Thiocyanate Determination using Technique of Flow Injection-Gas Diffusion Spectrophotometry with Cerium (IV)-Ninhydrin Reagent.

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Abstract. Numerous food plants contain thiocyanate, which can inhibit iodine uptake of thyroid gland and induce to the risk of iodine deficiency disorders (IDD). This research is focused on the development of new method for thiocyanate determination based on the formation of red hydrindantin using spectrophotometric flow injection (FI)–gas diffusion technique. In this technique, thiocyanate is oxidized by cerium (IV) in the acid donor stream to form hydrogen cyanide which diffuses through the membrane in the gas diffusion cell and reacts selectively with alkaline ninhydrin in the acceptor stream to form a red hydrindantin detected by spectrophotometer at 485 nm. The common operational conditions including sample volume, the length of mixing coil, and the flow rate as well as chemical parameters (concentration of cerium (IV) and ninhydrin, and pH reaction) were optimized with respect to sensitivity, analysis time, and linearity. The optimum condition notified by highest absorbance with sharp single peak and fast analysis was obtained under flow rate of 2 mL min\(^{-1}\), 300 µL sample volume, 75 cm mixing coil, 0.01 M cerium (IV), 1 % ninhydrin, and pH of 11. The interfering ions frequently exist with thiocyanate were also studied. Under the optimized conditions, the method showed linear for thiocyanate concentration from 5-30 mgL\(^{-1}\) and sampling rate of 120 s/h with a satisfactory reproducibility (RSD < 2% at n=3). This method has been successfully applied to determine thiocyanate in saliva from smoker and non-smoker and the results showed good agreement with those obtained from standard spectrophotometry method.

1. Introduction
Thiocyanate is neurotoxic to humans and at high concentrations in the blood can inhibit the activity of various enzymes as well as impaired thyroid gland function [1]. Continued ingestion of thiocyanate can cause goiter, mental retardation, poor growth, stunting and infant mortality [2]. Many plant foods such as cassava, cabbage, turnips, broccoli, brussel sprouts, and cauliflower were reported as source of thiocyanate [3]. Thiocyanate can also be found in the body fluids, such as blood, saliva, and urine as detoxification product of cyanide from several types of vegetables containing cyanogenic glucoside such as cabbage, cassava, squash, radishes, mustard greens, eggplant, papaya leaves, cassava, and...
bamboo shoots [4]. Therefore, the existence of thiocyanate in the body fluids was reported for monitoring cyanide poisoning resulted from cyanogenic glucoside consumption [5].

The most popular spectrophotometric method for thiocyanate determination is using iron (III) reagent [6]. The short linear calibration of 0.1-2 mgL\(^{-1}\) and low precision for biological samples of this method was improved by introducing multi commutated approach for determining thiocyanate in human saliva with high precision (RSD of 1.0 %); but this method only can be used for high concentration (0.5 to 10.0 mmol L\(^{-1}\) SCN) [7]. Keyvanfard (2013) reported flow injection spectrophotometric method for the determination of thiocyanate based on inhibitory effect of thiocyanate on the oxidation of janus green by bromate and monitored by measuring janus green absorbance at 554 nm. The calibration graph was linear over the range 0.02–1.0 μg mL\(^{-1}\), low detection limit of 0.016 μg mL\(^{-1}\) and sample throughput of 25 sample per hour [8].

Indirect spectrophotometric methods based on the oxidation of thiocyanate to cyanide were also reported. Lundquist (1995) described spectrophotometric method using anion exchanger, followed by elution using perchlorate oxidizing agent and König reaction by the use of carcinogenic reagent of isonicotinic acid and 1,3-dimethyl-barbituric acid. The method offers sensitive method for thiocyanate with linear measurement up to 29 mg L\(^{-1}\) and limit of 0.05 mg L\(^{-1}\) [9]. Sulistyarti (2010) has improved the method for thiocyanate determination in flow system based on the oxidation of thiocyanate by permanganate to cyanide, followed by the formation of tetracyanonickelate(II) complex in the presence of nickel-ammonia reagent [10]. The complex was detected using spectrophotometer at 267 nm and this method showed much longer linear measurement up to 50 mg L\(^{-1}\) with RSD of 1.34 %, low detection limit of 0.07 mg L\(^{-1}\), and analysis rate of 20 samples per hour.

