be $-0.30 \pm 0.25$ and the shifts in potentials were
correlated with the nature of substituents and pH of the solution. Lawrence et al.\textsuperscript{52} used
this technique to the determination of ethylene thiourea (ETU) and related compounds
viz. N-methyl ETU, thioimidazol and thiohydantoin in rat urine. The determination at
DME have been made at pH 3.0 in potassium nitrate supporting electrolyte. Owing to the
ability of thioureas (containing sulphur in $-\text{SH}$ form) to form partially insoluble
complexes with mercury, cathodic stripping voltammetry has been developed as a
technique for the determination of some thiourea containing agrochemicals by Smyth and
Osteryoung\textsuperscript{53}. The limits of detection at $10^{-7} - 10^{-3}$M levels were, however, found to be
lower than by polarographic methods based on anodic waves observed at DME.
Thioureas have also been determined polarographically following complexation with
copper(II) ions or by liberation of sulphur atom and subsequent determination of the latter
as hydrogen sulphide\textsuperscript{54}. Lee et al.\textsuperscript{55} developed a arsenic incorporated lead dioxide
electrode (As/PbO₂) for studying the oxidation of thiourea and subsequently determined the latter by a constant-potential amperometry using above electrode in a thin layer electrochemical cell. The use of organic phase enzyme based biosensors (OPEB) based on the change in the electrocatalytic current of peroxide reduction by the biosensor has been reported for the determination of urea and ethylene thiourea⁵⁶.

**Spectrophotometric methods:**

A spectrophotometric method for the determination of thiourea based on reaction with nitric acid and bromine has been developed⁵⁷. The cyanogen bromide formed gives an intense dye with pyridine and barbituric acid, which is measured at 589 nm. Another colorimetric method uses a modified Grote-reagent (sodium nitroprusside reduced with hydroxylamine and oxidized with bromine). Absorption at 610 nm is determined and compared with standard curves⁵⁸. Sodium nitroprusside as a suitable reagent for spectrophotometric determination of thiourea has also been mentioned by other workers⁵⁹,⁶⁰. Anisimova et al.⁶¹ developed a spectrophotometric method for the determination of thiourea based on reaction with pentacyanoferrate(II) which can be obtained by the base hydrolysis of sodium nitroprusside at pH ≥ 12, followed by the acidification of the solution at pH 6. The coloured complex is measured at 590 nm and thiourea in the range 10⁻⁴ to 10⁻⁶ M can be determined by this procedure. Thiourea in copper electrolyte has also been determined spectrophotometrically⁶²,⁶³. In the first method thiourea is separated by precipitation/coprecipitation and determined spectrophotometrically using a mixed reagent containing iodine and starch. The second method consists in extracting thiourea with ethyl acetate from a acetic acid-sodium acetate buffer and then determined spectrophotometrically at 450 nm. Chiba⁶⁴ determined 1-amidino-2-thiourea, a nitrification inhibitor in fertilizers, by UV spectrophotometry. The compound was extracted with water and then determined at 262 nm.

**Catalysis of iodine-azide reactions:**

Sodium azide reacts with iodine only in the presence of compounds possessing in their molecules the atom of dinegative sulphur. Sulphur compounds act as inductor of this

\[ 2 \text{NaN}_3 + I_2 \rightarrow 3 \text{N}_2 + 2 \text{NaI} \]
reaction. The amount of iodine consumed or nitrogen evolved is proportional to the amount of sulphur compound present in the sample. This reaction has found wide applications in the determination of thioureas. The determination is concluded by either measuring the consumed iodine or nitrogen evolved. That thiourea can be determined by enthalpimetry with the use of catalytic iodine-azide reaction, has also been mentioned.

**Acidimetric methods:**

In the presence of mercuric acetate and in non-aqueous media thiourea can be titrated with strong acids. Alicino, and Bayer and Posgay titrated thiourea and allyl thiourea directly with perchloric acid in acetic acid. Although, the role of mercuric acetate is not certain, it has been formulated as follows:

\[
2 \text{H}_2\text{N} \equiv \text{C} \equiv \text{SH} + \text{Hg(OAc)}_2 \rightarrow \left[ \text{H}_2\text{N} \equiv \text{C} \equiv \text{S} \right]_2 \text{Hg} + 2 \text{AcOH}
\]

One mole of thiourea consumes an equivalent of acid.

**Thioureas as pesticides:**

Schroeder reviewed the biological applications of thioureas and it was revealed that a large number of these compounds exhibit fungicidal, herbicidal and insecticidal activities. Of the various thioureas exhibiting pesticidal properties, thiophanate-methyl and thiophanate find maximum use as fungicides. They exhibit a rather broad anti fungal spectrum and are extensively used against various crops, fruits and vegetable diseases. In view of their wide applications, they have been determined by a variety of techniques.

Miller and coworkers have developed a colorimetric method for the determination of thiophanate-methyl in milligram and sub-milligram amounts and subsequently adapted it for its analysis in its formulation and residues on foodstuffs. For milligram amounts, the aqueous suspension of the fungicide was shaken with copper(II)-triethanolamine-alkali reagent, the precipitate formed filtered off and unconsumed copper(II) in the filtrate was reacted with pyridine-thiocyanate. The coloured product was extracted in chloroform and measured spectrophotometrically at 435 nm. For sub-milligram amounts, copper-thiophanate methyl complex formed in colloidal solution, was measured directly at 290 nm. Alternatively, unconsumed copper(II) can also be
determined colorimetrically by diethyldithiocarbamate procedure\textsuperscript{76}. In an IUPAC report\textsuperscript{77} on the review of methods for the residue analysis of thiophanate-methyl/thiophanate, the compound was recommended to be heated with methanol-hydrochloric acid and ethanolic silver nitrate respectively, thereby covering it to carbendazim. The latter was measured by spectrofluorimetric/spectrophotometric techniques. Mestress et al.\textsuperscript{78} however, determined thiophanate-methyl residues in fruits and vegetables by converting the compound into 2-methyl benzimidazolo-2-yl-carbamate (MBC) by heating with dimethylformamide and ammonia. The product was extracted into hydrochloric acid and measured spectrophotometrically at 282 nm. Ono et al.\textsuperscript{79} have determined this fungicide and its degradation product MBC by UV spectrophotometry on 16 different crops. The residue sample was shaken with methanol, which extracts, both of them. The extract was acidified with hydrochloric acid and again shaken with dichloromethane, when thiophanate-methyl was selectively extracted. The extract was heated with copper(II) acetate in aqueous-acetic acid (1:1 v/v), thereby converting the fungicide into MBC. After clean up, the latter was determined spectrophotometrically at 282 nm. Gnegi et al.\textsuperscript{80} also gave a similar method for the determination of thiophanate-methyl in residues on vegetables and fruits.

Methods based on the transformation of thiophanate-methyl into carbendazim with subsequent determination of the latter by GC-MS and HPLC have also been reported in literature. Anastassiades and Scherbaum\textsuperscript{81} determined thiophanate-methyl residues in citrus fruits by converting the fungicide to carbendazim under alkaline catalysis. Carbendazim was derivatised using pentafluorobenzyl bromide (PFBB) and potassium carbonate as catalysts and determined by GC-MS technique. Fruit samples were extracted with acetone and liquid-liquid partition was carried out with a mixture of cyclohexane-ethyl acetate (1:1 v/v) to extract the fungicide. After clean up, the compound was converted into carbendazim, derivatised and determined gas chromatographically using mass spectroscopic detection. The method was subsequently modified\textsuperscript{82,83} by extracting the fungicide involving super critical fluid extraction with carbon dioxide and further analysis performed without additional cleanup either by GC-MS after derivatisation or directly by HPLC using diode array detection. The authors claimed that this approach is faster and cost effective than traditional solvent-based approaches.

The conversion of thiophanate-methyl into carbendazim/MBC and determination of latter by HPLC using UV detector has been made use of in determining this fungicide
in residues on soil, air and agricultural products. The method has also been extended
to the determination of thiophanate methyl/degradation products in the presence of
benomyl fungicide in fruits by other workers. Duan and Hao used this technique for
simultaneous determination of thiophanate-methyl, aldicarb, primicarb, carbaryl,
carbofuran, benomyl and carbendazim. Li and Sun separated thiophanate-methyl and
thiram from mixture preparations and determined by HPLC on C18 column at 270 nm
with isopropanol-water (40:60 v/v) as mobile phase. The use of this technique for the
determination of above fungicides has also been reported by other workers.

Thin layer chromatography has been applied for the determination of thiophanate-
methyl and its degradation products. Ito and coworkers have used gas chromatography equipped with flame photometric detector for the determination of
thiophanate-methyl and its metabolites. Baker and Hoodless have reviewed the
analytical methods for the detection and determination of residues of systemic fungicides
including thiophanate-methyl. The applications of reverse phase HPLC, liquid
chromatography/atmospheric pressure chemical ionization mass spectrometry for the
determination of this fungicide in residues have also been reported in literature.

**Thioureas as drugs:**

Another important application of thioureas is their use as drugs. They are the
active ingredients of many drug formulations as they possess antithyroid, anticonvulsant,
antitumor and sedative properties. The antithyroid activity of compounds bearing thiourea
function was discovered in 1943 with the introduction of thiouracil for the treatment of
thyrotoxicosis. Thiouracil was replaced by its most effective 6-methyl and 6-propyl
derivatives in 1953 for their clinical use in human hyperthyrodism. The compounds
prevent iodination of the precursors of thyroxin and triodothyroxin. Thiouracils are the
condensation products of thiourea and sodium derivatives of β-ketonic esters

\[
\text{HN} - \text{C} - \text{SH} + \text{HC} = \text{CR} \cdot \text{ONa} \rightarrow \text{HS} - \text{N} = \text{C} - \text{OEt} + \text{NaOH} + \text{EtOH}
\]

(ethyl formyl acetate). Radioiodine-labelled thiourea has also been synthesized and
evaluated as a radiopharmaceutical for establishing the viability of ocular melanoma after
radiation treatment. Rehenium(III) complexes of the type [(ReL₆)-L] X₃, with L =
thiourea, N'-methyl thiourea, N-ethyl thiourea, n-ethylene thiourea and X = Cl or PF₆⁻ have been reported as suitable precursors for the synthesis of some nuclear medicines\cite{105}.

Thiourea can be acylated by malonic acid to give thiobarbituric acid. The efficiency and low toxicity of thiobarbituric acids containing all kinds of substituents in all possible positions have been synthesized. The derivatives with one or preferably two alkyls in the 5-position have received considerable attention as they show valuable physiological properties. They are prepared by reacting thiourea with alkyl malonic esters

\[
\text{EtO} - \text{CO} \rightarrow \text{S} = \text{C} \rightarrow \text{NH}_2 \rightarrow \text{EtO} - \text{CO} \quad \text{EtO} - \text{CO}
\]

The best known of these compounds is thiopentone or thiopental (with R-ethyl; R'1-methyl butyl). The sodium salt of thiopentone (commercially known as thiopentone sodium) is a very quick acting thiobarbiturate containing sodium 5-ethyl-5-(1-methyl butyl)-2-thiobarbiturate and anhydrous sodium carbonate. It is used in drug preparations as hypnotics and sedatives and is given intravenously for the production of light anesthesia. Recently, the use of thiourea and its derivatives in some drug preparations for the treatment of tumor and AIDS control has also been reported\cite{106,107}. The activity of these drugs has been thoroughly tested and the investigations are in process for incorporation in the drug schedule.

Because of the extensive applications of thiourea-based drugs, their analytical chemistry has attracted a lot of attention. Titrimetric methods have found maximum applications for the determination of these compounds in drug formulations. However for their trace determination in biological fluids, various chromatographic and spectrophotometric methods have been used.

Thiouracils react quantitatively with silver nitrate; the reaction thus enables their determination. Several variations of this basic method have been employed by various

\[
\text{S} = \text{C} \rightarrow \text{NH} \quad \text{N} = \text{C} \rightarrow \text{R} \quad \text{N} = \text{C} \rightarrow \text{R} \quad \text{N} = \text{C} \rightarrow \text{R}
\]

workers in developing titrimetric methods for the determination of above drug compounds. The commonly employed method for the analysis of thiouracils in commercial drugs, however, utilizes the titration of liberated nitric acid with standard sodium hydroxide\cite{109}. The method suffers from the drawback that it cannot be used in the
presence of sucrose, lactose, calcium carbonate or steric acid (drug excipients) and consequently cannot be applied as such for the assay of these drugs in tablet formulations. To overcome this difficulty, an alternative procedure based on the measurement of excess silver nitrate in acidic medium with ammonium thiocyanate has been proposed. The above argentometric method has subsequently been adapted to the assay of thiopental in drug formulations. In these determinations, one mole of thiobarbiturate consumes four moles of silver. The use of mercuric acetate has also been reported in the pharmacopial assay of thiouracils in drug formulations including tablets. That these compounds ionize and assume mercapto form and form insoluble mercuric salts with the reagent in 2:1 molar ratio, has been made the basis of the method. A weighed quantity of the sample is dissolved in dilute sodium hydroxide solution to form the corresponding sodium salt (ionic mercapto form), mixed with sodium acetate and acetic acid. The buffered solution is then titrated with mercuric acetate using diphenylcarbazone as an indicator to a colour change from red to violet. Amperometric titrations with the reagent have also been successful. The mercurimetric method is more accurate and precise than argentometric method and is relatively free from interferences. Lactose, however, interferes with the end-point, but this interference can be eliminated by preliminary treatments. Based on the formation of a slightly soluble mercury salt of thiopentone sodium (a thiobarbiturate) with mercury on a hanging mercury drop electrode (HMDE) surface, a simple and extremely sensitive cathodic stripping voltammetric method for the determination of thiopentone sodium has been described by Ali et al. The reduction current of the formed salt was measured by linear sweep and differential pulse cathodic stripping voltammetry. The method has been successfully applied to the determination of thiopentone sodium in pharmaceuticals, urine and human serum samples. The polarographic determination of these compounds has also been reported in the literature.
That thiouracils are oxidized with bromine solution and the excess bromine can be determined iodometrically forms the basis of a titrimetric method for the determination of propyl thiouracil in tablets. Zima et al.\textsuperscript{112} determined thiouracils with coulometrically generated iodine or bromine. The authors recommended that for titration with iodine, pH 7 is optimum. The determination with bromine can be carried out in acidic medium. Thiobarbiturates have also been determined oxidimetrically with bromine and N-bromosuccinimide\textsuperscript{113}. Jogdanker et al.\textsuperscript{114} studied the oxidation of thiopentone with potassium iodate using starch indicator and proposed iodate-starch solution as a chromogenic spray reagent for the TLC detection and determination of drug.

Thiouracils and thiobarbiturates are weak monobasic acids and have been determined by titration with bases/alkalies in non-aqueous solvents. The use of sodium hydroxide in ethanol and sodium methoxide in anhydrous dimethyl formamide medium has been reported for the purpose. Thiopentone sodium furnishes sodium hydroxide when dissolved in water and latter can be measured by titration with standard hydrochloric acid. This forms the basis of the official pharmacopial method for the assay of thiopentone sodium injections\textsuperscript{109}. This method is however based on the measurement of sodium content of the drug. For determining the thiopentone content also, the titrated solution is extracted with successive quantities of chloroform. The chloroform is removed by evaporation and resulting residue is dried and weighed to give the content of thiopentone in the drug.

Spectrophotometric assay methods for above drug compounds have been reported in literature\textsuperscript{115}. The reaction between thiouracils and nitrous acid to form coloured thiocyanic acid and the use of 2,6-dichloroquinone, chloramide and cobalt(II) acetate as chromogenic agents have been described in developing such methods. UV spectrophotometry has also been applied for the determination of thiouracils and thiobarbiturates\textsuperscript{116}. The methods are based on the measurement of the absorbance of ether extract of thiouracil and thiopentone at 234 and 288 nm respectively. The methods have been used to determine these drugs in body fluids.

When it becomes necessary to distinguish between several thiouracils/thiobarbiturates (used medicinally) and their analysis at trace level in body/biological fluids is required, chromatography proves to be an efficient technique. The chromatographic techniques viz. TLC, GC and HPLC, are the most widely used and their application in such analysis have been reported by various workers. De Bradander et al.\textsuperscript{117} determined above drugs by GC-MS techniques after high performance TLC
separation in biological materials. The determination is based on the fluorescent induction of 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole derivatives of drugs with cysteine. Pensabane et al.\textsuperscript{118} have described the use of GC with nitrogen-phosphorus detection (NPD) and tandem mass spectrometric confirmation of thyrostats viz. thiouracil, methyl thiouracil and propyl thiouracil in meat tissues to check their tolerance levels. The drug compounds after extraction with acetonitrile-water media were derivatised with N-methyl-N-(trimethylsilyl) trifluoroacetamide and determined by GC. Schilt and workers\textsuperscript{119} determined these thyrostats in cattle urine after selective preconcentration on a mercurated affinity column followed by derivatisation by alkylation. Blanchflower et al.\textsuperscript{120} determined five thiouracils in thyroid and urine after extraction with ethyl acetate and clean-up on silica gel phase cartridge using LC-MS system. Buick et al.\textsuperscript{121} standardized HPLC methodology for the detection and quantification of thiouracil, methyl thiouracil, propyl thiouracil and phenyl thiouracil drug residues in cattle serum and thyroid tissues. Esteve Romero et al.\textsuperscript{122} used micellar electrokinetic chromatography for the determination of thiouracil and its alkyl derivatives in dried animal feed.

