

Full Paper

Flow-through Bulk Optode for Spectrophotometric Determination of Thiocyanate and Its Application to Water and Saliva Analysis

M^a Soledad García, Joaquín A. Ortuño, Concepción Sánchez-Pedreño*, M^a Isabel Albero and M^a José Fernández

Department of Analytical Chemistry, Faculty of Chemistry, University of Murcia, 30071 Murcia, Spain; E-mails: msgarcia@um.es (for MSG), jortuno@um.es (for JAO), spedreno@um.es (CS-P), mialbero@um.es (forMIA)

* Author to whom correspondence should be addressed; E-mail: spedreno@um.es

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Abstract: A flow-through spectrophotometric bulk optode for the flow-injection determination of thiocyanate is described. As active constituents, the optode incorporates the lipophilized pH indicator 5-octadecanoyloxy-2-(4-nitrophenylazo)phenol and methyltridodecyl ammonium chloride, dissolved in a plasticized poly(vinyl)chloride membrane entrapped in a cellulose support. The optode is applied, in conjunction with the flow injection technique, to the determination of thiocyanate at pH 7.5 (TRIS/H₂SO₄). The sensor is readily regenerated with a 10⁻² M NaOH carrier solution. The analytical characteristics of this optode with respect to thiocyanate response time, dynamic measurement range, reproducibility and selectivity are discussed. The proposed FI method is applied to the determination of thiocyanate in waters from different sources and in human saliva samples in order to distinguish between smokers and non-smokers.

Keywords: Flow-through optode; spectrophotometry; thiocyanate; water, saliva.

1. Introduction

Many areas of applied analytical chemistry, such as process control and clinical, food and environmental sampling, require a fast and simple method for determining the concentrations of ionic species in aqueous systems. The use of optical sensors is considered a simple, quick and inexpensive

method of analysis. The development of optical chemical sensors (optodes) as viable alternative to other types of sensor is of great interest [1-4] and several optodes have been applied to the trace analysis of heavy metals in processes control and environmental and medical analysis [3-7]. The basic principles and techniques of optical ion sensing have been described. [8] One type of optode makes use of a plasticized polymeric membrane and is based on the reversible mass transfer of analyte from the sample into the bulk of the sensing layer. This type of optical sensor was named “bulk optode membrane” by Seiler and Simon, who described the basic principles and techniques that can be used with it [9].

Several ionophores and appropriate lipophilic pH indicator dyes introduced into the membranes have been used to design optical cation sensing systems [5]. This pH indicator approach has also been adapted to the design of optical anion optode membranes [6, 7]. In the case of anion determination, optical transduction is based on the protonation and deprotonation of the pH indicator dye related with the anion concentration through co-extraction of the anion/proton pair to and from the membrane. If the membrane contains no anion-selective ionophore, the selectivity of the optode membrane depends on the lipophilicity of the anions (Hofmeister series).

The performance of reversible bulk optodes is probably best tested in flow injection systems (FI), a configuration that provides greater flexibility and the possibility of automation, in addition to a wider applicability to real samples [2]. However, to our knowledge, only one method for the determination of thiocyanate using a flow through bulk optode has been proposed [10].

Thiocyanate possesses many industrial and biological applications and although its toxicity is not comparable with cyanide, it is harmful to aquatic life. Determination of its concentration at low levels in water and industrial samples is therefore important.

Clinical studies have shown that the urinary and saliva thiocyanate concentration is higher in smokers than in non-smokers and its assay can be used to distinguish the two groups in epidemiologic studies [11-13].

Many methods for the determination of thiocyanate have been reported, including chromatographic [13-15], spectrophotometric [11,12,16-18], spectrofluorimetric [19], potentiometric [20-22] and voltammetric [23] methods.

In previous papers, we described flow-through bulk optode membranes for the FI-determination of various inorganic ions [24-28]. In this paper, we present a flow-through bulk optode for FI-spectrophotometric determination of thiocyanate using the lipophilized pH indicator 5-octadecanoyloxy-2-(4-nitrophenylazo) phenol and methyltridodecyl ammonium chloride as the active constituents in a plasticized poly(vinyl) chloride membrane entrapped in a cellulose-based support.

