Determination of thiourea in fruit juice by a kinetic spectrophotometric method

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ABSTRACT

A catalytic kinetic method is described for determination of trace levels of thiourea based on its catalytic effect on the oxidation of Janus green (JG) by potassium iodate in hydrochloric acid media. The reaction was monitored by measuring the decrease in absorbance of the dye at 610 nm after 25 min. The effect of some factors on the reaction speed was investigated. The developed method allowed the determination of thiourea in range of $0.01–12.00$ mg L^{-1} with good precision, accuracy and the detection limit was 0.008 mg L^{-1}. Most of foreign species do not interfere with the determination. The method was found to be sensitive, selective and was applied to the determination of thiourea in fruit juices and orange peel.

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1. Introduction

Thiourea (TU) has found many applications in medicine, industry and other areas of chemistry [1]. TU and its derivatives are classical additives for the electro deposition of copper and other metals [2–13], corrosion inhibitors, and vulcanization accelerators, components of fertilizers, pharmaceuticals, pesticides and herbicides [14–17].

These substances are known to be toxic and hazardous; their toxic and hazardous effects seem to arise from a disturbance of carbohydrate metabolism [18] and could result in chronic goitrogenic and other glandular difficulties in humans [19]. Furthermore, TU and its derivatives have also been screened as allergenic [20] and carcinogenic factors [21], and it was shown that their presence inhibits nitrification in soil and water [22].

The direct exposure of workers or researchers and contamination of environment with thiourea should be avoided, because thiourea has been labeled as having carcinogenic activity. Hypothyroidism was induced in animals by using thiourea [23].

For these reasons, it has become increasingly important to monitor TU and related compounds, therefore appropriate methods for their determination in various media are worthy of development.

It is worthy of note that only a few methods are presented in the literature for the determination of thiourea. These are based on direct reflectance spectrometric measurements [23–25], Raman spectroscopy [26], mass spectrometry [27], FTIR [28,29], voltammetry [16], potentiometry [30], amperometry [31], titrimetry with iodine [32], potassium dichromate [33] and Masson and Race reagent [34], gas chromatography after benzoylation [35], high-performance liquid chromatography [36], flow injections methods [37–39] and kinetic methods [40,41]. Catalytic kinetic spectrophotometric method is a suitable technique for determination of trace levels of thiourea because of its high sensitivity and selectivity and ease of instrumentation.

Based on our knowledge, there is a kinetic catalytic spectrophotometric method for the determination of thiourea [41]. This method is based on oxidation of Janus green (JG) in acid phosphoric media and presence of Triton X-100 surfactant. The reaction was monitored spectrophotometrically by tracing the formation of green-colored oxidized product of JG. This method needs a surfactant or micellar media to access the high sensitivity. Introduction of a surfactant in the environment will always lead to contamination with this surfactant and is, therefore, of little use when the compound itself gives rise to environmental concern [42]. Because surfactants make the environment contaminated and are considered as a big threat, we realize if the reaction is done in a higher temperature, without utilizing surfactants, we will achieve more ideal sensitivity. In addition, this method suffers from interferences of some transition metals and anions.

This paper describes an original method for the highly sensitive, selective and precise determination of trace levels of thiourea in fruit juices and orange peel by spectrophotometry. This method is based on thiourea catalytic effect on the oxidation of JG with potassium iodate (KIO$_3$). The method has wide linear dynamic range ($0.01–12.00$ mg L^{-1}, low detection limit (0.008 mg L^{-1}) and free of most of interferences.
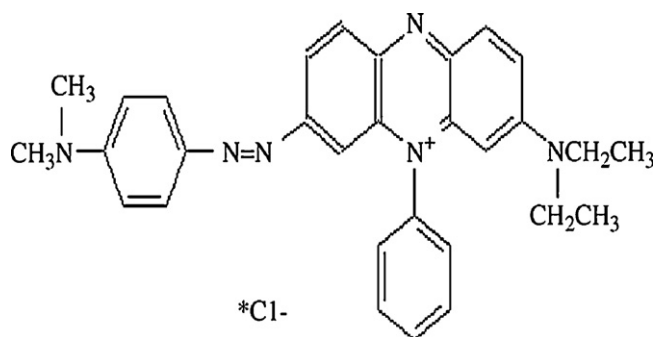
Fig. 1. Structure of Janus green.