The use of capillary zone electrophoresis (CZE) for the separation and quantification of three thiouracils viz. 2 thiouracil, methyl thiouracil and propyl thiouracil has also been reported in literature\textsuperscript{123,124}.
PRESENT WORK
Organic compounds containing thiourea function have attained a special importance as synthetic compounds of considerable agricultural, pharmaceutical and analytical importance. Thiophanate-methyl (I) and thiophanate (II) are commercial fungicides based on a bis-thiourea. They are protective, curative and systemic in action and exhibit a rather broad antifungal spectrum against Cercospora leaf spot, powdery mildew, Schlerotinia rot and Botrytis mold of various crops as well as apple scab and sheath blight of rice. Though non-phytotoxic, they have a long residual effect. The wide use of these agrochemicals necessitates convenient, reliable and sensitive methods for their analysis not only at formulation level but at residual level too. Thiouracil (III), methyl thiouracil (IV) and thiopentone sodium (V) are important drugs containing thiourea function. The analysis of above drugs for their active ingredient contents is an important part of their commercial analysis.

In view of the wide use of these compounds, they have been determined by a number of methods and techniques viz. HPLC, GC, UV-VIS spectrophotometry and capillary zone electrophoresis. Most of these methods based on above techniques deal with their determination in various substrates viz. environment, biological fluids etc. However, the analysis of these thiourea derivatives in their formulated products with regards to quality control has attracted little attention despite the fact that the need of quality assurance is also extremely essential to obtain reliable residue data. The method commonly employed for the determination of thiophanate-methyl in formulations and residues involves its reaction with an excess of copper(II)-triethanolamine-alkali (alkali-amine-copper) reagent. The insoluble product is removed by filtration and unconsumed
copper(II) in the filtrate is reacted with pyridine-thiocyanate reagent. The coloured product is extracted in chloroform and absorbance of the extract measured at 435 nm. In another method, the sample is reacted with copper(II) and absorbance of the product formed in colloidal suspension, measured at 390 nm (a shoulder between 370 and 400 nm in the spectrum of the colloidal suspension). The first method besides being indirect (based on the measurement of excess reagent) is tedious and time consuming; it involves steps like filtration and solvent extraction, which if not performed with utmost care could result in the loss of material and thus vitiate the results. With regards to the second method, it may be mentioned that methods based on the absorption of colloidal suspensions are always feared to be of doubtful accuracy because a large error generally arises from the difficulty of preparing and maintaining all standards and unknown suspensions in a uniform and reproducible degree of dispersion.

The pharmacopoeia method\textsuperscript{109} for the assay of thiouracils in drug formulations involves the titration of drug sample in sodium acetate-acetic acid buffer (pH-6) with standard mercury(II) acetate using diphenyl carbazone as an indicator to a colour change from red to violet. Thiopentone sodium is assayed for both sodium and thiopentone by the commonly employed IP method\textsuperscript{109}. Sodium is determined by titrating the aqueous drug solution with standard sulphuric acid with methyl red as indicator. The liberated carbon dioxide from the sodium carbonate constituent is removed by boiling before the final end point is taken. The resultant neutral solution is then acidified with sulphuric acid and extracted with successive quantities of chloroform. Each extract is washed with water. The chloroform from the combined extract is removed by evaporation and resulting residue is dried and weighed to give the content of thiopentone in the drug. The above methods are, however, tedious, time consuming and require larger sample size of the material for analysis. These drugs have also been determined spectrophotometrically\textsuperscript{108} based on the measurement of their ether extracts at 228 – 234 nm. The purity of the solvent and interferences from drug excipients at the above analytical wavelengths are the problems associated with these methods.

In view of the above, there is a dire need of new/ superior methods possessing advantages over the existing/ commonly employed methods in terms of simplicity, rapidity and accuracy of procedures for the analysis of above compounds. Our efforts have led to the development of such methods by making use of polarographic and spectrophotometric techniques of analysis.
(A) Polarographic Methods:

As is well known, an important application of polarography for the study and analysis of organic sulphur compounds is because of the existence of sulphur in different oxidation states and consequently their susceptibility to electrochemical oxidation-reduction. A polarographic method of analysis is based on the study of electrolysis at mercury capillary electrode and subsequent measurement and interpretation of resulting current-voltage curve. The shape of the curve obtained in electrolysis with dropping mercury electrode (DME) enables to detect as well as to determine quantitatively the constituents of the solution. Polarographic determinations can be performed even at larger dilutions and with smaller volumes of solution. The polarographic method is, therefore, economical in both time and material used. In the context of growing emphasis on the accuracy of results and trace determinations, pulse polarography provides a significant and attractive alternative to the prevalent techniques in the field. Pulse polarography, a relatively inexpensive technique coupled with high sensitivities (10^{-9}M) attainable, could be of immense use in developing suitable method of analysis for above thiourea-based products.

Both thiourea fungicides as well as drugs are characterized by the presence of a thiourea group. The ease with which thiourea group gets oxidized to the disulphide in

\[
\begin{align*}
2 \text{RNH}_\text{H}_2\text{N} &\rightarrow 2 \text{RNH} \quad \text{RNH} \quad \text{HN} \quad \text{HN} \quad \text{HN} \\
C = S &\rightarrow C - S - S - C \quad \text{HN} \quad \text{HN} \quad + 2 \text{H}^+ + 2 \text{e}^{-}
\end{align*}
\]

acidic medium could serve as an important handle for their analysis. Though this oxidation behaviour for some thioureas has been investigated voltammetrically\textsuperscript{2,47-51,125}, the determination of above commercial products using polarography however, does not appear to have attracted much attention. That these compounds (except thiopentone sodium) are only sparingly soluble in water, could be the cause for not doing so. However, prompted by the observation that these compounds are freely soluble in acetonitrile and the latter is not only resistant to oxidation or reduction but is miscible with water/ aqueous acidic solutions, we succeeded in evolving pulse polarographic methods for their determination. A thorough study of experimental conditions and instrumental parameters to study the oxidation behaviour of thiourea group (present in these compounds) at DME has been made to obtain relatively well-defined polarograms and consequently reproducible results. Best results were obtained in aqueous acetonitrile (3:2v/v) in the presence of sulphuric acid (to maintain the pH at 2-3) and potassium
perchlorate (0.01 M) as supporting electrolyte. Under the above conditions, a linear baseline having plateau parallel to the potential axis was obtained over a wide range of potentials. The polarograms of thiophanate-methyl, thiophanate, thiouracil, methyl thiouracil and thiopentone sodium and formulated products based on them viz. fungicides and drugs, were run resulting in well defined, diffusion-controlled anodic waves with half wave potentials \( E_{1/2} \) of -0.125, -0.130, 0.110, 0.123 and 0.097 V (vs SCE) respectively. The effect of mercury column height \( V^h \) on the diffusion current \( i_d \) was also studied. A linear plot of \( i_d \) vs \( \sqrt{h} \) indicated the diffusion-controlled nature of the reaction.

The proportionality between the diffusion current (wave/peak height) and the concentration of the compound under study forms the basis of quantitative polarographic analysis. The above compounds have been determined by linear calibration plots using normal pulse polarography (NPP) and differential pulse polarography (DPP). On recording their voltammograms, it was found that wave height (in NPP mode) and peak height (in DPP mode) was proportional to the concentration of these compounds.

The pulse polarographic methods have been applied with success not only to the analysis of various fungicide/drug formulations (based on thiourea derivatives) for their active ingredient contents but also in analyzing residues containing them. In the latter context, the use of DPP has been described for analyzing thiophanate-methyl residues on foodstuffs, which is of particular importance in monitoring pollution arising out of the use of this fungicide.

(B) Spectrophotometric Methods:

Spectrophotometry is a simple, sensitive and relatively inexpensive technique and the methods based on this technique have continued to flourish and find wide-acceptance. It may be emphasized that spectrophotometric methods are widely used in monitoring environmental pollution and analytical assessment of industrial processes\(^{126-128}\). Our efforts in evolving spectrophotometric methods for the analysis of commercial products based on thiourea derivatives have been quite successful.

Though complex formation reactions of thioureas with metals to yield coloured complexes enabling colorimetric determination of these metals, has been known since long, analytical methods based on this behaviour for the determination of thioureas are however, lacking in literature. In the course of our investigations in this direction, we have found that nickel(II) could prove a suitable reagent for the determination of
thioureas and commercial products based on them. In methanol-dimethylformamide (DMF) media and in the presence of triethylamine, thioureas react with nickel(II) in 2:1 molar ratio to form greenish yellow complexes absorbing at 360 nm. This observation has been made the basis of a spectrophotometric method for the determination of thiourea derivatives. The colour which develops immediately on mixing the reagents is stable for at least 2 h. The analysis can be accomplished either by direct colorimetry or by photometric titrations. The direct colorimetric method consists in reacting each thiourea derivative with nickel(II) acetate in methanol-DMF media in the presence of triethylamine. The absorbance of the coloured solution is measured at 360 nm against a reagent blank and the results calculated on the basis of calibration graphs prepared separately. The photometric titration method consists in titrating thioureas in the same media photometrically at 360 nm against a standard nickel(II) reagent. The absorbance at this wavelength goes on increasing till the sample-reagent molar ratio 2:1 is achieved. Thereafter, it attains almost constant values. An inverted L-shaped titration curve is obtained; the end-point is found by extrapolation of the linear segments. It may be mentioned here that whereas the photometric titration methods are more rapid (no calibration curve is required), accurate and precise than direct colorimetric methods, the latter are, however, more sensitive. The above spectrophotometric methods have successfully been applied to the analysis of above thiourea derivatives in their commercial products and residues. In the latter context, the direct colorimetric method has been used for the determination of thiophanate-methyl fungicide in residues on grains and apples.

It is important to mention here that the proposed methods (both polarographic as well as spectrophotometric) developed for the determination of thiophanate-methyl, thiophanate, thiouracils and thiopentone sodium (with thiourea group present in them serving as the basis of analysis), have first been tested on a good number of reference compounds i.e. thiourea derivatives, in order to establish the generality and versatility of the methods. These methods have, thus, the potential of further extension to other commercial products being marked or in the process of trial.
EXPERIMENTAL PROCEDURES
Determination of some thiourea derivatives as such, in their formulated products and residues.

A. Pulse-polarographic determination of thiourea derivatives and fungicide and drug compounds based on them.

(a) Measurement of half wave/peak potentials ($E_{1/2}$/$E_p$)

Polarograms of known concentrations of each thiourea derivative and fungicide/drug compounds and their commercial formulations at dropping mercury electrode (DME) in aqueous acetonitrile (3:2 v/v) in the presence sulphuric acid (to maintain pH at 2-3) and potassium perchlorate (0.01 M in water) as supporting electrolyte were recorded. The general procedure for recording a polarogram was as follows:

A known volume of supporting electrolyte solution (20 ml, 0.01 M potassium perchlorate in water) and 0.5 ml of 2 M sulphuric acid (to adjust pH at 2-3) were taken in the polarographic cell containing 20 ml acetonitrile. Triton-X-100 (1 ml, 0.02% in acetonitrile) was added as suppressor and the final volume made to 50 ml with supporting electrolyte. Nitrogen gas was bubbled through the solution for 15 min. A polarogram of the supporting electrolyte alone (using NPP and DPP) was recorded with the following instrumental parameters:

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>SETUP</th>
<th>PARAMETERS</th>
<th>SETUP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial applied voltage</td>
<td>+400 mV vsSEC</td>
<td>Drop time</td>
<td>1 sec.</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>10 μA/V for NPP</td>
<td>Scan rate</td>
<td>NOR</td>
</tr>
<tr>
<td></td>
<td>1 μA/V for DPP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C.C. compensation</td>
<td>5</td>
<td>Acquisition</td>
<td>Fast</td>
</tr>
<tr>
<td>I.R. Compensation</td>
<td>0</td>
<td>O/P Zero</td>
<td>485</td>
</tr>
<tr>
<td>Height of Hg column</td>
<td>150 cm</td>
<td>Time constant</td>
<td>20 m sec</td>
</tr>
<tr>
<td>Height of Hg Pool</td>
<td>3.5 cm</td>
<td>Temperature</td>
<td>22±5°C</td>
</tr>
<tr>
<td>Pulse amplitude</td>
<td>50 mV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polarocord Parameters</td>
<td>X-axis = 100mV/cm</td>
<td>Y-axis = 200 mV/cm.</td>
<td></td>
</tr>
</tbody>
</table>

Thereafter, aliquots of standard solutions in acetonitrile of each thiourea derivative, fungicide/drug compound were taken and polarograms recorded in the usual way keeping the above mentioned parameters same. A typical normal pulse and differential pulse polarogram of phenyl thiourea, as a representative of thiourea derivatives, is shown in Fig 1. The values of half-wave potential ($E_{1/2}$) and peak potential ($E_p$) for each thiourea
Fig. 1

(a)

(b)

Typical normal pulse polarogram (a) and differential pulse polarogram (b) of phenyl thiourea at 22±1°C

1: supporting electrolyte, 2: phenyl thiourea
derivative are given in Table 1. The E_{1/2} and Ep values of pure fungicide/drug compounds as well as and their formulated products were also determined in same way. Typical polarograms one for each compound, are shown in Figs. 2-4. In Table II are given values of E_{1/2} and Ep of these compounds.

(b) Effect of height of mercury column on diffusion current

Polarograms of standard solutions of the thiourea derivatives, fungicide/ drug compounds at a fixed concentration but at various heights of mercury column were recorded in the usual way. Typical plot showing linear relationship between i_d and \( \sqrt{H_{\text{Hg}}} \) indicating diffusion-controlled nature of electrode reaction are represented in Figs. 5 and 6. The values of \( i_d/\sqrt{H_{\text{Hg}}} \) are given in Tables III and IV.

(c) Preparation of calibration graphs for thiourea derivatives

Stock standard solution (10^{-3}M) of each derivative was prepared in acetonitrile. Aliquots (0.1-1.0 ml) of each solution were taken in the polarographic cells and diluted to 20 ml with acetonitrile. Each solution was mixed with 20 ml of potassium perchlorate (0.01 M in water) followed by the addition of 2 M sulphuric acid (0.5 ml) to adjust the pH of the solution at 2-3. Triton-X-100 (1ml, 0.02% in acetonitrile) was added as suppressor. The final volume of each solution was made to 50 ml with potassium perchlorate. Nitrogen gas was bubbled through each solution for 15 min. and polarograms were recorded in the same manner as described under general procedure (in (a) above). The calibration graphs were constructed by plotting diffusion/ peak current against concentration of each compound in the usual way. Some results are given in Tables V and VI.

(d) Preparation of calibration graphs for pure fungicide and drug compounds

Aliquots of standard solutions (10^{-3} M) of each of the pure compound in acetonitrile were taken in polarographic cells and diluted to 20 ml with the same solvent. Each solution was mixed with potassium perchlorate (20 ml, 0.01 M in water), sulphuric acid (0.5 ml, 2 M) and Triton-X-100 (1 ml, 0.02% in acetonitrile) and final volume made to 50 ml with potassium perchlorate. Polarograms of each solution were recorded and calibration curves plotted in the same manner as described above. The results of analysis are summarized in Tables VII-X.
Typical normal pulse polarogram (a) and differential pulse polarogram (b) of thiophanate-methyl and thiophanate at 22±1°C
1: supporting electrolyte, 2: thiophanate-methyl; 3, thiophanate
Typical normal pulse polarogram (a) and differential pulse polarogram (b) of thiouracil and methyl-thiouracil at 22±1°C.

1: supporting electrolyte, 2: thiouracil; 3: methyl-thiouracil.
Fig. 4
(a)  
![Graph(a)]

(b)  
![Graph(b)]

Typical normal pulse polarogram (a) and differential pulse polarogram (b) of thiopentone sodium at 22±1°C
1; supporting electrolyte, 2; thiopentone sodium
Fig. 5

Diffusion current (μA)

Height of mercury column (√hₚ) cms
(e) Formulation analysis

One fungicide formulation (containing 70% thiophanate-methyl, W.P.) and two drug formulations (methyl thiouracil tablets containing 50 mg active ingredient per tablet and interval sodium injection containing 0.5g of thiopentone sodium per vial) were used. The procedures for bringing each fungicide and drug formulation in the measurable form are:

For fungicide formulation, a single large sample of the formulation was weighed, shaken with acetonitrile and filtered. The residue (if any) was washed 2-3 times with acetonitrile. The filtrates and washings were diluted to a known volume with acetonitrile. In case of methyl thiouracil tablets however, a known number of tablets (say 20) were weighed and finally ground. Stock solution was prepared by dissolving accurately weighed amount in acetonitrile and filtered. The residue, if any, was washed 2-3 times with acetonitrile. The filtrate and washings were diluted to a known volume with the same solvent. For injection formulation, the contents of ten vials were weighed and mixed thoroughly for the sake of uniformity of the material. A single large sample of the drug was weighed and dissolved in known volume of acetonitrile. Aliquots of the extracts/solutions of each of the above fungicide/ drug formulation were taken and processed for analysis by the experimental procedure described above. The results of analysis are given in Table XI.