A new reaction based for sensitive, selective, fast and simple method for cyanide determination has been reported using ninhydrin [11-14]. This reaction has been used for thiocyanate analysis in sequential injection technique [15], but it has not been applied in simple flow injection system. Based on the review from the previous reports [9,10,15], it was known that thiocyanate in the presence of oxidizing agent is changed to cyanide. Therefore, this work was focused on the development of new flow injection spectrophotometric method for determination thiocyanate in human saliva based on the oxidation of thiocyanate with cerium (IV) to form cyanide which reacts selectively with ninhydrin to form hydrindantin, which presents radiation absorption at 485 nm.

2. Method

2.1 Chemicals and Reagents
All chemicals were of analytical reagent grade, and all solutions were prepared with demineralized water except stated. A stock thiocyanate solution (1000 mg L\(^{-1}\) SCN\(^{-}\)) was prepared by dissolving the appropriate amount of NH\(_4\)SCN (Merck) into demineralized water. Standard thiocyanate solutions of lower concentrations were prepared by diluting the stock thiocyanate solution. Stock solution of 0.1 M cerium (IV) was made by dissolving cerium (IV) sulphate (CeSO\(_4\), Merck) in 1 M H\(_2\)SO\(_4\) (Merck) and the lower concentrations were prepared by dilution from the stock solution using 1 M H\(_2\)SO\(_4\). Solution of 1% ninhydrin (Merck) was prepared by dissolving 1 gram of ninhydrin into 100 mL of carbonate buffer (Na\(_2\)CO\(_3\) and NaHCO\(_3\), Merck) solution conditioned at the appropriate pH.

2.2 Apparatus
The flow injection manifold (Figure 1) consisted of a peristaltic pump (Minipuls, Gilson), with the thiocyanate standard solution injected via a six-way PTFE rotary valve (Rheodyne® model 5020). Teflon tubing (0.5 mm i.d., Supelco) was used as flow lines and reaction coil for mixing sample and reagents. The T-piece connector was used for merging the reagent streams. The reaction product was carried through the quartz flow-through cell (Hellma, Germany) with 1.00 cm optical path, which was located into the spectrophotometer (Shimadzu 1601 UV/VIS spectrophotometer) measurement compartment and connected with a personal computer (PC) where the FI grams were recorded as a
peak as the function of time and data processing system using the laboratory-developed software as an interfacing system.

2.3 Flow injection procedure

The flow system integrated two lines (Figure 1), where the thiocyanate sample is injected into the donor stream containing cerium (IV) in acid (H₂SO₄). In this donor stream, thiocyanate is mixed with cerium (IV) and oxidized to cyanide which is directly converted to hydrogen cyanide. Then, the hydrogen cyanide diffuses through a teflon membrane in the gas diffusion cell into acceptor stream containing ninhydrin in carbonate buffer to form red hydridantin which is detected using spectrophotometer at wavelength of 485 nm.

![Figure 1](image1)

**Figure 1.** Schematic of the flow injection system (P= peristaltic pump; GD= gas diffusion cell equipped with a PTFE membrane (M); 485 = Spectrophotometry at 485 nm; I = rotary injection valve; C = mixing coil; donor stream = Cerium (IV) in acid; acceptor stream = ninhydrin in carbonate buffer); W = waste).

3. Results and Discussion

The parameters that influence the reproducibility, sensitivity, and the analysis time were studied to establish the optimum conditions for the determination of thiocyanate.