2. Experimental

2.1. Apparatus

A double beam UV–vis spectrophotometer Varian model 300 Bio with 10 mm quartz cell was used for the recording the absorption spectra. A spectrophotometer (unic, Model 4802) was used to measure the absorbance change at 610 nm. A thermostat water bath was used to keep the reaction temperature at 55 °C. Eppendorf Vary-pipettes (10–100 μL and 100–1000 μL) were used to deliver accurate volume. All glassware and storage bottles were soaked in 10% HNO_3 overnight and thoroughly rinsed with water prior to use.

2.2. Reagents

All the reagents of analytical grade were used without further purification. Doubly distilled water was used to prepare all solutions throughout the study. A 1000 mg L^{-1} thiourea stock standard solution was prepared by dissolving 0.10 g of thiourea (Merck, Darmstadt, Germany) in water and diluting to 100 mL in a volumetric flask. Working standard solutions were obtained by appropriately diluting the stock solution before use. A 1.00×10^{-3} M JG solution was prepared by dissolving 0.0511 g of JG (Merck) in 100 mL water. A 0.02 M iodate stock's solution was prepared by dissolving 0.4280 g KIO_3 (Merck) in 100 mL water. A 0.20 M hydrochloric acid solution was prepared by diluting the required volume of 37% acid (Merck).

2.3. Recommended procedures for the determination of thiourea

All the working solution kept at (55 ± 0.5) °C in water bath for 25 min. Into a 25 mL volumetric flask, 2.5 mL hydrochloric acid (0.20 M), 0.9 mL JG (1.00×10^{-3} M) and appropriate amount of thiourea (0.01–12.00 mg L^{-1}) were transferred. Then 3.0 mL of KIO_3 (0.02 M) were added and solution was diluted with water to 25 mL, shaken and left in water bath. The absorbance of this catalyzed reaction was labeled as A_s and measured at 610 nm. The same procedure was repeated without addition of thiourea to get the blank signal (uncatalyzed reaction) and the signal was labeled as A_b. The calibration graph was constructed by plotting $\Delta A = A_b - A_s$ vs. thiourea concentration.

3. Results and discussions

3.1. The absorption spectra

JG (Fig. 1) is a basic dye of mono-azo group used in cytology for specific supervital staining of mitochondoria [43]. It is cathionic dye, which tends to form ion pair with bulky anions, so it is used as an excellent carrier for the transport of copper as Cu (SCN)$_4$$^{2-}$ ion through a bulk liquid membrane [44]. JG is a color reagent, which
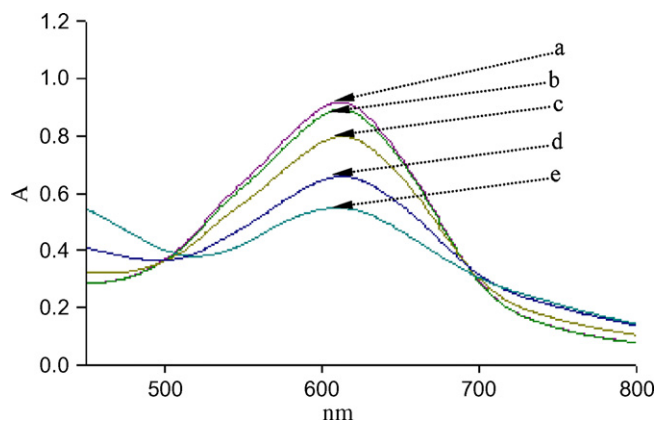

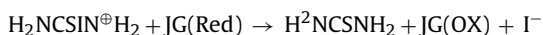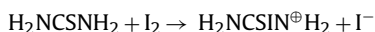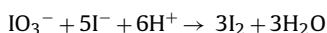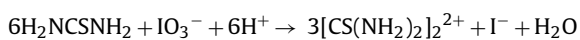Fig. 2. Variation of absorbance spectra of Janus green with thiourea concentration.

is oxidized by oxidizing agents such as bromate and iodate at slow reaction [45] to give a green product. On the other hand, in the presence of thiourea, the oxidation of JG by iodate is rapid. This cause a rapid change from blue to green, which can be monitored spectrophotometrically at 610 nm.