(f) Residue analysis of thiophanate-methyl (on grains and apple fruits)

(i) Recovery experiments:

A known weight of grains (barley and wheat) were mixed with various amounts of the thiophanate-methyl (in acetonitrile). The samples were well mixed and extracted with 4-5 installments of 2-3 ml acetonitrile. The combined extracts were diluted to 20 ml with acetonitrile. Each extract was transferred into the polarographic cell, mixed with potassium perchlorate (20ml, 0.01 M), sulphuric acid (0.5 ml, 2M) and Triton-X-100 (1ml, 0.02% in acetonitrile) and made to 50 ml with potassium perchlorate and processed for differential pulse polarographic analysis in the manner described above. The amount of the fungicide was determined from the calibration curves. The results are given in Table XII.

In case of apple fruits, known weight of the sample taken in glass containers were sprayed with various amounts of thiophanate-methyl (in acetonitrile). The samples were
well mixed and blended mechanically in the presence of acetonitrile and filtered through Buchner funnel fitted with glass sinter. The residue of each sample was washed 4-5 times with sufficient amount of acetonitrile and combined extracts cleaned up on silica gel column using cyclohexane-ethylacetale (1:1 v/v) mixture as eluting solvent. The eluate was concentrated by evaporation on hot water bath. The residue was dissolved in acetonitrile (20 ml) and processed for analysis as described above. The results are given in Table XII.

(ii) Residue analysis:
Grains (barley, wheat) and apple fruits were sprayed with fungicide formulation (aqueous dispersion) at a concentration of 0.2 – 0.6 g/l at a rate of 100 ml/kg commodity. Sprayed samples were dried in the sun and from these lots, samples of 15-20g were drawn for residue analysis and processed as described under “recovery experiment”. The results are given in Table XIII.

B. SPECTROPHOTOMETRIC METHODS

1. Direct colorimetric procedure:

(a) Preparation of calibration graphs for thiourea derivatives:

Aliquots (0.1 – 1.5 ml) of solutions in dimethylformamide of each derivative were taken in 5 ml-measuring flasks and the volume made to 3 ml with the same solvent. Each solution was mixed with triethylamine (1ml, ~1M in dimethylformamide) and nickel(II) acetate (1ml, ~0.001N in methanol) and the final volume made to 5 ml with dimethylformamide. The absorbance of yellowish green solution so obtained was measured at 360nm (spectrum of this solution illustrated in Fig 7) against a reagent blank. The absorbance values were plotted against the concentration of derivatives used and calibration curves prepared. Some results are given in Table XIV.

(b) Preparation of calibration graphs for pure fungicide and drug compounds:

Aliquots (0.1 – 1.5) of solutions in dimethylformamide of each pure compound were taken in 5 ml-measuring flasks. The subsequent experimental details are the same as given above for thiourea derivatives. The results are summarized in Tables XV and XVI.
Fig. 7

Absorption Spectra
A: Reagent Blank
B: Ni(II)-Thiourea complex
(c) Formulation analysis:

Aliquots of dimethylformamide extracts of each formulation were taken and processed for analysis as described above for pure compounds. The results of analysis are given in Table XVII.

(d) Residue analysis of thiophanate-methyl (on grains and apple fruits)

(i) Recovery experiment:

A known weight of grains (barley and wheat) were mixed with various amounts of thiophanate-methyl (in dimethylformamide). The samples were well mixed and extracted with 4-5 installments of 2-3 ml acetonitrile. The extracts were evaporated to dryness on a water bath. The residue was dissolved in dimethylformamide and transferred to 25 ml measuring flask. Aliquots of each solution were then mixed with triethylamine (1 ml, ~1 M in dimethylformamide) and nickel(II) acetate (1 ml, ~ 0.001N in methanol) and the final volume made to the mark with dimethylformamide. The colour which developed instantaneously was measured at 360 nm against a reagent black. The results are given in Table XVIII.

In case of apple fruits, known weight of samples taken in containers were sprayed with various amounts of thiophanate-methyl (in dimethylformamide). The samples were well mixed and blended mechanically in the presence of acetonitrile and filtered through Buchner funnel fitted with glass sinter. The residue of each sample was washed 4-5 times with sufficient amount of acetonitrile and combined extracts were cleaned up on silica gel column using cyclohexane-ethylacetate (1:1 v/v) mixture as eluting solvent. The eluate was concentrated by evaporation. To the residue, were added dimethylformamide (20 ml), triethylamine (1 ml, ~ 1M in dimethylformamide) and nickel (II) acetate (1 ml, ~ 0.001N in methanol) and the final volume made to 25 ml with dimethylformamide. The analysis was concluded by measuring the absorbance of yellowish green colour at 360 nm against a reagent blank. The results are given in Table XVIII.

(ii) Residue analysis:

Grains (barley, wheat) and apple fruits were sprayed with fungicide formulation (aqueous dispersion) at a concentration of 1.2–6.0 g/l at a rate of 100 ml/kg commodity. Sprayed samples were dried in the sun and from these lots; samples of 15-20 gm were drawn for residue analysis and processed as described above under recovery experiment. The results are given in Table XIX.
2. Photometric titration procedure
   
   (a) Determination of thiourea derivatives:

   Aliquots of solutions of dimethylformamide of each derivative were taken, mixed with triethylamine (1 ml, ~ 1 M in dimethylformamide) and the volume made to 5 ml with dimethylformamide. The titration was commenced by adding standard nickel(II) acetate solution in small instalments, stirring the solution magnetically (with a specially designed stirring disc) each time and measuring the absorbance at 360 nm. For plotting the titration curve, the absorbance values were corrected to the initial volume of the system by multiplying the absorbance reading by a factor $V + \frac{v}{V}$, where "$V$" is the initial volume, "$v$" is the volume of the reagent added for a particular absorbance reading being measured. The plot of absorbance versus ml of nickel(II) acetate solution added for each derivative was then made and the best straight lines drawn between the points taken before and after the equivalence point. The intersection of linear segments was taken as the end-point. The profile of a typical photometric titration is shown in Fig 8. The results are summarized in Table XX.

(b) Determination of pure fungicide and drug compounds:

   Aliquots of solutions in dimethylformamide of each pure compound were taken and the photometric titrations were performed in the same manner as described above for thiourea derivatives. The results are given in Tables XXI and XXII.

(c) Formulation analysis:

   Suitable aliquots of the extracts of fungicide and drug formulations in dimethyl formamide were taken and processed for analysis as described above for pure compounds. The results of analysis are given in Table XXIII.
RESULTS
Table I: Half-wave potentials ($E_{1/2}$) and peak potentials ($E_p$) of thiourea derivatives

<table>
<thead>
<tr>
<th>Thioureas</th>
<th>$E_{1/2}$ (in V) (vs SCE)</th>
<th>$E_p$ (in V) (vs SCE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenyl</td>
<td>-0.155</td>
<td>-0.130</td>
</tr>
<tr>
<td>o-Tolyl</td>
<td>-0.165</td>
<td>-0.140</td>
</tr>
<tr>
<td>o-Methoxy phenyl</td>
<td>-0.193</td>
<td>-0.168</td>
</tr>
<tr>
<td>p-Methoxy phenyl</td>
<td>-0.195</td>
<td>-0.170</td>
</tr>
<tr>
<td>o-Ethoxy phenyl</td>
<td>-0.197</td>
<td>-0.172</td>
</tr>
<tr>
<td>p-Ethoxy phenyl</td>
<td>-0.200</td>
<td>-0.175</td>
</tr>
<tr>
<td>o-Phenylene-bisthiourea</td>
<td>-0.183</td>
<td>-0.158</td>
</tr>
<tr>
<td>Pure compound</td>
<td>$E_{1/2}$ (in V) (vs SCE)</td>
<td>$E_p$ (in V) (vs SCE)</td>
</tr>
<tr>
<td>----------------------</td>
<td>---------------------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td>Thiophanate-methyl</td>
<td>-0.125</td>
<td>-0.100</td>
</tr>
<tr>
<td>Thiophanate</td>
<td>-0.130</td>
<td>-0.105</td>
</tr>
<tr>
<td>Thiouracil</td>
<td>0.110</td>
<td>0.085</td>
</tr>
<tr>
<td>Methyl thiouracil</td>
<td>0.123</td>
<td>0.098</td>
</tr>
<tr>
<td>Thiopentone sodium</td>
<td>0.097</td>
<td>0.072</td>
</tr>
</tbody>
</table>
Table III: Effect of height of mercury column on diffusion current
Concentration of each thiourea derivative taken = 25 μg ml⁻¹.

<table>
<thead>
<tr>
<th>Height of mercury column (cms)</th>
<th>√h</th>
<th>Phenyl</th>
<th>o-Tolyl</th>
<th>o-Methoxy phenyl</th>
<th>p-Methoxy phenyl</th>
<th>o-Ethoxy phenyl</th>
<th>p-Ethoxy phenyl</th>
<th>o-Phenylenebisthiourea</th>
</tr>
</thead>
<tbody>
<tr>
<td>152.0</td>
<td>12.32</td>
<td>0.50</td>
<td>0.48</td>
<td>0.49</td>
<td>0.47</td>
<td>0.53</td>
<td>0.51</td>
<td>0.54</td>
</tr>
<tr>
<td>146.0</td>
<td>12.08</td>
<td>0.51</td>
<td>0.49</td>
<td>0.50</td>
<td>0.47</td>
<td>0.50</td>
<td>0.50</td>
<td>0.54</td>
</tr>
<tr>
<td>132.0</td>
<td>11.48</td>
<td>0.49</td>
<td>0.49</td>
<td>0.51</td>
<td>0.48</td>
<td>0.50</td>
<td>0.51</td>
<td>0.54</td>
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<tr>
<td>126.0</td>
<td>11.22</td>
<td>0.51</td>
<td>0.48</td>
<td>0.49</td>
<td>0.47</td>
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<tr>
<td>112.0</td>
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<td>0.49</td>
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<td>0.51</td>
<td>0.48</td>
<td>0.53</td>
<td>0.52</td>
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</tr>
</tbody>
</table>
Table IV: Effect of height of mercury column on diffusion current.
Concentration of each fungicide and drug compound taken = 20µg ml⁻¹.

<table>
<thead>
<tr>
<th>Height of mercury column (cms)</th>
<th>( \sqrt{h} )</th>
<th>( i_d/\sqrt{h} )</th>
<th>Thiophanate-methyl</th>
<th>Thiophanate</th>
<th>Thiouracil</th>
<th>Methyl Thiouracil</th>
<th>Thiopentone Sodium</th>
</tr>
</thead>
<tbody>
<tr>
<td>150</td>
<td>12.25</td>
<td>0.52</td>
<td>0.49</td>
<td>0.50</td>
<td>0.53</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td>140</td>
<td>11.83</td>
<td>0.52</td>
<td>0.48</td>
<td>0.51</td>
<td>0.54</td>
<td>0.51</td>
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</tr>
<tr>
<td>130</td>
<td>11.41</td>
<td>0.51</td>
<td>0.49</td>
<td>0.50</td>
<td>0.53</td>
<td>0.52</td>
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<tr>
<td>120</td>
<td>10.96</td>
<td>0.51</td>
<td>0.49</td>
<td>0.51</td>
<td>0.54</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>110</td>
<td>10.49</td>
<td>0.52</td>
<td>0.48</td>
<td>0.49</td>
<td>0.53</td>
<td>0.51</td>
<td></td>
</tr>
</tbody>
</table>
### Table V: Determination of thiourea derivatives: Normal pulse polarographic procedure

<table>
<thead>
<tr>
<th>Thioureas</th>
<th>Values are mean of three determinations with standard deviation (±)</th>
<th>Mean diffusion current, $i_d$, μA</th>
<th>Amount found, μg</th>
<th>Mean diffusion current, $i_d$, μA</th>
<th>Amount found, μg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenyl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>o-Tolyl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>o-Methoxy phenyl</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>p-Methoxy phenyl</td>
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<tr>
<td>o-Ethoxy phenyl</td>
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<td></td>
</tr>
<tr>
<td>p-Ethoxy phenyl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>o-Phenylene-bisthiourea</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Amount taken = 4.5 μg  
** Amount taken = 18.0 μg
Table VI: Determination of thiourea derivatives: Differential pulse polarographic procedure

<table>
<thead>
<tr>
<th>Thioureas</th>
<th>Values are mean of three determinations with standard deviation (±)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean peak current, $i_p$, $\mu$A</td>
</tr>
<tr>
<td>Phenyl</td>
<td>1.25</td>
</tr>
<tr>
<td>o-Tolyl</td>
<td>1.40</td>
</tr>
<tr>
<td>o-Methoxy phenyl</td>
<td>2.25</td>
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<tr>
<td>p-Methoxy phenyl</td>
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</tr>
<tr>
<td>p-Ethoxy phenyl</td>
<td>2.45</td>
</tr>
<tr>
<td>p-Ethoxy phenyl</td>
<td>2.00</td>
</tr>
<tr>
<td>o-Phenylene-bisithiourea</td>
<td>1.42</td>
</tr>
</tbody>
</table>

* Amount taken = 1.10 $\mu$g
** Amount taken = 5.50 $\mu$g
<table>
<thead>
<tr>
<th>Amount taken, ( \mu g )</th>
<th>Thiophanate-methyl found, ( \mu g )</th>
<th>Thiophanate found, ( \mu g )</th>
<th>Mean diffusion current, ( i_d, \mu A )</th>
<th>Mean diffusion current of thiophanate, ( i_d, \mu A )</th>
<th>Mean diffusion current, ( i_d, \mu A )</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.8</td>
<td>12.0</td>
<td>6.84, 0.047</td>
<td>14.0</td>
<td>6.78, 0.050</td>
<td></td>
</tr>
<tr>
<td>13.6</td>
<td>24.5</td>
<td>13.73, 0.083</td>
<td>28.4</td>
<td>13.64, 0.088</td>
<td></td>
</tr>
<tr>
<td>27.2</td>
<td>54.0</td>
<td>27.48, 0.166</td>
<td>58.2</td>
<td>26.93, 0.154</td>
<td></td>
</tr>
<tr>
<td>54.4</td>
<td>101.0</td>
<td>54.38, 0.328</td>
<td>110.2</td>
<td>54.34, 0.324</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean of three determinations with standard deviation (\( \pm \)).
Table VIII: Determination of thionophate-methyl and thionophate: Differential pulse polarographic procedure

<table>
<thead>
<tr>
<th>Amount taken, μg</th>
<th>Mean peak current, ( i_p ) μA</th>
<th>Mean peak current, ( i_p ) μA</th>
<th>Mean peak current, ( i_p ) μA</th>
<th>Mean peak current, ( i_p ) μA</th>
<th>Thionophate found, ( \mu g )</th>
<th>Thionophate found, ( \mu g )</th>
<th>( \mu g )</th>
<th>( \mu g )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.7</td>
<td>1.15</td>
<td>1.30</td>
<td>1.72</td>
<td>0.012</td>
<td>1.71</td>
<td>0.014</td>
<td>3.44</td>
<td>0.024</td>
</tr>
<tr>
<td>3.4</td>
<td>2.40</td>
<td>2.46</td>
<td>3.39</td>
<td>0.028</td>
<td>6.86</td>
<td>0.054</td>
<td>6.74</td>
<td>0.050</td>
</tr>
<tr>
<td>6.8</td>
<td>4.50</td>
<td>4.60</td>
<td>6.74</td>
<td>0.050</td>
<td>13.54</td>
<td>0.088</td>
<td>13.62</td>
<td>0.084</td>
</tr>
<tr>
<td>13.6</td>
<td>7.52</td>
<td>7.68</td>
<td>13.62</td>
<td>0.084</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table IX: Determination of thiouracil, methyl thiouracil and thiopentone sodium: Normal pulse polarographic procedure

<table>
<thead>
<tr>
<th>Amount taken, µg</th>
<th>Mean diffusion current, i, µA</th>
<th>Thiouracil found, µg</th>
<th>Mean diffusion current, i, µA</th>
<th>Methyl thiouracil found, µg</th>
<th>Mean diffusion current i, µA</th>
<th>Thiopentone sodium, found, µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>14.0</td>
<td>2.52 0.018</td>
<td>18.0</td>
<td>2.51 0.014</td>
<td>8.13</td>
<td>2.48 0.019</td>
</tr>
<tr>
<td>5.0</td>
<td>28.0</td>
<td>5.05 0.040</td>
<td>40.2</td>
<td>4.96 0.042</td>
<td>11.9</td>
<td>5.06 0.041</td>
</tr>
<tr>
<td>7.5</td>
<td>40.8</td>
<td>7.58 0.064</td>
<td>70.0</td>
<td>7.42 0.062</td>
<td>35.1</td>
<td>7.46 0.060</td>
</tr>
<tr>
<td>10.0</td>
<td>60.0</td>
<td>10.08 0.071</td>
<td>86.0</td>
<td>9.92 0.068</td>
<td>40.63</td>
<td>9.93 0.072</td>
</tr>
</tbody>
</table>

Values are mean of three determinations with standard deviation (±)
**Table X: Determination of thiouracil and methyl thiouracil: Differential pulse polarographic procedure**