3.1 Optimisation of FI Parameters

3.1.1. Optimisation of sample volume. The volume of sample injected into the flow system affected the product of reaction and dispersion in the flow stream. The most suitable amount of the analyte injected was determined from the sample volume that gives the greatest sensitivity and reproducible results. The influence of the sample volume on the sensitivity was monitored based on the absorbance of product reaction (red hydridantin) and investigated by injecting volumes of sample solution in the range 100–500 µL of 15 mg L⁻¹ thiocyanate standard solution into the FI manifold. It can be seen from Figure 2 that peak height (absorbance of hydridantin) increased with increasing sample volume and reached a plateau at 300 µL, and further increase of sample volume did not increase the absorbance. Therefore, the sample volume of 300 µL was selected as an optimum condition.

![Figure 2](image2)

**Figure 2.** Effect of sample volume on the absorbance of hydridantin.
(Conditions: Thiocyanate 15 mg/L; cerium (IV) 0.01 M; ninhydrin 1 % in carbonate buffer pH 10; mixing coil 50 cm; and flow rate 2 mL/min).
3.1.2. Optimisation of mixing coil. The effects of various mixing coil length were investigated over the range 25-125 cm in order to achieve complete oxidation of thiocyanate by cerium (IV) with minimum dispersion. The longer mixing coil will give better mixing attain the maximum product of reaction, and thus increase sensitivity. On the other hand, the longer mixing coil can also increase dispersion which results to decrease sensitivity. Figure 3 shows that increasing mixing coil length up to 75 cm increased the absorbance as expected; however, the absorbance declines for mixing coil longer than 75 cm. Thus, a 75 cm mixing coil is considered to be suitable due to its best sensitivity.

![Figure 3](image)

Figure 3. Effect of mixing coil length on the absorbance of hydrindatin.
Conditions: Thiocyanate 15 mg/L; cerium (IV) 0.01 M; ninhydrin 1 % in carbonate buffer pH 10; sample volume 300 µL; and flow rate 2 mL/min.

3.1.3. Optimisation of flow rate. The flow rate in FI system influences significant to the absorbance signals. The higher flow rate the shorter time for each sample passing through the system, thus gives high sample throughput. However, it often results in a low precision of the signal and low sensitivity due to the incomplete reaction. With a low flow rate, the residence time for each sample is long and thus it gives adequate time for reaction and increase sensitivity. On the other hand, under lower flow rate the dispersion is large, which can reduce the sensitivity and sample throughput. The effects of flow rate for both streams were investigated in the range of 0.5–2.5 mL min\(^{-1}\). The effect of flow rate on the absorbance is shown in Fig. 4; in which the absorbance decreases with increasing flow rate. Flow rate of 1.5 mL/min showed best sensitivity with acceptable peak shape; however, 2 mL min\(^{-1}\) was chosen for further experiment as it gave acceptable sensitivity, perfect peak shape (high precision), and the best analysis time. So, a flow rate of 2.0 mL min\(^{-1}\) of both streams was chosen and used for subsequent measurements.

![Figure 4](image)

Figure 4. Effect of flow rate on the absorbance of hydrindatin. Conditions: Thiocyanate 15 mg/L; cerium (IV) 0.01 M; ninhydrin 1 % in carbonate buffer pH 10; sample volume 300 µL; and flow rate 2 mL/min).
3.1.4. Optimisation of Reagents. The necessity for optimization of reagent concentrations (cerium (IV) and ninhydrin) as well as pH is to obtain sufficient reagent concentration for enhancing the product reaction thus permits the quantitative reaction for spectrophotometric determination of the thiocyanate.

3.1.5 Optimisation of cerium (IV) concentration. The concentration of cerium (IV) should be sufficient to oxidize thiocyanate to cyanide which is then in the presence of ninhydrin forming red hydridantin. Thus, various concentrations of the cerium (IV) reagent was optimized over the range of 0.002-0.012 M. It was found that the absorbance increased very significantly with increasing cerium (IV) concentration in the range of 0.002–0.008 M and remained constant for higher concentration (Fig. 5). Hence, the 0.01 M was chosen as an optimum cerium (IV) concentration and was used for further investigations.