As shown in Fig. 2, the absorption spectra, both catalyzed (Fig. 2b–e) and uncatalyzed (Fig. 2a), reach their maximum at 610 nm reaction systems, and their difference also reaches its maximum at 610 nm. As a result, we selected 610 nm as the measuring wavelength.

Therefore, by measuring the decrease in absorbance of JG for 25 min from initiation of the reaction, the thiourea contents in the sample can be measured.

3.2. The indicator reaction

The possible mechanisms of the JG oxidation by thiourea given by Abbasi et al. [41]. According to these authors, this mechanism seems to contribute to the following reactions in its simplest form:

$$6H_2NCSNH_2 + IO_3^- + 6H^+ \rightarrow 3[CS(NH_2)_2]_2^{2+} + I^- + H_2O$$

$$IO_3^- + 5I^- + 6H^+ \rightarrow 3I_2 + 3H_2O$$

$$H_2NCSNH_2 + I_2 \rightarrow H_2NCSIN^{\oplus}H_2 + I^-$$

$$H_2NCSIN^{\oplus}H_2 + JG(Red) \rightarrow H^2NCSNH_2 + JG(OX) + I^-$$

where Red is reduced form and OX is the oxidized form of JG. According to this proposed mechanism, the generation of $H_2NCSIN^{\oplus}H_2$ can increase the rate of oxidation of JG.

3.3. Method optimization

The effect of reaction variables (reaction medium, oxidant concentration, indicator concentration, temperature and time) were studied by changing each variable in turn while keeping all others constant. The optimum values of the variables were maintained in each determination.

3.3.1. Effect of reaction medium
Preliminary investigation showed that inhibition effect can be observed in reaction media, thus some efforts was made for choosing the best type of reaction media. Several reaction media such as sulfuric acid (H_2SO_4), hydrochloric acid (HCl), phosphoric acid (H_3PO_4), boric acid (H_3BO_3), sodium hydroxide (NaOH), ammonium chloride–ammonia (NH_4Cl–NH_3),
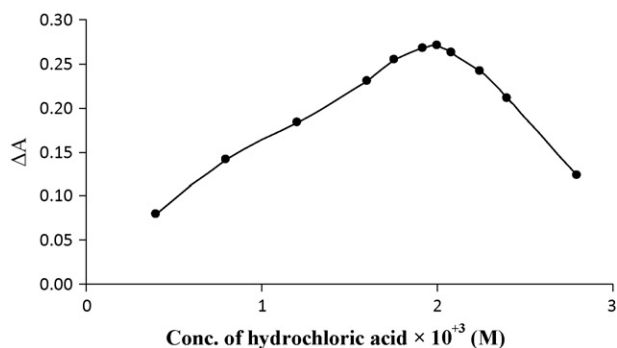
Fig. 3. Effect of acidity on the ΔA value.

potassium acid phthalate–NaOH, acetic acid–sodium acetate (CH_3COOH–CH_3COONa), hexamethylenetetraamine–hydrochloric acid ((CH_2)$_6$$N_4$–HCl), sodium borate ($Na_2B_4O_7$), citric acid–NaOH and sodium bicarbonate–sodium hydroxide ($NaHCO_3$–NaOH) had been tested. From the experiment result, it can be seen that ΔA reached its maximum in the medium of hydrochloric acid. Therefore, a solution of 0.2 M hydrochloric acid was prepared and effect of hydrochloric acid concentration on the ΔA was studied in the range of 4.0×10^{-3}–2.8×10^{-2} M. According to the results (Fig. 3) when 2.0×10^{-2} M of acid is used ΔA has a maximum sensitivity. Therefore, we selected 2.0×10^{-2} M of hydrochloric acid as optimum acidity value.