<table>
<thead>
<tr>
<th>Amount taken, µg</th>
<th>Thiouracil found, µg</th>
<th>Methyl thiouracil found, µg</th>
<th>Thiopentone sodium found, µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.25</td>
<td>1.27 ± 0.010</td>
<td>1.54 ± 0.010</td>
<td>1.17 ± 0.011</td>
</tr>
<tr>
<td>2.50</td>
<td>2.52 ± 0.019</td>
<td>3.82 ± 0.018</td>
<td>2.39 ± 0.020</td>
</tr>
<tr>
<td>3.75</td>
<td>3.78 ± 0.028</td>
<td>4.90 ± 0.026</td>
<td>3.10 ± 0.031</td>
</tr>
<tr>
<td>6.25</td>
<td>6.20 ± 0.040</td>
<td>9.50 ± 0.044</td>
<td>5.42 ± 0.042</td>
</tr>
</tbody>
</table>

Values are mean of three determinations with standard deviation (±).
Table XI: Assay results of some commercial formulations containing thiophanate-methyl, methyl thiouracil and thiopentone sodium

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Maker's specification</th>
<th>Recovery of active ingredient*, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NPP</td>
</tr>
<tr>
<td>Thiophanate-methyl</td>
<td>70%</td>
<td>98.6 ± 0.54</td>
</tr>
<tr>
<td>Methyl thiouracil</td>
<td>50 mg per tablet</td>
<td>99.2 ± 0.42</td>
</tr>
<tr>
<td>Thiopentone sodium</td>
<td>0.5 gm per vial</td>
<td>98.6 ± 0.50</td>
</tr>
</tbody>
</table>

* Values are mean of three determinations with standard deviation (±)
Table XII: Recovery of thiophanate-methyl from fortified samples: *Differential pulse polarographic method*

<table>
<thead>
<tr>
<th>Active ingredient added, µg</th>
<th>Recovery*, %</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wheat</td>
<td>Barley</td>
<td>Apple fruit</td>
</tr>
<tr>
<td>3.42</td>
<td>97.4, 0.60</td>
<td>96.4, 0.68</td>
<td>94.2, 0.60</td>
</tr>
<tr>
<td>5.14</td>
<td>96.1, 0.50</td>
<td>98.0, 0.060</td>
<td>93.2, 0.48</td>
</tr>
<tr>
<td>8.55</td>
<td>95.4, 0.58</td>
<td>96.8, 0.62</td>
<td>98.0, 0.50</td>
</tr>
<tr>
<td>11.97</td>
<td>90.5, 0.52</td>
<td>92.1, 0.58</td>
<td>94.6, 0.58</td>
</tr>
</tbody>
</table>

* Values are mean of three determinations with standard deviation (±)
<table>
<thead>
<tr>
<th>Strength of spray used, g/l</th>
<th>Residue found (ppm)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wheat</td>
<td>Barley</td>
<td>Apple fruit</td>
</tr>
<tr>
<td>0.2</td>
<td>1.35</td>
<td>1.70</td>
<td>1.08</td>
</tr>
<tr>
<td>0.3</td>
<td>2.02</td>
<td>2.67</td>
<td>1.56</td>
</tr>
<tr>
<td>0.4</td>
<td>2.37</td>
<td>3.89</td>
<td>2.01</td>
</tr>
<tr>
<td>0.5</td>
<td>3.27</td>
<td>4.14</td>
<td>2.80</td>
</tr>
</tbody>
</table>

Table XIII: Results of residue analysis (ppm) of treated samples
Table XIV: Determination of thiourea derivatives with nickel (II): Direct colorimetric procedure

<table>
<thead>
<tr>
<th>Thioureas</th>
<th>Values are mean of five determinations with standard deviation (±)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount found*, µg</td>
</tr>
<tr>
<td>Phenyl</td>
<td>4.96, 0.030</td>
</tr>
<tr>
<td>o-Tolyl</td>
<td>4.98, 0.024</td>
</tr>
<tr>
<td>o-Methoxy phenyl</td>
<td>5.01, 0.041</td>
</tr>
<tr>
<td>p-Methoxy phenyl</td>
<td>4.95, 0.028</td>
</tr>
<tr>
<td>o-Ethoxy phenyl</td>
<td>4.94, 0.030</td>
</tr>
<tr>
<td>p-Ethoxy phenyl</td>
<td>5.02, 0.032</td>
</tr>
<tr>
<td>o-Phenylene-bisthiourea</td>
<td>4.97, 0.037</td>
</tr>
</tbody>
</table>

* Amount taken, 5 µg
** Amount taken, 40 µg
Table XV: Determination of thiophanate-methyl and thiophanate with nickel(II): Direct colorimetric procedure

<table>
<thead>
<tr>
<th>Amount taken, µg</th>
<th>Thiophanate-methyl</th>
<th>Thiophanate</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0</td>
<td>2.96, 0.024</td>
<td>2.98, 0.028</td>
</tr>
<tr>
<td>6.0</td>
<td>5.96, 0.038</td>
<td>6.04, 0.044</td>
</tr>
<tr>
<td>12.0</td>
<td>12.10, 0.051</td>
<td>11.94, 0.050</td>
</tr>
<tr>
<td>24.0</td>
<td>23.78, 0.174</td>
<td>23.86, 0.152</td>
</tr>
<tr>
<td>48.0</td>
<td>47.66, 0.274</td>
<td>47.72, 0.284</td>
</tr>
</tbody>
</table>

Values are mean of five determinations with standard deviation (±)
Table XVI: Determination of thiouracil, methyl thiouracil and thiopentone sodium with nickel (II): Direct colorimetric procedure

<table>
<thead>
<tr>
<th>Amount taken, µg</th>
<th>Values are mean of five determinations with standard deviation (±)</th>
<th>Amount found, µg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Methyl thiouracil</td>
<td>Thiouracil</td>
</tr>
<tr>
<td>5.0</td>
<td>4.96, 0.034</td>
<td>4.98, 0.027</td>
</tr>
<tr>
<td>10.0</td>
<td>9.93, 0.052</td>
<td>9.98, 0.042</td>
</tr>
<tr>
<td>20.0</td>
<td>19.96, 0.124</td>
<td>19.94, 0.178</td>
</tr>
<tr>
<td>40.0</td>
<td>39.94, 0.196</td>
<td>40.01, 0.280</td>
</tr>
<tr>
<td>50.0</td>
<td>49.98, 0.310</td>
<td>49.84, 0.340</td>
</tr>
</tbody>
</table>
Table XVII: Assay results of some commercial formulations containing thiophanate-methyl, thiouracil and thiopentone sodium.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Maker’s specification</th>
<th>Recovery of active ingredient*, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Present Method</td>
</tr>
<tr>
<td>Thiophanate-Methyl (fungicide)</td>
<td>70%</td>
<td>98.6 ± 0.50</td>
</tr>
<tr>
<td>Methyl thiouracil (drug)</td>
<td>50 mg per tablet</td>
<td>98.8 ± 0.34</td>
</tr>
<tr>
<td>Thiopeptone-sodium injection (drug)</td>
<td>0.5 gm per vial</td>
<td>99.0 ± 0.62</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Comparison Method</td>
</tr>
<tr>
<td></td>
<td></td>
<td>99.2 ± 0.74</td>
</tr>
<tr>
<td></td>
<td></td>
<td>99.0 ± 0.64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>98.4 ± 0.84</td>
</tr>
</tbody>
</table>

* Values are the mean of five determinations with standard deviation (±)
<table>
<thead>
<tr>
<th>Active ingredient added, μg</th>
<th>Wheat</th>
<th>Barley</th>
<th>Apple fruit</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.0</td>
<td>97.8, 0.38</td>
<td>95.8, 0.46</td>
<td>94.6, 0.38</td>
</tr>
<tr>
<td>20.0</td>
<td>96.4, 0.48</td>
<td>97.8, 0.51</td>
<td>96.0, 0.50</td>
</tr>
<tr>
<td>30.0</td>
<td>98.0, 0.42</td>
<td>96.8, 0.58</td>
<td>94.2, 0.48</td>
</tr>
<tr>
<td>40.0</td>
<td>96.8, 0.50</td>
<td>98.4, 0.48</td>
<td>92.1, 0.42</td>
</tr>
<tr>
<td>50.0</td>
<td>95.6, 0.52</td>
<td>96.8, 0.42</td>
<td>90.0, 0.40</td>
</tr>
</tbody>
</table>

*Values are the mean of five determinations with standard deviation (±)*
Table XIX: Results of residue analysis (ppm) of treated samples

<table>
<thead>
<tr>
<th>Strength of spray used, g/l</th>
<th>Residue found ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wheat</td>
</tr>
<tr>
<td>6.0</td>
<td>42.00</td>
</tr>
<tr>
<td>4.8</td>
<td>30.42</td>
</tr>
<tr>
<td>2.4</td>
<td>17.10</td>
</tr>
<tr>
<td>1.2</td>
<td>8.09</td>
</tr>
</tbody>
</table>
Table XX: Determination of thioureas with nickel (II): Photometric titration procedure

<table>
<thead>
<tr>
<th>Thioureas</th>
<th>Amount found*, µg</th>
<th>Amount found**, µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenyl</td>
<td>9.94, 0.064</td>
<td>40.38, 0.121</td>
</tr>
<tr>
<td>o-Tolyl</td>
<td>10.06, 0.058</td>
<td>40.40, 0.174</td>
</tr>
<tr>
<td>o-Methoxy phenyl</td>
<td>9.92, 0.048</td>
<td>39.88, 0.152</td>
</tr>
<tr>
<td>p-Methoxy phenyl</td>
<td>9.86, 0.054</td>
<td>39.94, 0.184</td>
</tr>
<tr>
<td>o-Ethoxy phenyl</td>
<td>9.90, 0.060</td>
<td>39.96, 0.160</td>
</tr>
<tr>
<td>p-Ethoxy phenyl</td>
<td>10.08, 0.074</td>
<td>39.92, 0.192</td>
</tr>
<tr>
<td>o-Phenylene-bisthiourea</td>
<td>9.90, 0.074</td>
<td>39.86, 0.187</td>
</tr>
</tbody>
</table>

* Amount taken, 10 µg
** Amount taken, 40 µg
Table XXI: Determination of thiophanate-methyl and thiophanate with nickel (II): Photometric titration procedure

<table>
<thead>
<tr>
<th>Amount taken, µg</th>
<th>Thiophanate-methyl found, µg</th>
<th>Thiophanate found, µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.0</td>
<td>12.02, 0.074</td>
<td>11.96, 0.080</td>
</tr>
<tr>
<td>24.0</td>
<td>23.86, 0.181</td>
<td>24.12, 0.154</td>
</tr>
<tr>
<td>36.0</td>
<td>35.80, 0.164</td>
<td>35.72, 0.180</td>
</tr>
<tr>
<td>48.0</td>
<td>47.92, 0.247</td>
<td>47.88, 0.252</td>
</tr>
</tbody>
</table>
Table XXII: Determination of thiouracil, methyl thiouracil and thiopentone sodium with nickel (II): Photometric titration procedure

<table>
<thead>
<tr>
<th>Amount taken, µg</th>
<th>Amount found, µg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Thiouracil</td>
</tr>
<tr>
<td>10</td>
<td>10.08, 0.070</td>
</tr>
<tr>
<td>20</td>
<td>19.98, 0.120</td>
</tr>
<tr>
<td>40</td>
<td>39.70, 0.292</td>
</tr>
<tr>
<td>60</td>
<td>60.16, 0.310</td>
</tr>
</tbody>
</table>

Values are mean of five determinations with standard deviation (+)
Table XXIII: Assay results of some commercial formulation containing thiophanate-methyl, methyl thiouracil, and thiopentone-sodium.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Maker’s specification</th>
<th>Recovery of active ingredient*, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Present method</td>
</tr>
<tr>
<td>Thiophanate-methyl (fungicide)</td>
<td>70%</td>
<td>98.2 ± 0.48</td>
</tr>
<tr>
<td>Methyl thiouracil tablet (drug)</td>
<td>50 mg per tablet</td>
<td>99.0 ± 0.38</td>
</tr>
<tr>
<td>Thiopentone sodium injection (drug)</td>
<td>0.5 gm per vial</td>
<td>99.4 ± 0.40</td>
</tr>
</tbody>
</table>

* Values are mean of five determinations with standard deviation (±)
The importance of determination of organic compounds in industry and research is well known. The approach utilizing the reaction characteristics of various functional groups present in organic compounds, is extremely selective for the analysis of these compounds. The methods based on this approach not only still persist but have continued to flourish and remain indispensable. The great advantages of this approach has been demonstrated in the present work in the analysis of some industrial products viz fungicide and drugs based on thioureas. The reactions of thiourea group (present in these industrial compounds) have been utilized to evolve new methods possessing advantages over the existing/commonly-employed methods in terms of simplicity, rapidity and cost-effectiveness. The proposed methods, as already stated, have first been tested on a good number of thiourea derivatives before applying them to their real samples viz. thiophanate-methyl, thiophanate, thiouracil, methyl thiouracil and thiopentone sodium in order to establish the generality and wide applicability of these methods.

The facile oxidation of thiourea group to formamidine disulphide in acidic medium, could form the basis of an important approach to the analysis of thiourea derivatives and commercial products based on them. This could be achieved either chemically with the use of suitable oxidants or electrochemically by using some sensitive electroanalytical techniques. In the context of growing emphasis on the accuracy of results and trace determination especially of pesticide residues in agriculture, pulse polarography has emerged as a suitable technique among the electrochemical methods of analysis. In the present work, the polarographic oxidation of thiourea derivatives using the above technique was studied at DME in aqueous acetonitrile medium in the presence of sulphuric acid (to maintain the pH at 2-3) and potassium perchlorate (0.01-0.3M) as a supporting electrolyte. Acetonitrile has been particularly chosen as the solvent in this method because of the easy solubility of the thiourea derivatives (which are otherwise slightly soluble in water) in it, the miscibility of the solvent with aqueous/aqueous acidic solutions and, above all, its resistance to electrochemical oxidation/reduction. The above experimental conditions with respect to pH and concentration range of electrolyte were finalised on the basis of studies carried out over a wide range of pH values and concentrations of supporting electrolyte (potassium perchlorate) respectively. Under these experimental conditions, well-defined polarograms were obtained in case of each thiourea derivative. The voltammograms of thiourea derivatives and thiophanate-methyl, thiophanate, thiouracil, methyl thiouracil and thiopentone sodium under the above
experimental conditions in normal and differential pulse modes are shown in Figs. 1-4. The half-wave/ peak potential \( (E_{1/2}/Ep) \) values are given in Tables I and II. That the oxidation of above compounds is diffusion-controlled is evident from the linear relationship obtained between diffusion current \( (i_d) \) and square root of the mercury column height \( (\sqrt{h}) \) (Figs 5 and 6; Tables III and IV).

The analysis has been accomplished both by normal pulse polarography (NPP) and differential pulse polarography (DPP) using the linear calibration plots. Calibration graphs were prepared between concentration of each thiourea derivative taken and diffusion current measured in terms of wave height (using NPP) and peak height (using DPP). The plots were linear up to 18.24, 20.40, 21.96, 21.90, 23.74, 23.76 and 26.88 \( \mu g \) ml\(^{-1} \) (using NPP) and 6.08, 6.88, 7.32, 7.26, 7.92, 7.98 and 8.96 \( \mu g \) ml\(^{-1} \) (using DPP) respectively for phenyl, tolyl-, o-methoxy, p-methoxy, o-ethoxy, p-ethoxy and o-phenylene-bisthiourea. The results recorded in Tables V and VI show that these compounds with sample sizes of 4.5 and 18.0 \( \mu g \) (using NPP); and 1.1 and 5.5 \( \mu g \) (using DPP) could be determined with overall standard deviations of 0.030 and 0.090; and 0.009 and 0.031 respectively.

The above pulse-polarographic methods have subsequently been extended to the analysis of commercial products based on thiourea derivatives. Before applying the methods to the analysis of formulated products of thiourea derivatives, they have first been used for the determination of pure compounds of above products. The maximum RSD's calculated from the pooled data of all the determinations using thiophanate-methyl and thiophanate in the ranges 6.8 - 54.4 \( \mu g \) (using NPP) and 1.7 - 13.6 \( \mu g \) (using DPP) were 0.74 and 0.82% respectively (Tables VII and VIII). The same in the determinations using thiouracil, methyl thiouracil and thiopentone sodium in the ranges 2.5 - 10.0 \( \mu g \) (using NPP) and 1.25 - 6.25 \( \mu g \) (using DPP) were 0.85% and 0.88% respectively (Tables IX and X). On applying the method to the formulations, the recoveries were in the range 98.6 - 99.2% of the nominal contents with RSD's in the range 0.42 - 0.54% (Table XI). The results have, however, been compared to an independent method\(^{75,109} \). The method using differential pulse mode for the determination of thiophanate-methyl is quite sensitive and can be applied for the analysis of as little as 0.34 \( \mu g \) ml\(^{-1} \) of thiophanate-methyl. The method has subsequently been extended to the residue analysis of this fungicide on grains and apple fruits. The recoveries from fortified samples (wheat, barley and apple fruits) were good. They ranged from 90.5 - 98.0% with RSD's in the range
The results of residue analysis of treated samples are given in Table XIII.

The above pulse polarographic methods besides being simple, rapid, accurate and sensitive are advantageous because of their specificity particularly in the case of drugs where the excipients of the drug formulations interfere in the commonly employed methods, do not do so in the above methods. Sucrose, lactose, calcium carbonate or steric acid even when present upto 200-folds excess do not cause interference using the pulse polarographic methods.