![Figure 5. Effect of cerium (IV) concentration on the absorbance of hydridantin. Conditions: Thiocyanate 15 mg/L; cerium (IV) 0.002-0.012 M; ninhydrin 1 % in carbonate buffer pH 10; mixing coil 75 cm, sample volume 300 µL; and flow rate 2 mL/min.](image)

3.1.6. Optimisation of ninhydrin concentration. Commonly, the amount of reagent required for complete reaction is greater than stoichiometric reaction to shift the equilibrium to the product. The effect of different ninhydrin concentrations over the range of 0.4-1.2 % was examined. As shown in Fig. 6, it was found that the absorbance of red hydridantin increased with increasing ninhydrin concentrations from 0.4 to 1 %, above of which the absorbance slightly decreased. Therefore, 1 % ninhydrin was selected as optimal.

![Figure 6. Effect of ninhydrin concentration on the absorbance of hydridantin. Conditions: Thiocyanate 15 mg L⁻¹; cerium (IV) 0.01M; ninhydrin 0.4-1.2 % in carbonate buffer pH 10; mixing coil 75 cm, sample volume 300 µL; and flow rate 2 mL min⁻¹.](image)
3.1.7. Optimisation of pH. The formation of red hydrindantin is affected by pH of solution. Nagaraja (2002) reported that the hydrindatin is colourless under neutral condition, deep-red colour under slight alkaline (pH 8-12), and deep-blue colour under strong alkaline (high pH >12). The effect of pH over the range of 8-12 was examined and as shown in Fig. 7, it was obtained that the absorbance of red hydrindantin increased with increasing pH from 8-11 and remain constant up to pH 12. Hence, the pH 11 was considered to be optimum for the proposed FI method, which was used throughout the experiments.

![Figure 7](image.png)

**Figure 7.** Effect of pH on the absorbance.

Conditions: Thiocyanate 15 mg/L; cerium (IV) 0.01M; ninhydrin 1 % in carbonate buffer pH 8-12; mixing coil 75 cm, sample volume 300 µL; and flow rate 2 mL/min.

3.1.8. Analytical characteristics. Using the proposed FI manifold for thiocyanate determination (Fig. 1) and optimum conditions in Table 1, a linear calibration was obtained in the concentration range of 5-30 mg L\(^{-1}\) which can be expressed by the regression equation \(y = 0.0932x - 0.1184\) (R\(^2\) = 0.992), where \(y\) is absorbance of red hydrindantin and \(x\) is the concentration of thiocyanate in mg L\(^{-1}\) (Fig. 8). The relative standard deviation for triplicate injections was less than 2 % with LOD of 3 mg L\(^{-1}\) and short analysis time of typically 30 seconds/sample (120s/h). The typical FI-gram with high reproducibility of the method is shown in Figure 8.

| No. | Parameter       | Range studied     | Optimum value |
|-----|-----------------|-------------------|---------------|
| 1   | Sample volume   | 100-500 µL        | 300 µL        |
| 2   | Mixing coil     | 50 – 125 cm       | 75 cm         |
| 3   | Flow rate       | 1-2.5 mL/min      | 2 mL/min      |
| 4   | Ce(SO\(_4\))\(_2\) | 0.002-0.012 M    | 0.01 M        |
| 5   | Ninhydrin       | 0.4-1.2%          | 1 %           |
| 6   | pH ninhydrin    | 8-12              | 11            |
Figure 8. FI-gram of thiocyanate determination. Conditions: Thiocyanate 5-40 mg L\(^{-1}\) under optimum conditions as in Table 1.