3.3.2. Effect of oxidant

The effects of five oxidants such as hydrogen peroxide, ammonium peroxodisulfate, potassium iodate, potassium bromate and potassium periodate were tested on ΔA. When potassium iodate (KIO_3) was used as the oxidant, ΔA reached its maximum. The influence of iodate concentration on the reaction was tested. The rate of oxidation reaction is proportional to the iodate concentration, so the signal ($\Delta A = A_b - A_s$) was increased until 2.4×10^{-3} M iodate. After that, the rate of JG-iodate in uncatalyzed solution was raised more than increasing rate of catalyzed reaction. Therefore, the net absorbance signal (ΔA) was decreased. It can be seen that the best concentration of iodate is 2.4×10^{-3} M. The results are shown in Fig. 4.

3.3.3. Effect of indicator

When the other reaction factors were fixed, the effect of JG dosage on ΔA was studied. When the dosage of JG increased, ΔA increased correspondingly; when concentration of JG was more than 3.6×10^{-5} M, ΔA would decrease. This may be to the aggregation of the dye at higher concentrations. Thus, 3.6×10^{-5} M was
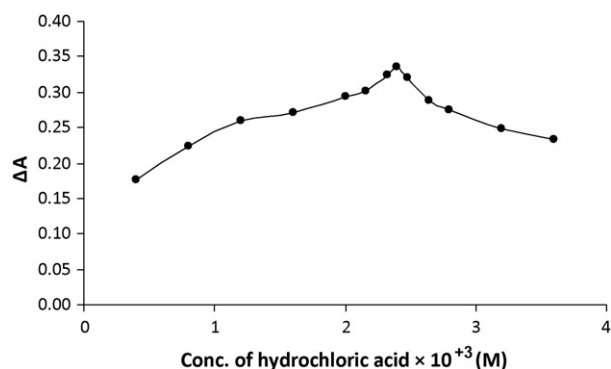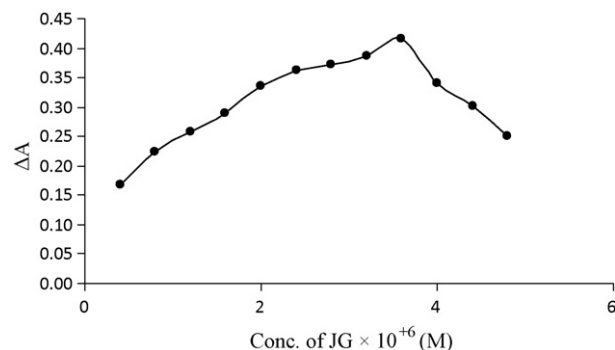

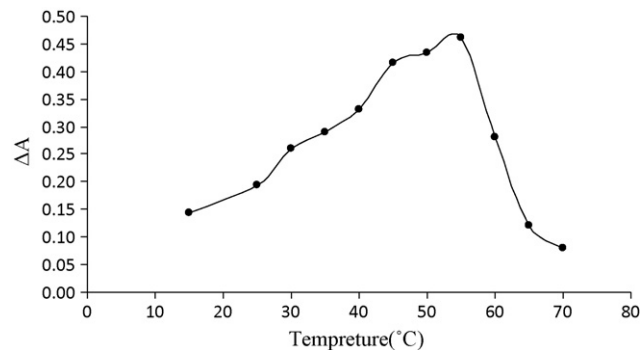**Fig. 5.** Effect of Janus green concentration on the ΔA value.

Fig. 6. Effect of temperature on the reaction speed.

selected as the optimum dosage of indicator. The results are shown in Fig. 5.

3.3.4. Effect of temperature

The influence of reaction temperature on the catalytic determination of thiourea was studied in range of 15–70 °C. As can be seen from Fig. 6, (ΔA) increased rapidly with increasing reaction temperature up to 55 °C and decreased at higher temperatures. This trend is due to the fact that upon increasing temperature, the rate of uncatalyzed reaction was increased, and therefore the difference of absorbance value between catalyzed and uncatalyzed reaction (ΔA) was decreased. Thus, 55 °C was chosen for further experiments.