The polarographic behaviour of thiourea group at DME is believed to be due to oxidation of mercury involving one-electron transfer per thiourea group (similar to that of the mercapto group (-SH) present in thiols). The consistency of $E_{1/2}$ at various concentrations of thioureas supports the mechanism given above. Further, support to this electrode process comes from the observation (a) When the solution obtained at the end of the polarographic analysis is subjected to polarographic measurements again, a reversible cathodic wave corresponding to one-electron reaction is obtained. (b) When one solution containing 1.0 mM of each thiourea derivative alone and the other containing 1.0 mM thiourea derivative plus 0.5 mM mercuric chloride were polarographed under identical conditions, the latter yielded a well-defined cathodic wave corresponding to one-electron reduction having $E_{1/2}$ value equal to that of anodic wave obtained using solution containing thiourea derivative alone. These observations clearly indicate that the electrode reaction is solely due to the anodic dissolution of mercury forming its compound with thiourea derivatives. That thiols, 2-mercaptoethanol and reduced glutathion undergo similar reaction, has also been reported by some workers. In our laboratory similar behaviour has been found in case of dithiocarbamates whose oxidation mechanism is similar to mercaptans.
Another attractive feature of the proposed methods is that $E_{1/2}/E_p$ values for the thiourea derivatives are sufficiently spaced to make their characterisation/identification possible if they happen to be present in admixtures.

Despite the development of many competitive analytical techniques, spectrophotometry continues to be very popular. The inherent ease and simplicity of spectrophotometric methods coupled with the availability of inexpensive and reliable instruments are undoubtedly the important reasons for their popularity. The technique has been found quite useful in the present investigations. An extremely sensitive spectrophotometric method for the micro determination of above compounds and easily adaptable to the residue analysis of thiophanate-methyl fungicide on foodstuffs has been developed. The method is based on the formation of greenish yellow complex (showing $\lambda_{\text{max}}$ at 360 nm, Fig 7) formed through complexation of the thiourea moiety of above compounds with nickel(II) in the presence of triethylamine. The colour which develops instantaneously on mixing the reagents is stable for at least 2h. The method can be employed for the estimation of as little as 1 $\mu$g ml$^{-1}$ of each thiourea derivative. The results recorded in Table XIV show that overall standard deviations calculated from the pooled data of all the determinations made with 5 and 40 $\mu$g of each thiourea derivative are 0.032 and 0.225 respectively. Before applying the method to the analysis of commercial products of thiourea derivatives, it has first been used for the determination of pure compounds of above products. The maximum RSD's calculated from the pooled data of all the determinations made with 3 – 48 $\mu$g of thiophanate-methyl and thiophanate were 0.93% (Table XV). The same in the determination of thiouracil, methyl thiouracil and thiopentone sodium with sample sizes in the range 5 – 50 $\mu$g were 0.89% (Table XVI). When the method was applied to the analysis of commercial formulations of above compounds, the recoveries were in the range 98.6 – 99.0% of the nominal contents with RSD’s in the range 0.34 – 0.62% (Table XVII). The method has subsequently been extended to the residue analysis of thiophanate-methyl on grains and apple fruits. The recoveries from fortified grains (wheat and barley) and apple fruits ranged from 90.0 – 98.4% with RSD’s in the range 0.38 – 0.58% (Table XVIII). The results of residue analysis of treated samples are summarized in Table XIX.

The above method is quite sensitive ($\varepsilon$, $7.3 \times 10^3$ 1 mol$^{-1}$ cm$^{-1}$ on the basis of fungicide content), as little as 0.5 $\mu$g ml$^{-1}$ of the fungicide can be determined. The method is advantageous in that it is simple and rapid; it does not involve time-consuming
operations such as precipitation, filtration and solvent extraction (of coloured products) used in commonly employed method\textsuperscript{25}.

The rapidity and well-established stoichiometry of the colour reaction coupled with the stability of the colour prompted us to use this reaction for the photometric titrimetric determination of thiourea derivatives. In photometric titrations performed at 360 nm, the absorbance increases linearly up to the equivalence point (corresponding to thiourea derivative-metal molar ratio of 2:1) and thereafter, it attains almost constant values (Fig 8). The method has subsequently been applied to the determination of thiourea derivatives in commercial formulations. The overall standard deviations calculated from the pooled data of all the photometric titrations performed with 10 and 40 µg of each derivative have been found to be 0.062 and 0.167 respectively (Table XX). The maximum RSD’s in the determination of pure thiophanate-methyl, thiophanate, thiouracil, methyl thiouracil and thiopentone sodium were 0.75, 0.64, 0.73, 0.80 and 0.73% respectively (Tables XXI- XXII). When applied to the determination of these compounds in their commercial formulations, the recoveries were in the range 98.2 – 99.4% with RSD’s in the range 0.38 – 0.48 (Table XXIII). The photometric titration procedure possesses an edge over the direct colorimetric procedure in that no calibration curve is required and this makes the method rapid. However, the latter are more sensitive.

The results obtained show that each molecule of thiourea derivative including thiouracil, methyl thiouracil and thiopentone sodium reacts with 0.5 mole of nickel(II) in the presence of triethylamine. That thioureas form complexes with metals where thiourea

\[
2 \text{-HN-C=S} \quad \text{+} \quad 2 \text{-HN-C-SH+Ni}^{2+} \quad \text{+} \quad 2 \text{H}^+ \rightarrow \left[\left(\text{-HN-C-S}_{2}\text{-Ni}\right)\right] + 2 \text{H}^+
\]

moiety is bound to metal through sulphur atom (and not through nitrogen atom) is well established\textsuperscript{2}. It may be mentioned that formation of above type of complexes (ML\textsubscript{2}) as a result of reaction of not only of thioureas but also other sulphur ligands viz. thiols, 1,2-dithiols, N,N-diphenyl-N’-(4-phenyl-benzoyl) thiourea with metal ions like copper(II), nickel(II) and cobalt(II) is also well known\textsuperscript{13,115,131-134}. The above stoichiometry is further supported by high accuracy and precision obtained in photometric titrations which are marked by a well-defined intersection at sample to metal molar ratio of 2:1. However, with thiophanate-methyl and thiophanate, molar ratio of 1:1 is obtained which is in
conformity with the presence of two thiourea moieties. The most plausible course of reaction is:

\[
\begin{align*}
\text{NH–C–NH–C–OR} & \quad \text{SH} \quad \text{O} \quad + \text{Ni}^{2+} \\
\text{NH–C=N} – \text{C–OR} & \quad \text{SH} \quad \text{O} \quad + 2\text{H}^+ \\
\text{NH–C–N} – \text{C–OR} & \quad \text{SH} \quad \text{O} \\
\end{align*}
\]

This mechanism is supported by following literature citation and experimental observations.

(1) That alkoxy carbonyl thiourea group \( \text{–NH–C–NH–C–OR} \) (present in thiophanates) acts as a uninegative bidentate ligand and coordinates to metal through oxygen and protonated sulphur, is well known\(^{135}\).

(2) The infra red spectrum of the isolated products show red shifts for both \( \text{C=O} \) (1680 cm\(^{-1}\)) and \( \text{C=S} \) (1500 cm\(^{-1}\)) bands with the simultaneous appearance of new bands at 1560 cm\(^{-1}\) (\( \text{C-O} \)) and 1430 cm\(^{-1}\) (\( \text{C-S} \)) clearly suggesting the participation of alkoxy carbonyl oxygen and thiocarbonyl sulphur in complex formation.

(3) The estimation of nickel complexometrically\(^{136}\) and thioureas/fungicides oxidimetrically with cerium(IV)\(^{137,138}\) in the isolated products (nickel(II)-thiourea/fungicide complexes) further provide support to above stoichiometries.

It is important to mention here that triethylamine plays an important role in the proposed method. By acting as a base, it takes up the protons released in the above colour forming reactions and thereby drives the reaction to quantitative completion. Thioureas

\[
\text{Et}_3\text{N} + \text{H}^+ \quad \longrightarrow \quad \text{Et}_3\text{NH}^+
\]

as such are very weak nucleophiles and consequently their complexes with metals are not formed readily. Triethyl amine (a base) activates them by abstracting hydrogen from thiol sulphur and thus makes them strong nucleophiles. The role of amines and for that matter
any base in activating thioureas (including their synthetic reactions) in characterizing alkyl halide is well known.

\[ RX + CS(NH_2)_2 \rightarrow RS-C_S^{\text{NH}}NH_2 HX \]

With regard to the above spectrophotometric methods, it may be emphasized that the instantaneous development of colour and its sufficient solution stability, nonrequirement of extraction of the coloured product (a factor common in colorimetric analysis) and well established stoichiometry of the colour reaction are some of the special attributes of these methods.
Organic compounds containing thiourea function find many industrial, agricultural, pharmaceutical and analytical applications. Thiophanate-methyl and thiophanate (fungicides) and thiouracil, methyl thiouracil and thiopentone sodium (drugs) are important commercial formulations based on thiourea compounds. Their wide use necessitates convenient, reliable and rugged methods for their determinations, not only in their formulations for the purpose of quality control but in residues (particularly in case of fungicides) as well. Our efforts have led to the development of pulse polarographic and spectrophotometric methods based on the electrochemical oxidation and coloured complex formation reaction of thiourea function present in them. In order to ensure wide applicability of these methods to more and more fungicides and drugs (based on thiourea derivatives), it was thought worthwhile to standardize these methods on a large number of thiourea derivatives before applying them to the real samples.

Advantage has been taken of the facile electrochemical oxidation of thiourea group in acidic medium in developing a pulse polarographic method for the determination of thiourea derivatives and subsequently extend the same to formulated products based on them. Owing to the limited solubility of these compounds (except thiopentone sodium) in water/aqueous acidic media, their polarographic determination in mixed solvent media (through the use of suitable solvent-electrolyte system) is advantageous. As a result of wide study covering various solvents and supporting electrolytes to obtain relatively well defined polarograms, best results have been obtained in aqueous acetonitrile (3:2 v/v) media in the presence of sulphuric acid (to maintain pH at 2-3) and potassium perchlorate (0.01M) as supporting electrolyte. Acetonitrile has been chosen as the solvent in view of the free solubility of water -insoluble thiourea derivatives in it, its miscibility with aqueous/aqueous acidic solutions and resistance to electrochemical oxidation/reduction. The polarographic oxidation of these compounds was studied at DME. Well defined diffusion-controlled anodic waves at —0.125, -0.130, 0.110, 0.123 and 0.097V (vs SCE) were obtained for thiophanate-methyl, thiophanate, thiouracil, methyl thiouracil and thiopentone sodium respectively and subsequently made the basis of analysis of above compounds. The analysis has been accomplished by NPP and DPP using linear calibration plots. The methods have been subsequently adapted to the analysis of these compounds in various fungicide and drug formulations and in analyzing thiophanate-methyl (fungicide) residues on foodstuffs (grains and apple fruits). In view of the threat posed to human health by consumption of contaminated foodstuffs resulting from large-scale application of pesticides on crops, the residue analysis is of quite importance for
continuously monitoring the products for pesticide residues. The residues on foodstuffs are extracted with acetonitrile and then chromatographed on silica-gel column. After concentration of the eluate by evaporation, the residue is dissolved and polarographed in DPP mode.

That thioureas react with nickel(II) in 2:1 molar ratio in methanol-DMF media and in the presence of triethylamine to form intense greenish yellow complexes, has been made use of in developing spectrophotometric methods for the micro/trace determination of thiourea derivatives and their commercial products. The colour which develops instantaneously on mixing the reagents is stable for atleast 2h and shows λmax at 360 nm. The method can be employed for the estimation of as little as 1μg ml⁻¹ of each thiourea derivative. The smooth and quantitative nature of the colour reaction, its well-established stoichiometry and excellent solution stability of the reagent as well as colour have further been utilized in developing a photometric titration procedure for the determination of above compounds. In photometric titrations performed at 360 nm, an inverted L-shaped titration curve has been obtained. The end-point has been found by extrapolation of linear segments. The methods have successfully been applied to the analysis of commercial products based on thiourea derivatives. The spectrophotometric method for the determination of thiophanate-methyl is quite sensitive (E, 7.3 x 10³ l mol⁻¹ cm⁻¹); as little as 0.5 μg ml⁻¹ of the fungicide can be determined. The method has subsequently been applied to the analysis of this fungicide in residues on foodstuffs. The residues were extracted with acetonitrile and then cleaned-up using ethyl acetate – cyclohexane (1:1 v/v) as eluting solvent. After the concentration of the eluate, the residue was dissolved in DMF, mixed with triethylamine and nickel(II) and resulting colour was evaluated at 360 nm.

The instantaneous development of colour and its stability, well-established stoichiometry of colour reaction and non-extraction of colored products are some of the attractive features of the above spectrophotometric method.
REFERENCES
15. Quraishi, M.A., Ansari, F.A. and Jamal, D., Material Chemistry and Physics., 2003, 77, 687
84. Qian, C. and Shan, G., Huanjing Huaxue, 1991, 10, 76.
86. Miyamoto, F. and Saeki, M., Chiba-Ken Eisei Kenkyusha Kenkyu Kokoku, 1996, 20, 12.
89. Ishii, Y., Nippon Noyaku Gakkaishi, 1990, 15, 211.


CHAPTER – II

SECTION – B
DETERMINATION OF SOME AMINES AND ISOTHIOCYANATES (AS THIOUREA DERIVATIVES) IN THEIR FORMULATIONS AND RESIDUES
INTRODUCTION
In the course of our investigations on the determination of thioureas, we found that the reactions which are used in the preparation of these compounds are of considerable analytical utility. Special mention in this context may be made of the reactions of amines with isothiocyanates. The transformation of amines with isothiocyanates and vice-versa to respective thiourea derivatives is so smooth and quantitative that this reaction could form the basis of an important approach for the determination of amines as well as isothiocyanates. The simplicity and reliability of polarographic and spectrophotometric methods already developed for the determination of thiourea derivatives prompted us to extend the advantages of these methods to the analysis of amines and isothiocyanates as well. In these efforts a large number of primary/secondary amines and isothiocyanates have been determined by making use of their mutual reaction to form respective thiourea derivatives. These methods have subsequently been applied to the analysis of above compounds in their formulations and in residues (on foodstuffs). The proposed methods possess significant advantages over the commonly employed methods for these compounds/commercial products. A brief account of the methods available in literature for the analysis of amines and isothiocyanates are given below.

**Amines (amino function)**

Among the various nitrogen functions, the amino function is the most important in terms of number of compounds of commercial and biological significance. The biological importance of amines and amino acids is well known. Appreciable portion of huge industries such as those connected with pharmaceuticals, coatings, adhesives, cosmetics, textiles and dyestuffs utilize amines or amino derivatives at many stages. Amines also serve as the starting materials for the preparation of a variety of commercially important synthetic compounds such as dithiocarbamates, amides, thioamides, ureas, thioureas, aminophthalimides and pyrazole, benzodiazepino, cyclodecano and cyclotridecano derivatives.

The amino function involves primary, secondary and tertiary amines (RNH₂, R₂HN, R₃N). It has been determined by numerous methods involving a variety of procedures and techniques. The work on analysis of this function has been reviewed in several books/monographs. Broadly, the methods for determining amino function depend on one of the following fundamental procedures, i.e. neutralisation, nitrosation, acetylation, diazotisation and Schiff's base formation.
The alkyl amines are stronger bases than ammonia, whereas aromatic amines are weaker. Almost any amine can be titrated either in water or in certain organic solvents, like acetic acid, nitromethane, methylene chloride or chlorobenzene. The aliphatic amines are usually basic enough to be titrated directly in aqueous solutions using standard acids. The aromatic amines or other weakly basic amines i.e. symmetrical aliphatic diamines, can not be titrated in water but can be successfully titrated in non-aqueous solvents.

The primary amines react with nitrous acid to liberate nitrogen which is measured gasometrically or analyzed by gas chromatography. The classic Van Slyke method utilized this reaction for the determination of amino acids and proteins. Various improvements have been made for its quantitative conversion to nitrogen. This reaction has also been applied to the determination of secondary amines as well. The latter react with nitrous acid in a different way, but the 1:1 stoichiometry is preserved. The determination has been carried out by direct titration of the secondary amine with standard sodium nitrite or by measuring the nitrosamine spectrophotometrically. The use of nitrosyl bromide (in case of primary amines) and silica sulphuric acid (formed from the reaction of chlorosulphuric acid and silica gel) and sodium nitrite (in case of secondary amines) have been described as best nitrosation reagents.

The reactions of primary and secondary amines with anhydrides viz. acetic anhydride, succinic anhydride and o-sulphobenzoic anhydride can be used to determine these amines. The determination is accomplished by measuring the liberated acid alkalimetrically or measuring the residual anhydride.

Aromatic amines have reactions specific to them which can be used for their analysis. For example, primary aromatic amines can be diazotised with nitrous acid, which can be measured. The formation of diazonium salts through reaction of aromatic

\[
\text{RNH}_2 + \text{HNO}_2 \rightarrow \text{ROH} + \text{N}_2 + \text{H}_2\text{O}
\]

\[
\text{RNH}_2 + \text{HNO}_2 \rightarrow \text{RN}=\text{O} + \text{H}_2\text{O}
\]
amines with sodium nitrites is also known. Segmou et al. used this reaction for the indirect determination of nitrite in water samples. The unreacted aromatic amines were determined electrochemically and utilized as an indicator for nitrite. Some aromatic amines couple with diazonium compounds and this also forms the basis of an analytical method for their determination.

\[
\text{H}_2\text{N}-\text{C}_\text{H}_2\text{N}^+ + \text{ClN}=\text{N}^+ \rightarrow \text{H}_2\text{N}-\text{C}_\text{H}_2\text{N}=\text{N}^+ + \text{HCl}
\]

The reaction of primary amines with a carbonyl group to form Schiff's base (azomethane group) and the measurement of the Schiff's base or water produced form the basis of a method for the determination of primary amines. The Schiff's base produced by condensation of an amine with an aldehyde in strong acid solution, which could be oxidized to give coloured material has been utilized by various workers for the colorimetric determination of amines. Milla et al. determined ethylene diamines in some pharmaceutical preparations based on the colorimetric determinations of an orange chelate of a Schiff's base produced by the reaction of amine with pyridine-2-carboxyaldehyde.