3.1.9. *Interference Study.* The effect of interfering ions, such as bicarbonate ion (hydrogen carbonate ion) and chloride were studied by involving various concentration of interfering ions, whilst the concentration of thiocyanate was kept constant. The results depicted in Table 2 showing that that the proposed FI-method is selective, where the presence of bicarbonate ion up to 75 mgL\(^{-1}\) and chloride up to 30 mgL\(^{-1}\) did not significantly interfere the measurement shown by the recovery of close to 100 %. However, higher concentration of chloride of 75 mg L\(^{-1}\) decreased the recovery of thiocyanate measurement down to 77.05 %.

| Ions added to 15 mgL\(^{-1}\) SCN | [Ions] mgL\(^{-1}\) | Measured SCN, mgL\(^{-1}\) | % Recovery* |
|----------------------------------|-----------------|-----------------|-------------|
| HCO\(_3\)\(^-\)                  | 0               | 15.32           | 102.15      |
|                                  | 15              | 15.20           | 101.36      |
|                                  | 30              | 14.42           | 96.11       |
|                                  | 75              | 13.77           | 91.80       |
| Cl\(^-\)                         | 0               | 15.35           | 102.31      |
|                                  | 15              | 15.10           | 100.66      |
|                                  | 30              | 13.78           | 91.87       |
|                                  | 75              | 11.56           | 77.05       |

*Average from triplicates with RSD < 2 %.

3.1.10. *Validity of FI-Method.* Validity of the method was done by applying the FI-method for determining thiocyanate in synthetic and real samples, than comparing the results to those obtained from the standard spectrophotometry method. The validation test using synthetic samples is depicted in Table 3, in which the data showed comparable results to all samples between the proposed FI method and spectrophotometric standard method. The validity of the method using real sample was done by determining thiocyanate in the saliva of smoker and non-smoker. As shown in Table 4, the proposed FI-method performed good agreement with those obtained from the standard method supported by the value of t-test calculated < t-test tabulated (at \(\alpha\) of 0.05, \(n = 3\)).
Table 3. Validation test using synthetic samples.

| SCN- Sample (mg L⁻¹) | FIA-GD [SCN⁻] (mg L⁻¹) | % R   | Standard Spectrophotometry [SCN⁻] (mg L⁻¹) | % Recovery |
|----------------------|------------------------|-------|-----------------------------------------|------------|
| 5                    | 5.068 ± 0.03           | 101.35| 5.152 ± 0.144                           | 103.03     |
| 10                   | 10.46 ± 0.054          | 104.59| 10.010 ± 0.167                          | 100.09     |
| 15                   | 15.052 ±0.047          | 100.35| 14.916 ± 0.083                          | 99.44      |

Table 4. Validation test using saliva sample.

| Saliva sample        | [SCN⁻] measured by FI-Spectrophotometry (mg L⁻¹) | [SCN⁻] measured by Standard Spectrophotometry (mg L⁻¹) |
|----------------------|---------------------------------------------------|------------------------------------------------------|
| Smoker               | 8.008 ± 0.027                                     | 8.278 ± 0.028                                        |
| Smoker*              | 19.949 ± 0.018                                    | 19.514 ± 0.008                                       |
| Non-smoker           | 4.208 ± 0.018                                     | 4.354 ± 0.015                                        |
| Non-smoker*          | 14.376 ± 0.025                                    | 14.262 ± 0.030                                       |

*3 replicates with RSD ≤ 3 %.
*Added with 10 mg L⁻¹ SCN⁻.

4. Conclusion

The proposed FI spectrophotometric method has proved to be the simple and selective method for the determination of thiocyanate. This proposed method is based on the formation of red hydrindatin monitored at 485 nm. This method is fast and it can be useful for routine analysis of thiocyanate in body fluids. The obtained results showed good agreement between the proposed FI-method and the standard spectrophotometric method as the Student’s t-test showed no significant difference at 95 % confidence level.

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