3.3.5. Effect of time

As shown in Fig. 7, when the reaction time is between 5 and 25 min, ΔA is linearly correlating to the reaction time. After 25 min, the change in ΔA is very little with time, when the time is longer than 70 min ΔA will be constant and have no tendency to
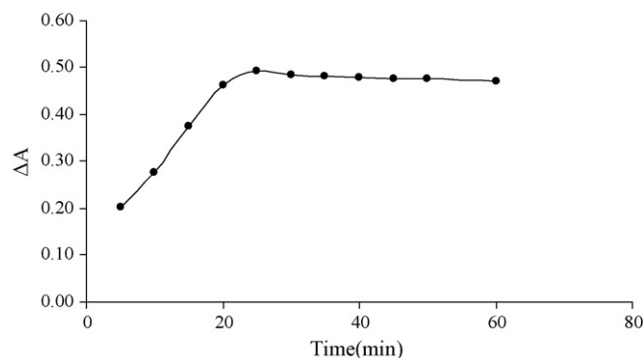

Fig. 4. Effect of oxidant concentration on the ΔA value.

Fig. 7. Effect of time on the ΔA value.

Table 1
Effect of diverse ions on the determination of 1.0 mg L^{-1} of thiourea.

Species	Tolerance limit ($C_{ion}/C_{thiourea}$)
K^+, Na^+, Mg^{2+}, Cd^{2+}, Ca^{2+}, Zn^{2+}, Ni^{2+}, Ba^{2+}, Mn^{2+}, Cr^{3+}, Bi^{3+}, Al^{3+}, Mn^{4+} Fructose, glucose, citric acid, ethanole, methanole, tartaric acid, EDTA, urea NO^-_3, Cl^-, ClO^-_4, $H_2PO^-_4$, F^-, $C_2O_4^{2-}$	2000
Li^+, NH^+_4, Cu^{2+}, Fe^{2+}, Co^{2+} Br^-, BrO_3^-, NO_2^-, HCO_3^-, CH_3COO^-, SO_4^{2-}, $S_2O_3^{2-}$,	1000
Pb^{2+}, Fe^{3+}, La^{3+} SCN^-, PO_4^{3-}	500
Hg^{2+}, Sn^{4+} CN^-, I^-	100
Ag^+, ascorbic acid	10

Table 2
Determination of thiourea in real samples when acid hydrochloric (0.2): 2.5 mL, JG (1.0×10^{-3} M): 0.9 mL, KIO$_3$ (0.02): 3.0 mL, time:25 min and temperature: (55 ± 0.5) °C.

Sample (mg L^{-1})	Amount added	Amount found ($n = 4$)	Average recovery (%)	Relative error (%)	Reference[a] method ($n = 5$)
Orange juice	5.0	4.87 ± 0.06	97.4	1.64	5.02 ± 0.07
	10.0	9.62 ± 0.09	96.2	0.85	10.12 ± 0.10
	15.0	14.51 ± 0.11	96.7	0.37	15.07 ± 0.13
Lemon juice	5.0	4.91 ± 0.08	98.2	1.84	5.06 ± 0.09
	10.0	9.72 ± 0.10	97.2	2.09	10.13 ± 0.11
	15.0	14.31 ± 0.13	95.4	0.98	15.08 ± 0.15
Orange peel	5.0	5.05 ± 0.05	101.0	3.33	5.02 ± 0.07
	8.0	7.81 ± 0.07	97.6	1.47	8.73 ± 0.08
	10.0	9.77 ± 0.09	97.7	0.76	10.02 ± 0.10

[a] Standard pulse polarographic method, Ref. [15].

increase. Therefore, 25 min was selected as the optimum reaction time.

3.4. Analytical data

Under the optimized experimental conditions, the assay of thiourea was carried out.