Primary and secondary amines react with carbon disulphide to form dithiocarbamates. Tertiary amines on the other hand fail to do so. That the dithiocarbamates formed undergo oxidation and coloured complex formation reactions, has been made the basis of a variety of titrimetric, spectrophotometric and gas chromatographic methods for their determinations. Colour reaction of aliphatic amines with m-dinitrobenzene has been studied by Siddiqi and Pathania for their identification and spectrophotometric determination. The authors also reported the detection of methylamine in real water samples. The application of capillary electrophoresis technique has also been applied to the determination of biogenic amines/ amino drugs in biological fluids and human breast milk by some workers. Chiral recognition of secondary amines by using chiral crown (S,S)-3 and prudent (R,R)-4 has also been reported in literature.
Chromatographic methods viz. GC, HPLC, and HPTLC have found an extensive use in determination of amines. In the gas chromatographic determination of amines the relative ease of analyzing free amines follows the sequence:

Primary< Secondary< Tertiary

Although the analysis of tertiary amines presents no problems, the direct analysis of primary and secondary amines is usually achieved with column packing having supports treated with alkali to reduce adsorption or by derivatisation. Solid-phase micro extraction (SPME) coupled with GC with flame ionization detection (FID) to the determination of amines from gaseous samples has been reported by some workers.

The application of HPLC for the separation and determinations of amines including amino acids has also been reported in literature. Solid-phase micro extraction coupled with HPLC for the analysis of carcinogenic aromatic amines has been reported by Chang et al. HPLC coupled with UV detector, resonance raman detector, laser resonance spectrophotometric detector and electrogenerated chemiluminescence detector, have been reported by various workers for the determinations of various amines. Prechromatographic derivatisation of primary and secondary amines and amino acids by activated carbamates, polymeric anhydrides containing o-acetylsalicyl or phenyl isothiocyanate has also been reported by some workers. An isotope dilution liquid chromatography/tandem mass spectrometric detection method for DNA adducts of selected aryl amines has been developed by Means et al. Bang et al. employed blue chitin columns for the extraction of some carcinogenic heterocyclic amines from cooked meal in their liquid chromatographic-mass spectrophotometric (LC-MC) determination.

The use of high performance thin-layer chromatography (HPTLC) has also been described in the separation and determination of isomers of amines. The application of iodine-azide reaction in the HPTLC determination of non-sulphur amino-acids after derivatisation to respective thiourea derivatives has also been reported in literature.

Literature survey also reveals the application of voltammetry/polarography for the determination of aromatic amines. The use of this technique for the analysis of aromatic amines is, however, reviewed by some workers.
Dopamine hydrochloride — 3,4-dihydroxy phenethylamine hydrochloride is a commercial amino-based drug used as antihypertensive agent. For the treatment of hypertensive crises, it is given intravenously in the form of injection. The analysis of this drug is effected by titrating the drug in glacial acetic acid in the presence of mercuric acetate against 0.1 N perchloric acid using crystal violet solution as indicator. Each ml. of 0.1 N perchloric acid is equivalent to 0.01927g of C_9H_11NO_2.HCl. (dopamine hydrochloride).

Organoisothiocyanates (isothiocyanato function)

Organoisothiocyanates have been known over a century. The compounds are characterized by the presence of NCS group and are considered as esters of isothiocyanic acid i.e. H—NCS. Organoisothiocyanates are commonly found in plants viz. crees, radish, rape etc. and frequently possess physiological activity. Isothiocyanates have been widely used in biochemical analysis as a tool in the study of the structure and functions of proteins. They are also used extensively for identifying the amino terminal of amino acid for a peptide chain, to determine the amino acid sequence of peptides, as a label for proteins and polypeptides, and to induce chemical modifications of enzymes. Phenyl isothiocyanate is used extensively in sequencing peptides. Fluroscein isothiocyanate is used mainly as a reagent for attaching the fluroscein chromophore to amino acids of proteins for the purpose of micro determination. Recently, allyl and phenyl isothiocyanates have been shown to inhibit lung tumor in animals. It has also been established that dietary intake of benzyl isothiocyanate may protect against cancer. Some of these compounds particularly butyl, phenyl, allyl, phenyl ethyl and p-chlorophenyl isothiocyanate are known for their bactericidal and fungicidal activity. The use of organoisothiocyanates as intermediates in polymer synthesis is well known. In organic qualitative analysis, isothiocyanates are standard reagents for rapid identification of alcohols, phenols and primary and secondary amines.

The wide commercial importance of these compounds has prompted the appearance of many methods for their determination. The various chemical methods
developed for the determination of these compounds are based on the oxidation reactions of the \(-\text{N}=\text{C}^-\) bond with nucleophillic reagents or on the fission of their \(-\text{N}-\text{C}-\text{S}\) chain. The most widely used is the titrimetric method of Siggia and Hanna\(^1\) in which butylamine is reacted with the compound in dioxane and excess amine is titrated. This procedure has

\[
n\text{-C}_4\text{H}_9\text{NH}_2 + \text{RNCS} \rightarrow n\text{-C}_4\text{H}_9\text{NH CS NHR}
\]

been adapted to the micro level by Karten and Ma\(^2\). Chlorobenzene has also been used as the solvent in a micromethod\(^3\). Secondary amines such as di-n-butylamine\(^4\)-\(^5\), piperidine\(^6\) and diethyl amine\(^7\) have also been used in various solvents. Polyisothiocyanates used for the production of polyurethanes, have been determined by an ATSM method using di-n-butylamine in toluene\(^8\) and dimethylformamide\(^9\). Fischer\(^10\) determined isothiocyanates by reacting the sample with an excess of cystein or denatured ovalbumin of known thiol content. After incubating the reaction mixture, the unreacted

\[
\text{RNCS} + \text{HS-CH-COOH} \rightarrow \text{RNH-C-S-CH-COOH}
\]

excess of mercaptan was determined by back titration. Drobnica and Knoppova\(^11\) determined isothiocyanates and mustard oil glucosides (after liberating isothiocyanate from them) by mixing the sample in acetone with borate buffer (pH 10) and mercaptoacetic acid. The resulting solution was kept at 40\(^\circ\) for two hours, then mixed with hydrochloric acid and kept at 40\(^\circ\) for further two hours. The 3-substituted rhodamines produced were measured spectrophotometrically at 296 nm after extraction with ethylacetate. Ramose et al.\(^12\) isolated 3-methyl-3-butane and butyl isothiocyanates from capparis fluxuosa of Brazil and characterized and determined, these compounds by spectroscopic analysis (IR, MS, NMR) and chemical derivatisation. Reaction of fluorescein and isothiocyanate with thiols has also been used for the assay of isothiocyanates by Wilderspin and Green\(^13\).

Among the methods based on the desulphurisation reactions of thioureas formed as a result of the reaction between isothiocyanates and ammonia or amine, silver (I) and mercury (II) reagents have commonly been employed. The thiourea have been reported to react with silver(I) as :

\[
\text{RHNCSNH}_2 + 2\text{Ag}^+ \rightarrow \text{RHNCN} + \text{Ag}_2\text{S} + 2\text{H}^+
\]

Furst\(^14\) and Verma and Kumar\(^15\) determined isothiocyanates by titrating the alkylthiourea with silver nitrate amperometrically in aqueous ammonia-ammonium nitrate
buffer. The AOAC\textsuperscript{136} method for the determination of methyl isothiocyanate in pesticide formulations consists in transforming the isothiocyanate with n-butylamine in methanol into corresponding thiourea, keeping the solution for 20 minutes and titrating the thiourea formed with silver nitrate potentiometrically using Ag-AgCl electrode assembly.

In most of the methods\textsuperscript{137-146} employed for the determination of allyl isothiocyanate, the precipitated silver sulphide was filtered off and excess silver ions determined by Volhard’s method. Dieterich\textsuperscript{147,148} and Vuillemin\textsuperscript{149} developed gravimetric methods in which silver sulphide formed was weighed. Foster\textsuperscript{150} and Wronski\textsuperscript{151,152} described the determination of isothiocyanates by converting them to substituted thioureas in the usual way and titrating the latter with mercury (II) salts. Sontag and Kainz\textsuperscript{153} developed a method for the analysis of preserving agents for their isothiocyanate content. The latter was separated by steam distillation, converted to thiourea and titrated with cadmium acetate.

The method for the determination of isothiocyanate based on oxidimetric determination of substituted thioureas (formed as a result of their reaction with ammonia or amine), comes next to the silver procedures. Among the various oxidants, iodine, potassium bromate and potassium iodate or periodates have been quite oftenly used\textsuperscript{9,154-156}. Based on the property of thioureas to absorb in the UV regions, various spectrophotometric methods have been developed for the determination of isothiocyanates\textsuperscript{157-162}. Based on the hydrolysis in alcohol of isothiocyanates to give amines, Kemp\textsuperscript{164} developed a method for the determination of isothiocyanates by measuring the resulting amines. Skyler et al.\textsuperscript{165} applied this hydrolysis reaction to the

\[
\text{RNCS} + 3 \text{OH}^- \rightarrow \text{R NH}_2 + \text{HS}^- + \text{CO}_3^{2-}
\]

determination of isothiocyanatofluorescein and isocyanatofluorescein. The aminofluorescein formed from their alkaline hydrolysis was measured at 490 nm. Finholt et al.\textsuperscript{166} determined phenyl, \(\alpha\)-naphthyl and ethyl isocyanates by measuring the corresponding amines formed from their reduction with lithium aluminium hydride in other solvent. Wronski and Bald\textsuperscript{167} determined \(\alpha\)-naphthyl isothiocyanate by reducing it with Raney nickel. Chae and Tabatabai\textsuperscript{168} developed a colorimetric method for the determination of allyl isothiocyanate (produced in the thioglucosidase reaction from sinigrin). Methylene blue formed by the reaction of N, N-dimethyl p-phenylene diamine
with sulphide ions derived from hydrolysis of allyl isothiocyanate in xylene, was measured at 665 nm. Wronski\textsuperscript{169} reported that isothiocyanate possess hydrolysable sulphur which could be split off as sulphide. The measurement of the latter using o-hydroxymercuribenzoate and monopyridinium-3,6-dinitrophthalate reagents have been used in developing titrimetric\textsuperscript{169} and colorimetric methods\textsuperscript{170} for their determination. The oxidation of isothiocyanates to sulphate followed by its gravimetric or titrimetric determination, has been made the basis of a variety of methods for the determination of these compounds\textsuperscript{123}.

Anderson titrated\textsuperscript{171,172} alkyl silicon isothiocyanates by titration with standard alkali solution using acid-base indicators viz. phenolphthalein. In the titrimetric methods

\[
R_x Si[NCS]_{4-x} + (4-x) OH^- \rightarrow R_x Si(OH)_{4-x} + 4SC N^-
\]

involving the oxidation of isothiocyanates, the procedures generally follow back titration of the unused titrant. Breinlich\textsuperscript{174} carried out iodimetric titrations of allyl isothiocyanate in dilute alkali but iodine consumed was less than required to the proposed oxidation to isocyanates and sulphate. Wojahn\textsuperscript{137} also tried it under various conditions. He, however, failed to achieve more than 98% conversion and pointed out that alkyl thiourea might have been formed as an intermediate. Cavicchi\textsuperscript{156} and Nakamura\textsuperscript{174} used potassium bromate and permanganate oxidants. The excess oxidants were back titrated. Sharma and Sharma\textsuperscript{175} however, succeeded in direct titration of isothiocyanates in methanol – hydrochloric acid media with potassium bromate.

Gas chromatographic methods have been used for the analysis of isothiocyanates especially is residues on food and environment and also to study their degradation and emission in soils\textsuperscript{176-178}. Static headspace gas chromatographic (GC) methods\textsuperscript{179} for the analysis of methyl isothiocyanates and 1,3-dichloropropene (1,3-D) in soil and water have also been developed. Jiang et al.\textsuperscript{180} employed GC coupled with mass-spectrometry (GC-MS) for the analysis of allyl-3-methylthiopropyl-, 3-butenyl-, butyl-, phenyl- and 2-phenylethyl isothiocyanates in essential oil of Brassica Juncea in China. Literature survey also reveals the application of HPLC for determination of allyl isothiocyanate in vegetable samples\textsuperscript{181}.
PRESENT WORK
Amines and isothiocyanates, the compounds containing amino (\( \geq N \)) and isothiocyanato \((=\text{N}C\equiv\text{S})\) functions respectively, are of considerable industrial importance. Whereas the former find use as drugs, the latter find applications as pesticides predominantly as insecticides. Dopamine hydrochloride is a commercial drug containing amino function. Ditrapex is a commercial insecticide containing methyl isothiocyanate as the active ingredient. The analysis of the above products for their active ingredient contents is an important part of their commercial analysis.

Amines and isothiocyanates serve as the starting materials for the preparation of thioureas. The synthesis of many thiourea derivatives through amine–isothiocyanate reaction has been described in literature.\(^8\)\(^9\) In the course of our investigations, it was found that amine-isothiocyanate reaction besides serving as a means of preparing thioureas, shows promise of considerable analytical utility. The transformation of amines with excess of isothiocyanate and vice versa to the corresponding thiourea derivative in acetonitrile/dimethylformamide is smooth, rapid and quantitative at room temperature. These observations prompted us to make use of the formation of thiourea derivatives in the determination of amines and isothiocyanates, as well as commercial products viz. drugs and insecticide containing them.

The ease with which primary or secondary amines react with an excess of isothiocyanate and vice-versa in acetonitrile or dimethylformamide to quantitatively yield corresponding thiourea derivatives, and the simplicity and reliability of pulse-

\[
\text{RNH}_2 + \text{R'NCS} \rightarrow \text{RNHCS.NHR'}
\]

polarographic and spectrophotometric methods developed for the determination of thiourea derivatives, prompted us to extend the advantages of these methods to the determination of both amines as well as isothiocyanates. It is important to mention here again that both pulse-polarographic and spectrophotometric methods have first been tested on a good number of reference compounds viz. amines and isothiocyanates, before applying them to real samples viz. commercial drug and insecticide. This has been done to ensure the applicability of the methods to more and more commercial products containing amino and isothiocyanato function.

The observation that thiourea derivatives (formed from amines/isothiocyanates) react at DME in aqueous acetonitrile media in the presence of sulphuric acid (to keep pH at 2-3) and potassium perchlorate (0.01 M) as supporting electrolyte, to yield well-defined
analytically useful anodic waves, has been made the basis of pulse polarographic methods for the determination of above compounds. Whereas, in the transformation of amines/amino drug into thiourea derivatives, phenyl isothiocyanate has been found suitable, n-butylamine in turn is suitable for the transformation of isothiocyanates to the respective thiourea derivative. The excess of isothiocyanate or amine reagent do not interfere in the proposed methods under the experimental conditions. The analysis was accomplished by NPP and DPP using linear calibration plots. The methods have been subsequently applied to the analysis of above compounds in their commercial formulations for the purpose of quality control. Owing to the high sensitivity of DPP method, it has subsequently been applied for the determination ditrapex insecticide in residue and foodstuffs.

The reaction of thiourea derivatives formed from amines and isothiocyanates (through their mutual reaction) with nickel(II) acetate in methanol-DMF in the presence of triethylamine to form soluble greenish yellow nickel(II)-thiourea complexes, has been made the basis of spectrophotometric (both direct colorimetric as well as photometric titrimetric) methods for the determination of amines and isothiocyanates. The colour which develops instantaneously on mixing the reagents is stable for at least 2h and shows $\lambda_{\text{max}}$ at 360 nm in each case. The direct colorimetric method consists in adding to DMF solution of each amine an excess of phenyl isothiocyanate and to each isothiocyanate an excess of n-butylamine, followed by the addition of triethylamine and nickel(II) acetate (in methanol) and measuring the resulting colour at 360 nm. In photometric titrations, the resulting solution obtained by adding excess phenyl isothiocyanate to an amine and excess n-butylamine to an isothiocyanate is titrated photometrically with standard nickel(II) acetate (in methanol) at 360 nm in the presence of triethylamine. The absorbance increases till the quantitative formation of metal-thiourea complex and thereafter, it attains almost constant value. The end-point is point found from extrapolation of two linear segments. The above spectrophotometric methods have also been applied for analyzing commercial formulations containing both the insecticide as well as drug and residues containing insecticide only.
EXPERIMENTAL PROCEDURES
Determination of some amines and isothiocyanates (through transformation into corresponding thiourea derivatives) as such, in their formulated products and residues

A. Pulse-polarographic determination of amines and isothiocyanates and the drug/insecticide (based on them)

(a) Measurement of half-wave/peak potentials \((E_{1/2}/E_p)\)

A known concentration of each amine and dopamine hydrochloride (drug) in acetonitrile were taken and mixed with a drop (~50μl) of phenyl isothiocyanate and kept for 5 min to ensure the completion of reaction. Each solution was diluted to 20 ml with acetonitrile. For isothiocyanates, a known concentration of each isothiocyanate and ditrapex in acetonitrile were mixed with a drop (~50μl) of n-butylamine in acetonitrile. After 5 min, each solution was diluted to 20 ml with acetonitrile. Each of the above solution was transferred to polarographic cell containing potassium perchlorate (20 ml, 0.01 M in water), sulphuric acid (0.5 ml, 2M) to adjust pH at 2-3 and Triton-X-100 (1ml, 0.02%, in acetonitrile) and final volume made to 50 ml with potassium perchlorate. Polarograms (both NPP and DPP) of amines as well as isothiocyanates (as respective thiourea derivatives) were recorded with the following instrumental parameters:

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>SETUP</th>
<th>PARAMETERS</th>
<th>SETUP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Voltage</td>
<td>400mV vs SCE</td>
<td>Drop time</td>
<td>1 Sec.</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>10 μA/V for NPP</td>
<td>Scan rate</td>
<td>NOR</td>
</tr>
<tr>
<td></td>
<td>1 μA/V for DPP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C.C. compensation</td>
<td>5</td>
<td>Acquisition</td>
<td>Fast</td>
</tr>
<tr>
<td>I.R. compensation</td>
<td>0</td>
<td>0/P ZERO</td>
<td>485</td>
</tr>
<tr>
<td>Height of Hg column</td>
<td>150 cm.</td>
<td>Time constant</td>
<td>20 m Sec</td>
</tr>
<tr>
<td>Pulse amplitude</td>
<td>50 mV</td>
<td>Height of Hg pool</td>
<td>3.5 cm</td>
</tr>
<tr>
<td>Polarocord parameters</td>
<td>X axis= 100 mV/cm</td>
<td>Y axis= 200 mV/cm</td>
<td></td>
</tr>
</tbody>
</table>

Some typical polarograms each for n-butyl amine (a representative of all amines), dopamine hydrochloride, methyl isothiocyanate (a representative of isothiocyanates) are shown in Figs. 1-3. The values of half wave potentials \((E_{1/2})\) and peak potentials \((E_p)\) of above compounds are given in Tables I and II.
Typical normal pulse polarogram (a) and differential pulse polarogram (b) of n-butyl amine at 22±1°C.  
1, supporting electrolyte; 2, butyl amine.
Fig. 2

(a) Typical normal pulse polarogram (a) and differential pulse polarogram (b) of dopamine hydrochloride at 22±1°C
1, supporting electrolyte; 2, dopamine hydrochloride.
Typical normal pulse polarogram (a) and differential pulse polarogram (b) of methyl isothiocyanate at $22 \pm 1^\circ C$

1, supporting electrolyte; 2, methyl isothiocyanate.
(b) Effect of mercury column height on diffusion current:

Polarograms of standard solutions of each amine/ isothiocyanates and drug/insecticide compounds in acetonitrile at various height of mercury column were also recorded in usual way. The values of \( i_d / \sqrt{h} \) are given in Tables III and IV.

(c) Preparation of calibration graphs for amines and isothiocyanates

Stock standard solutions (10\(^{-3}\)M) of each pure amine or isothiocyanate were prepared in acetonitrile. Aliquots (0.1 - 1.0ml) of solutions of each amine in acetonitrile were mixed with a drop (~ 50 \(\mu\)l) of phenyl isothiocyanate. In case of isothiocyanates, aliquots (0.1-1.0ml) of solutions of each compound were in turn mixed with a drop of (~ 50 \(\mu\)l) of n-butylamine. Each solution was diluted to 20 ml with acetonitrile and kept for 5 min to ensure the completion of the reaction. To each solution were added potassium perchlorate (20 ml, 0.01M in water) and sulphuric acid (0.5 ml, 2 M) to adjust the pH of solution to 2. Triton-X-100 (1ml, 0.02% in acetonitrile) was also added as suppressor and final volume of each solution was made to 50 ml with potassium perchlorate. Nitrogen gas was bubbled for 15 min. through each solution and polarograms were recorded. The calibration graphs were constructed by plotting diffusion/peak current versus concentration of each compound in the usual way. Some results are given in Tables V – VIII.

(d) Formulation analysis

A single formulation each of dopamine hydrochloride injection (containing 40 mg ml\(^{-1}\) active ingredient) and ditrapex insecticide (containing 23.5% active ingredient) were used. A single large sample of each formulation was weighed and shaken with acetonitrile and filtered. The filtrate was diluted to known volume with the same solvent. Aliquots were then taken into the polarographic cell and processed for analysis in the same manner as described above for amines and isothiocyanates. Some results are given in Table IX.

(e) Residue analysis of ditrapex insecticide on grains

(i) Recovery experiment:

A known weight of grains (wheat and rice) were mixed with various amounts of ditrapex (in acetonitrile). The samples were extracted with 4-5 instalments of 2-3 ml of acetonitrile. The extracts were evaporated to dryness. The residue was dissolved in acetonitrile, and diluted to 20 ml with acetonitrile. Each extract was mixed with a drop of
n-butylamine (~50μl), kept for 5 minutes to ensure the completion of reaction and transferred into polarographic cell, containing potassium perchlorate (20 ml, 0.01 M), sulphuric acid (0.5 ml, 2M) and Triton-X-100 (1ml, 0.02% acetonitrile) and processed for differential pulse polarographic analysis in the same manner as described above. The results are given in Table X.

(ii) Residue analysis:

Grains (wheat and rice) were sprayed with insecticide formulation (aqueous dispersion) at concentration 1-6 g t⁻¹ at the rate of 100ml/kg commodities. Sprayed samples were dried in the sun and from these lots, samples of 15-20g were drawn for residue analysis and processed as described under recovery experiment. The results are given in Table XI.

B. SPECTROPHOTOMETRIC METHODS

1. Direct colorimetric procedure:

   Determination of amines and isothiocyanates and drug/insecticide based on them

   (a) Preparation of calibration graphs:

   Aliquots of (0.2 – 2.0 ml) of solutions in dimethylformamide of each compound were mixed with a drop (~50μl) of phenyl isothiocyanate (in case of amines/ amino drug compound) or n-butylamine (in case of isothiocyanates) and diluted to 3ml with dimethylformamide. After 5 min each solution was mixed with triethylamine (1 ml, 1M in DMF) and nickel(II) acetate (1ml, 0.001M in methanol). The absorbance of the greenish yellow colour measured at 360 nm. (the spectrum illustrated in Fig.4) against a reagent blank. The absorbance values were plotted against the concentration of amine/ amino drug and isothiocyanates/ ditrapex and calibration graphs were prepared. Some results are given in Tables XII and XIII.

   (b) Formulation analysis:

   Aliquots of dimethylformamide extracts of each drug and insecticide formulation were taken and processed for analysis as described above for pure compounds. The results of analysis were given in Table XIV
Absorbance Spectra
A: Reagent blank
B: Ni(II)-thiourea complex
(c) Residue analysis (for ditrapex insecticide)

(i) Recovery experiment:

A known weight of grains (wheat and rice) were mixed with various amounts of ditrapex (in dimethylformamide). The samples were extracted with 4-5 instalments of 2-3 ml acetonitrile. The extracts were evaporated to dryness, and residue dissolved in dimethylformamide and transferred to 25ml-measuring flasks. Each solution was then mixed with one drop of n-butylamine (~50μl), kept for 5 min (to ensure the completion of reaction), followed by the addition of triethylamine (1 ml, 1M in DMF), and nickel(II) acetate (1 ml 0.001 M in methanol) and colour which developed instantaneously was measured at 360 nm against a reagent blank. The results are given in Table XV.

(ii) Residue analysis:

Grains (wheat and rice) were sprayed with various strength (1-6 g/l) of insecticide formulation (aqueous dispersion) at a rate 100 ml/kg commodity. Sprayed samples were dried in the sun and from these lots samples of 15-20 g were drawn for residue analysis and processed as described under recovery experiment. The results are given in Table XVI.

2. Photometric titration procedure

(a) Determination of amines/ amino drug and isothiocyanates/insecticide

Aliquots of solutions in dimethylformamide of each amine/ amino drug compound, were taken mixed with one drop (~50μl) of phenyl isothiocyanate (for amines/ amino drug compound) or n-butylamine (for isothiocyanates) and diluted to 5 ml with same solvent. After 5 min each solution was mixed with triethylamine (1 ml, 1M in DMF) and titrated photometrically at room temperature (~24°C) with standard (0.001 M) nickel(II) acetate (in methanol) at 360 nm. Dilution corrections were applied and titration curves plotted in the usual way. The profile of a typical photometric titration is shown in Fig. 5. The results are summarized in Tables XVII and XVIII.

(b) Formulation analysis:

Suitable aliquots of dopamine hydrochloride and ditrapex in dimethylformamide were taken and processed for analysis as described above for pure compounds. The results of analysis are given in tables XIV.
RESULTS
Table I: Half wave potentials ($E_{1/2}$) and peak potentials ($E_p$) of amines as thiourea derivatives

<table>
<thead>
<tr>
<th>Amines</th>
<th>$E_{1/2}$ (in V) (vs SCE)</th>
<th>$E_p$ (in V) (vs SCE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl</td>
<td>-0.175</td>
<td>-0.150</td>
</tr>
<tr>
<td>Ethyl</td>
<td>-0.180</td>
<td>-0.155</td>
</tr>
<tr>
<td>n-Propyl</td>
<td>-0.185</td>
<td>-0.160</td>
</tr>
<tr>
<td>n-Butyl</td>
<td>-0.190</td>
<td>-0.165</td>
</tr>
<tr>
<td>Dimethyl</td>
<td>-0.194</td>
<td>-0.169</td>
</tr>
<tr>
<td>Diethyl</td>
<td>-0.205</td>
<td>-0.180</td>
</tr>
<tr>
<td>Di-n-butyl</td>
<td>-0.225</td>
<td>-0.200</td>
</tr>
<tr>
<td>Dopamine hydrochloride</td>
<td>-0.395</td>
<td>-0.370</td>
</tr>
</tbody>
</table>
Table II: Half wave potentials ($E_{1/2}$) and peak potentials ($E_p$) of isothiocyanates as thiourea derivatives.

<table>
<thead>
<tr>
<th>Isothiocyanates</th>
<th>$E_{1/2}$ (in V) (vs SCE)</th>
<th>$E_p$ (in V) (vs SCE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl</td>
<td>-0.135</td>
<td>-0.110</td>
</tr>
<tr>
<td>Ditrapex insecticide</td>
<td>-0.130</td>
<td>-0.105</td>
</tr>
<tr>
<td>(containing 23.5% methyl isothiocyanate)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n – Butyl</td>
<td>-0.225</td>
<td>-0.200</td>
</tr>
<tr>
<td>Allyl</td>
<td>-0.190</td>
<td>-0.165</td>
</tr>
<tr>
<td>Phenyl</td>
<td>-0.180</td>
<td>-0.155</td>
</tr>
</tbody>
</table>
Table III: Effect of height of mercury column on diffusion current.

Concentration of each compound taken = 20 μg

<table>
<thead>
<tr>
<th>Height of mercury column (cm)</th>
<th>√h</th>
<th>i_d/√h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Methyl amine</td>
</tr>
<tr>
<td>152.0</td>
<td>12.32</td>
<td>0.53</td>
</tr>
<tr>
<td>146.0</td>
<td>12.08</td>
<td>0.53</td>
</tr>
<tr>
<td>132.0</td>
<td>11.48</td>
<td>0.53</td>
</tr>
<tr>
<td>126.0</td>
<td>11.20</td>
<td>0.52</td>
</tr>
<tr>
<td>112.0</td>
<td>10.58</td>
<td>0.54</td>
</tr>
</tbody>
</table>
Table IV: Effect of height of mercury column on diffusion current.
Concentration of each compound taken = 20 µg

<table>
<thead>
<tr>
<th>Height of mercury column, (cm)</th>
<th>$\sqrt{h}$</th>
<th>$i_d/\sqrt{h}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Methyl isothiocyanate</td>
</tr>
<tr>
<td>150</td>
<td>12.25</td>
<td>0.49</td>
</tr>
<tr>
<td>140</td>
<td>11.83</td>
<td>0.50</td>
</tr>
<tr>
<td>130</td>
<td>11.40</td>
<td>0.49</td>
</tr>
<tr>
<td>120</td>
<td>10.95</td>
<td>0.50</td>
</tr>
<tr>
<td>110</td>
<td>10.49</td>
<td>0.49</td>
</tr>
</tbody>
</table>
Table V: Determination of amines (as thiourea derivatives): Normal pulse polarographic procedure

<table>
<thead>
<tr>
<th>Amines</th>
<th>Mean diffusion current, $i_d$, µA</th>
<th>Amount found*, µg</th>
<th>Mean diffusion current, $i_d$, µA</th>
<th>Amount found,**, µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl</td>
<td>19.60</td>
<td>1.21, 0.010</td>
<td>38.80</td>
<td>3.57, 0.028</td>
</tr>
<tr>
<td>Ethyl</td>
<td>20.16</td>
<td>1.19, 0.009</td>
<td>38.60</td>
<td>3.63, 0.030</td>
</tr>
<tr>
<td>n-Propyl</td>
<td>20.20</td>
<td>1.22, 0.008</td>
<td>39.50</td>
<td>3.58, 0.022</td>
</tr>
<tr>
<td>n-Butyl</td>
<td>20.60</td>
<td>1.22, 0.011</td>
<td>40.40</td>
<td>3.64, 0.024</td>
</tr>
<tr>
<td>Dimethyl</td>
<td>20.88</td>
<td>1.18, 0.009</td>
<td>40.80</td>
<td>3.59, 0.032</td>
</tr>
<tr>
<td>Diethyl</td>
<td>20.90</td>
<td>1.21, 0.007</td>
<td>40.88</td>
<td>3.58, 0.031</td>
</tr>
<tr>
<td>Di-n-butyl</td>
<td>21.40</td>
<td>1.19, 0.008</td>
<td>40.90</td>
<td>3.62, 0.028</td>
</tr>
<tr>
<td>Dopamine hydrochloride</td>
<td>19.40</td>
<td>1.21, 0.010</td>
<td>36.40</td>
<td>3.64, 0.020</td>
</tr>
</tbody>
</table>

* Amount taken = 1.2 µg
**Amount taken = 3.6 µg
Table VI: Determination of amines (as thiourea derivatives): Differential pulse polarographic procedure

<table>
<thead>
<tr>
<th>Amines</th>
<th>Mean peak current, $i_p$, $\mu A$</th>
<th>Amount found*, $\mu g$</th>
<th>Mean peak current, $i_p$, $\mu A$</th>
<th>Amount found,** $\mu g$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl</td>
<td>1.02</td>
<td>0.101, 0.001</td>
<td>3.02</td>
<td>0.404, 0.002</td>
</tr>
<tr>
<td>Ethyl</td>
<td>1.10</td>
<td>0.099, 0.002</td>
<td>3.06</td>
<td>0.402, 0.003</td>
</tr>
<tr>
<td>n-Propyl</td>
<td>1.08</td>
<td>0.101, 0.001</td>
<td>3.04</td>
<td>0.395, 0.001</td>
</tr>
<tr>
<td>n-Butyl</td>
<td>1.10</td>
<td>0.100, 0.001</td>
<td>3.08</td>
<td>0.396, 0.002</td>
</tr>
<tr>
<td>Dimethyl</td>
<td>1.15</td>
<td>0.098, 0.002</td>
<td>3.10</td>
<td>0.397, 0.003</td>
</tr>
<tr>
<td>Diethyl</td>
<td>1.20</td>
<td>0.101, 0.001</td>
<td>3.12</td>
<td>0.404, 0.003</td>
</tr>
<tr>
<td>Di-n-butyl</td>
<td>1.26</td>
<td>0.102, 0.001</td>
<td>3.13</td>
<td>0.402, 0.002</td>
</tr>
<tr>
<td>Dopamine hydrochloride</td>
<td>1.02</td>
<td>0.101, 0.001</td>
<td>3.04</td>
<td>0.404, 0.002</td>
</tr>
</tbody>
</table>

* Amount taken = 0.10 $\mu g$

** Amount taken = 0.40 $\mu g$
Table VII: Determination of organo isothiocyanates (as thiourea derivatives): *Normal pulse polarographic procedure*

<table>
<thead>
<tr>
<th>Isothiocyanates</th>
<th>Mean diffusion current, $i_d$, μA</th>
<th>Amount found*, μg</th>
<th>Mean diffusion current, $i_d$, μA</th>
<th>Amount found,**, μg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl</td>
<td>22.4</td>
<td>2.98, 0.020</td>
<td>80.50</td>
<td>8.92, 0.040</td>
</tr>
<tr>
<td>n-Butyl</td>
<td>23.8</td>
<td>2.97, 0.014</td>
<td>84.64</td>
<td>9.09, 0.042</td>
</tr>
<tr>
<td>Allyl</td>
<td>26.2</td>
<td>3.03, 0.018</td>
<td>86.24</td>
<td>9.02, 0.048</td>
</tr>
<tr>
<td>Phenyl</td>
<td>28.0</td>
<td>2.96, 0.020</td>
<td>88.24</td>
<td>8.98, 0.050</td>
</tr>
</tbody>
</table>

* Amount taken = 3μg

** Amount taken = 9μg
Table VIII: Determination of isothiocyanates (as thiourea derivatives): Differential pulse polarographic procedure

<table>
<thead>
<tr>
<th>Isothiocyanates</th>
<th>Mean peak current, $i_p$, μA</th>
<th>Amount found*, μg</th>
<th>Mean peak current, $i_p$, μA</th>
<th>Amount found,** μg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl</td>
<td>2.04</td>
<td>0.298, 0.002</td>
<td>5.06</td>
<td>0.892, 0.008</td>
</tr>
<tr>
<td>n-Butyl</td>
<td>2.12</td>
<td>0.297, 0.002</td>
<td>5.10</td>
<td>0.896, 0.007</td>
</tr>
<tr>
<td>Allyl</td>
<td>2.15</td>
<td>0.302, 0.001</td>
<td>5.12</td>
<td>0.906, 0.006</td>
</tr>
<tr>
<td>Phenyl</td>
<td>2.14</td>
<td>0.298, 0.002</td>
<td>5.18</td>
<td>0.905, 0.008</td>
</tr>
</tbody>
</table>

* Amount taken = 0.3 μg
** Amount taken = 0.9μg
Table IX: Assay results of some commercial formulations containing dopamine hydrochloride and methyl isothiocyanate.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Maker's specification*</th>
<th>Recovery of active ingredient**, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NPP</td>
</tr>
<tr>
<td>Dopamine hydrochloride injection (drug)</td>
<td>40 mg ml⁻¹</td>
<td>98.6 ± 0.48</td>
</tr>
<tr>
<td>Ditrapex (insecticide)</td>
<td>23.5 %</td>
<td>99.0 ± 0.50</td>
</tr>
</tbody>
</table>

* Maker's specification established by independent method \(^{103,136}\).