3.4.1. Calibration graph, detection limit and precision

Under the optimum experimental conditions, the difference in absorbance between blank and sample varied linearly with the concentration of thiourea in the range of 0.01–12.00 mg L^{-1} and fitted the equation: $\Delta A = 0.109C + 0.069$ with a square correlation coefficient (r^2) of 0.9990, where C is the thiourea concentration expressed in mg L^{-1}. The detection limit calculated from three times the standard deviation of the blank divided by the slope of the calibration graph was 0.008 mg L^{-1}. The relative standard deviation ($n = 7$) was

2.56% for the determination of 0.2 mg L^{-1} and 1.68% for the determination of 8.0 mg L^{-1} of thiourea.

3.4.2. Selectivity

In order to assess the application of the proposed method to environmental samples, the selectivity of the proposed method was evaluated by determining 1.0 mg L^{-1} of thiourea in the presence of varying amounts of various interfering species, which may accompany thiourea in fruit juice and orange peel. The tolerance limit was defined as the concentration of foreign species that produced a change in the ΔA less than 5%. The results are summarized in Table 1. It was found that many of these ions did not interfere, even when present in excess of 2000 to 20 fold. Positive interference was observed for Ag^+ because it could also catalyze the indicator reaction. The interference of Ag^+ could be successfully eliminated by masking with dithizone [46]. Ascorbic acid was found to inhibit the reaction slightly as in its presence; the recov-

Table 3
Some critical properties of this work with previous studies about determination of thiourea.

Number	Method	DL (mg L^{-1})	LR (mg L^{-1})	Reference
1	Piezoelectric	–	0.008–0.5	[1]
2	Voltammetry	–	0.3–76.1	[16]
3	Spectrophotometry	0.9	7.6–60.0	[23]
4	Mass spectrometry	0.001	0.01–5.0	[27]
5	FTIR	10.0	60.0–800.0	[28]
6	Amperometry	–	76.1–609.0	[31]
7	Flow injection fluorimetry	–	0.04–0.76	[37]
8	Stopped-flow technique	–	0.2–5.7	[39]
9	Catalytic Kinetic spectrophotometry	0.02	0.03–10.0	[41]
10	Adsorptive stripping voltammetry	2.5×10^{-6}	Upto 0.30	[48]
11	Spectrophotometry	–	4.0–32.0	[49]
12	Spectrophotometry	–	7.6–76.1	[50]
13	Flow injection amperometry	4.1	3.8–76.1	[51]
14	Chemiluminescence	7.6×10^{-4}	7.6×10^{-4}–7.6×10^{-2}	[52]
15	Catalytic kinetic spectrophotometry	0.008	0.01–12.00	This work

ery of thiourea was reduced by 30%. However, the interference of ascorbic acid can be removed by refluxing the sample for few minutes [47]. According to the result, method has a high selectivity.

3.4.3. Analytical application

Because of its high sensitivity and selectivity, the proposed method was directly applied to the determination of fruit juices and orange peel samples. Thiourea was determined in real samples using standard addition method. Fruits were purchased from a supermarket and juices were obtained by squeezing them [23]. The juices were filtered using Whatman 42 paper (semi-analytical) and centrifuged for 15 min at 3500 rpm. After refluxing (for removing the ascorbic acid interference [47]) for 10 min, 1 mL of this solution was transferred to a volumetric flask, diluted to 50 mL and analyzed following the recommended procedure. The dried orange peels were crushed into fine powder and dissolved in concentrated sulfuric acid. This solution wash filtered using Whatman 42 paper and refluxing for removing the ascorbic acid interference [47] for 10 min. then 5.0 mL of this solution transferred to a volumetric flask, diluted to 100 mL and analyzed following the recommended procedure.

The recovery results for the analysis of some real samples are shown in Table 2.

4. Conclusion

A simple, relatively rapid, low cost, highly sensitive and selective method is proposed for the determination of trace levels of thiourea based on its catalytic effect on the oxidation of JG in hydrochloric acid media. The method does not require any separation or pre-concentration steps and was applied to the determination of trace levels of thiourea in fruit juice, orange peel, directly. The results in Table 2 show that the method is accurate and gives good recoveries of added thiourea. Table 3 shows some critical properties of present work compared with some previous works. Comparison of present work with summarized results in this table show a good reproducibility as compared to other studies.

Acknowledgements

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