** Values are the mean of three determinations with standard deviation (±).
Table X: Percentage recovery of ditrapex from fortified samples

<table>
<thead>
<tr>
<th>Active ingredient added, µg</th>
<th>Recovery*, %</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wheat</td>
<td>Rice</td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>99.5, 0.48</td>
<td>99.2, 0.54</td>
<td></td>
</tr>
<tr>
<td>5.0</td>
<td>92.0, 0.54</td>
<td>90.5, 0.64</td>
<td></td>
</tr>
<tr>
<td>10.0</td>
<td>92.5, 0.68</td>
<td>95.4, 0.58</td>
<td></td>
</tr>
<tr>
<td>20.0</td>
<td>90.7, 0.57</td>
<td>92.5, 0.62</td>
<td></td>
</tr>
<tr>
<td>40.0</td>
<td>94.5, 0.48</td>
<td>94.4, 0.57</td>
<td></td>
</tr>
</tbody>
</table>

* Values are the mean of three determinations with standard deviation (±)
Table XI: Results of residue analysis (ppm) of treated samples

<table>
<thead>
<tr>
<th>Strength of the spray used, g/l</th>
<th>Residue found (ppm)</th>
<th>Wheat</th>
<th>Rice</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.0</td>
<td>40.24</td>
<td>34.20</td>
<td>44.06</td>
</tr>
<tr>
<td>4.8</td>
<td>30.60</td>
<td>18.02</td>
<td></td>
</tr>
<tr>
<td>2.4</td>
<td>16.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.2</td>
<td>7.95</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table XII: Determination of amines (as thiourea derivatives) with nickel (II): Direct colorimetric procedure

<table>
<thead>
<tr>
<th>Amines</th>
<th>Values are mean of five determinations with standard deviation (±)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount found*, µg</td>
</tr>
<tr>
<td>Methyl</td>
<td>5.02, 0.030</td>
</tr>
<tr>
<td>Ethyl</td>
<td>4.98, 0.040</td>
</tr>
<tr>
<td>n-Propyl</td>
<td>5.04, 0.038</td>
</tr>
<tr>
<td>n-Butyl</td>
<td>4.96, 0.024</td>
</tr>
<tr>
<td>Dimethyl</td>
<td>4.97, 0.040</td>
</tr>
<tr>
<td>Diethyl</td>
<td>5.01, 0.034</td>
</tr>
<tr>
<td>Di-n-butyl</td>
<td>5.04, 0.030</td>
</tr>
<tr>
<td>Dopamine hydrochloride</td>
<td>4.98, 0.032</td>
</tr>
</tbody>
</table>

* Amount taken = 5 µg  
** Amount taken 40 µg
Table XIII: Determination of isothiocyanates (as thiourea derivatives) with nickel (II): Direct colorimetric procedure

<table>
<thead>
<tr>
<th>Isothiocyanates</th>
<th>Values are mean of five determinations with standard deviation (±)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount found*, µg</td>
</tr>
<tr>
<td>Methyl</td>
<td>7.47, 0.030</td>
</tr>
<tr>
<td>n-Butyl</td>
<td>7.48, 0.040</td>
</tr>
<tr>
<td>Allyl</td>
<td>7.56, 0.034</td>
</tr>
<tr>
<td>Phenyl</td>
<td>7.53, 0.054</td>
</tr>
</tbody>
</table>

* Amount taken = 7.5µg  
** Amount taken = 45.0 µg
<table>
<thead>
<tr>
<th>Formulation</th>
<th>Maker's specification*</th>
<th>Recovery of active ingredient**, %</th>
<th>Direct colorimetric procedure</th>
<th>Photometric titrimetric procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine hydrochloride injection (drug)</td>
<td>40 mg ml⁻¹</td>
<td>99.3 ± 0.42</td>
<td>100.1 ± 0.30</td>
<td></td>
</tr>
<tr>
<td>Ditrapex (insecticide)</td>
<td>23.5 %</td>
<td>98.3 ± 0.58</td>
<td>99.2 ± 0.50</td>
<td></td>
</tr>
</tbody>
</table>

* Maker's specification established by independent method \(^{103,136}\).

** Values are the mean of five determinations with standard deviation (±)
Table XV: Percentage recovery of ditrapex from fortified samples.

<table>
<thead>
<tr>
<th>Active ingredient added, µg</th>
<th>Recovery*, %</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wheat</td>
<td>Rice</td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>99.5, 0.21</td>
<td>84.7, 0.20</td>
<td></td>
</tr>
<tr>
<td>4.0</td>
<td>96.8, 0.50</td>
<td>98.7, 0.69</td>
<td></td>
</tr>
<tr>
<td>6.0</td>
<td>94.7, 0.71</td>
<td>97.1, 0.64</td>
<td></td>
</tr>
<tr>
<td>8.0</td>
<td>84.7, 0.42</td>
<td>99.5, 0.58</td>
<td></td>
</tr>
<tr>
<td>10.0</td>
<td>96.8, 0.50</td>
<td>99.0, 0.62</td>
<td></td>
</tr>
</tbody>
</table>

*Values are the mean of five determinations with standard deviation (±)
Table XVI: Results of residue analysis (ppm) of treated samples

<table>
<thead>
<tr>
<th>Strength of the spray used, g/l</th>
<th>Residue found (ppm)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wheat</td>
<td>Rice</td>
</tr>
<tr>
<td>6.0</td>
<td>40.80</td>
<td>44.50</td>
</tr>
<tr>
<td>4.8</td>
<td>33.80</td>
<td>34.60</td>
</tr>
<tr>
<td>2.4</td>
<td>18.04</td>
<td>19.02</td>
</tr>
<tr>
<td>1.2</td>
<td>9.12</td>
<td>9.80</td>
</tr>
</tbody>
</table>
Table XVII: Determination of amines (as thiourea derivatives) with nickel (II): Photometric titration procedure

<table>
<thead>
<tr>
<th>Amines</th>
<th>Amount found*, µg</th>
<th>Amount found**, µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl</td>
<td>10.02, 0.050</td>
<td>40.10, 0.240</td>
</tr>
<tr>
<td>Ethyl</td>
<td>9.92, 0.055</td>
<td>39.84, 0.206</td>
</tr>
<tr>
<td>n-Propyl</td>
<td>10.06, 0.054</td>
<td>40.16, 0.212</td>
</tr>
<tr>
<td>n.-Butyl</td>
<td>9.89, 0.068</td>
<td>39.82, 0.204</td>
</tr>
<tr>
<td>Dimethyl</td>
<td>9.94, 0.060</td>
<td>40.20, 0.216</td>
</tr>
<tr>
<td>Diethyl</td>
<td>9.96, 0.062</td>
<td>39.89, 0.218</td>
</tr>
<tr>
<td>Di-n-butyl</td>
<td>10.10, 0.054</td>
<td>39.80, 0.220</td>
</tr>
<tr>
<td>Dopamine hydrochloride</td>
<td>9.98, 0.040</td>
<td>39.86, 0.208</td>
</tr>
</tbody>
</table>

* Amount taken = 10 µg
** Amount taken 40 µg
Table XVIII: Determination of organo isothiocyanates (as thiourea derivatives) with nickel (II): Photometric titration procedure

<table>
<thead>
<tr>
<th>Isothiocyanates</th>
<th>Values are mean of five determinations with standard deviation (±)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount found*, µg</td>
</tr>
<tr>
<td>Methyl</td>
<td>12.51, 0.040</td>
</tr>
<tr>
<td>n-Butyl</td>
<td>12.46, 0.054</td>
</tr>
<tr>
<td>Allyl</td>
<td>12.42, 0.044</td>
</tr>
<tr>
<td>Phenyl</td>
<td>12.48, 0.052</td>
</tr>
</tbody>
</table>

* Amount taken = 12.5 µg
** Amount taken 50.0 µg
DISCUSSION
The excellent performance of pulse polarographic and spectrophotometric methods developed for the determination of thiourea derivatives prompted us to extend the advantages of these methods to the analysis of amines and isothiocyanates after transforming them to above derivatives through their mutual addition reactions. The quantitative nature of transformation of amines through reaction with an excess of isothiocyanate and vice-versa into corresponding thiourea derivatives has already been established in our laboratory. In order to ensure wide applicability of these methods, it was thought worthwhile to standardise them first on a number of amines and isothiocyanates before applying them to commercial drug and insecticide based on them.

With regard to the polarographic determination of amines and isothiocyanates as their respective thiourea derivatives (obtained after transforming each amine with phenyl isothiocyanate and each isothiocyanate with n-butylamine), the voltamograms are shown in Figs 1-3. In Tables I and II are given the $E_{1/2}$ and $Ep$ values of above amines. That the electrode reaction at DME is diffusion controlled, is evident from the linear relationship obtained between diffusion current ($i_d$) and square root of mercury column height ($\sqrt{h_{Hg}}$) (Tables III and IV). The analysis has been accomplished by NPP and DPP using linear calibration plots. The overall standard deviation calculated from the pooled data of all the determinations made with 1.2 and 3.6 µg (using NPP) and 0.1 and 0.4 µg (using DPP) of the listed amines were 0.009 and 0.027; and 0.001 and 0.002 respectively (Tables V and VI). The same in the determination of listed isothiocyanates with sample sizes 3 and 9 µg (using NPP) and 0.3 and 0.9 µg (using DPP) were 0.018 and 0.044; and 0.002 and 0.007 respectively (Tables VII and VIII). The methods when applied to analyse commercial drug/insecticide formulations, the recoveries were in the range 98.6-99.8 % of the nominal content with RSD's in the range 0.4-0.5 % (Table IX). The method with differential pulse mode has further been applied with success to analyse ditrapex insecticide in its residues on wheat and rice grains. Recoveries from fortified grains samples ranged from 90.5-99.5% with RSD's in the range 0.48-0.68% (Table X). In Table XI are given the results of residue analysis (ppm) of the treated samples.

It is important here to mention here that acetonitrile has been found to be most suitable solvent for polarographic measurements because (i) it has high dielectric constant and consequently facilitate the solubility of amines/isothiocyanates; (ii) the transformation of amines with an excess of an isothiocyanate and vice-versa into the corresponding
thiourea derivative is smooth and quantitative in this solvent (that this is indeed so has already been established in our laboratory \(^{182-185}\)); and (iii) in the analysis of ditrapex insecticide in the residues on foodstuffs, it has been found to be a suitable extracting solvent. The polarographic method has an edge over the gas chromatographic method\(^{136}\) (of analysis of ditrapex insecticide) which is time-consuming and requires strict control of experimental conditions with regard to column temperature etc.

The spectrophotometric methods (both direct colorimetric as well as photometric titrimetric) for the determination of amines and isothiocyanates, based on the color reaction of thiourea derivatives (formed from their mutual reaction) with nickel (II) acetate (in methanol) in the presence of triethyl amine in DMF are simple, rapid and sensitive. The color is stable for at least 2 h and shows \(\lambda_{\text{max}}\) at 360 nm. (Fig 4) in each case. Using direct colorimetry, the amines with sample sizes 5 and 40 \(\mu\)g; and isothiocyanates with sample sizes 7.5 and 45 \(\mu\)g could be determined with overall standard deviations of 0.035 and 0.211; and 0.040 and 0.325 respectively (Tables XII and XIII). The method has successfully been applied to the analysis of each of commercial drug containing dopamine hydrochloride and ditrapex insecticide containing methyl isothiocyanate for their active ingredient contents. The recoveries were in the range 98.3-99.3% with RSD's in the range 0.4-0.6% (Table XIV). The method has also been applied with success to the analysis of ditrapex insecticide residues on grains. The recoveries from the fortified samples were in the range 84.7-99.5 % with RSD's in the range 0.20-0.71% (Table XV). The results of residue analysis (ppm) of treated samples are given in Table XVI.

The smooth and quantitative nature of the transformation of thiourea derivatives to the respective coloured nickel(II) complexes and the stability of the reagent as well as colour prompted us to work out a photometric titration procedure for their determination and consequently amines and isothiocyanates. In photometric titrations performed at 360 nm, an inverted L-shaped titration curve is obtained in each case (Fig.5). The overall standard deviations calculated from the pooled data of all the titrations performed with 10 and 40 \(\mu\)g of listed amines and; 12.5 and 50.0 \(\mu\)g of each isothiocyanate were 0.056 and 0.216; and 0.048 and 0.38 respectively (Tables XVII and XVIII). When applied to analysis of commercial drug and insecticide formulations, the recoveries were in the range 99.2-100.1% with RSD's in the range 0.3-0.5% (Table XIV).
SUMMARY
Amines and organoisothiocyanates, through their mutual addition reaction, are facile to easy transformation into the corresponding thiourea derivatives. The simplicity and reliability of pulse-polarographic and spectrophotometric methods developed for the determination of thiourea derivatives, prompted us to extend the same to the determination of amines and isothiocyanates (which are also the compounds of considerable commercial importance). Whereas, the former find wide use in pharmaceuticals/drugs, the latter find applications as agrochemicals, predominantly as insecticides. Dopamine hydrochloride is a commercial drug containing amino function. Ditrapex is a commercial insecticide containing an isothiocyanate. That amines react with an excess of isothiocyanate and vice-versa in acetonitrile or DMF and are smoothly, rapidly and quantitatively transformed into respective thiourea derivatives and the latter are susceptible to electrochemical oxidation at DME in polarography, and undergo colour reaction with nickel(II) acetate in methanol-DMF media in the presence of triethylamine, have been utilized in developing both polarographic as well as spectrophotometric methods for the determination of both amines and isothiocyanates. The same have subsequently been extended to the determination of dopamine hydrochloride (an amine) and ditrapex (an isothiocyanate) for their active ingredient contents.

The pulse polarographic determination of the above compounds/commercial products has been made in aqueous acetonitrile in the presence of sulphuric acid (pH, 2-3), and potassium perchlorate (as supporting electrolyte). Well-defined diffusion controlled anodic waves have been obtained in case of each compound/formulated product. The analysis has been accomplished both by NPP and DPP using linear calibration plots. The methods have been subsequently applied to the analysis of drug/insecticide in their commercial formulations and residues. In the latter context, ditrapex insecticide residues on foodstuffs have been determined using DPP.

The analysis of above amines and organoisothiocyanates as well as commercial products has also been carried out spectrophotometrically (both direct colorimetry as well as photometric titrimetry) by measuring the thiourea derivative (formed as a result of reaction of each amines with an excess of isothiocyanate and vice-versa) in methanol-DMF media as their soluble nickel(II) thiourea complexes absorbing at 360 nm. The colour which develops immediately on mixing the reagents is stable for at least 2h in each case. In photometric titrations, the absorbance at 360 nm increases till the quantitative formation of metal-thiourea complex and thereafter, it attains almost constant values. The
end-point is found by extrapolation of linear segments. An inverted L-shaped curve is obtained in each case. The spectrophotometric methods have not only been applied to the analysis of above compounds in their commercial formulations but also in analysing insecticide residues in foodstuffs.

Both the proposed polarographic and spectrophotometric methods for the determination of amines/isothiocyanates and their formulated products, besides being sensitive possess advantages over the commonly employed methods in terms of simplicity and rapidity of procedures. It is important to mention here that these methods offer the possibility of determination of amines/ amino drugs in the presence of basic compounds which otherwise interfere in their acidimetric determination.
REFERENCES


47. Dreb, E.N., *Drug Std.*, 1958, 26, 175.


60. Dilorenzo, A. and Russo, G., J. Gas Chromatogr. 1968, 6, 509.
64. Bonafford, C.E., J. Gas Chromatogr., 1968, 6, 438.