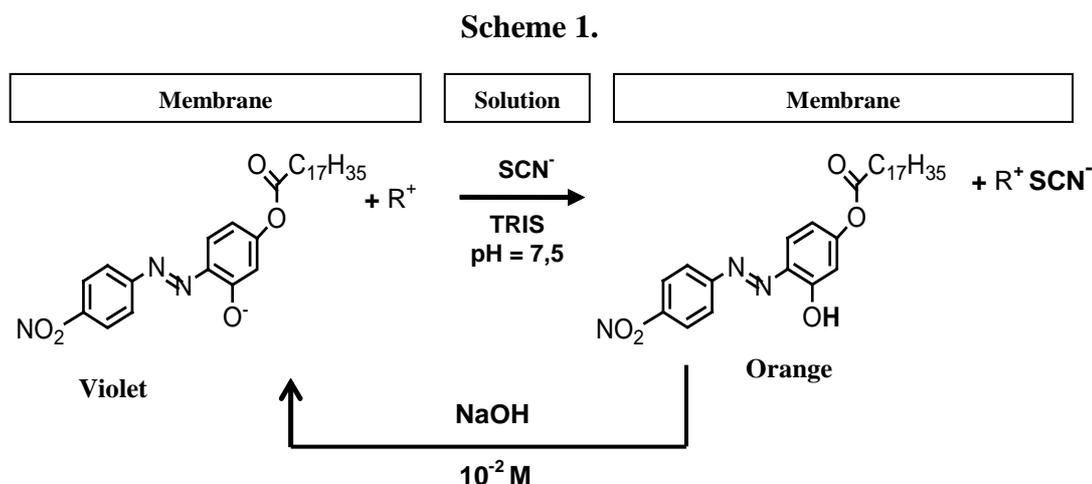
The flow-optode developed was applied with satisfactory results to the FI-spectrophotometric determination of thiocyanate in waters from different sources and human saliva.

2. Results and Discussion

2.1 Principle of operation

The flow-through optode membrane contains the lipophilic pH indicator 5-octadecanoyloxy-2-(4-nitrophenylazo) phenol ($\text{IND}^-_{\text{mem}}$) and the ionic additive methyltridodecylammonium (R^+_{mem}) chloride in the membrane. The indicator is violet in its basic form and orange in its acidic form.

The mechanism of the process is:



Upon exposure to a buffered solution of thiocyanate, the co-extraction of this anion and proton occurs simultaneously and the hydrogen ion combines with the pH indicator $\text{IND}^-_{\text{mem}}$ to give HIND_{mem} . The spectrum of the membrane will change accordingly. The degree of protonation of the pH indicator is dependent on the thiocyanate concentration. The sensor is readily regenerated with a carrier solution of 10^{-2} M NaOH.

2.2 Study of variables.

The coating solution and support type were those selected in a previous work [27], because they were found to provide better results for the determination of SCN^- .

Diffuse reflectance spectra of the optode membrane were shown in the above work [27]. Curves corresponding to the protonated and deprotonated form of pH indicator were obtained by pumping 10^{-2} M HClO_4 or 10^{-2} M NaOH solution until a constant diffuse reflectance value was reached. The greatest difference in reflectance was found at 550 nm and this wavelength was selected for all subsequent measurements of reflectance.

The FI variables studied were sample volume and flow rate, injecting a 10^{-3} M solution of SCN^- . The reactor length was kept as short as possible (40 cm). The signal was presented as normalized reflected radiation intensity $R_N = [R_{\text{max}} / R_{\text{min}}]_{\text{sample}} - [R_{\text{max}} / R_{\text{min}}]_{\text{blank}}$, where R_{max} is the maximum reflectance of the peak and R_{min} is the reflectance of the base line.

Figure 1A (1 and 2) shows the influence of sample volume on the analytical signal and on the peak width. As can be seen, increasing sample volumes increased the sensor response and increased the $W_{90\%}$ up to 250 μl with a corresponding decrease in the sampling frequency. A compromise sample volume of 350 μl was chosen for further experiments.

The effect of flow rate is shown in Fig. 1B (1 and 2), where it can be seen that the sensor response and the $W_{90\%}$ decreased considerably up to 0.44 ml min^{-1} . A flow rate of 0.44 ml min^{-1} was selected.

The effect of the sample pH was studied in the range 5.8-10.5 (TRIS/ H_2SO_4 buffer) in the same experimental conditions. As can be seen from Figure 1C (1 and 2), the response of the sensor

decreased when the pH increased, while the peak width increased considerably. A pH of 7.5 was selected for further studies as a compromise between sensitivity and sampling frequency.

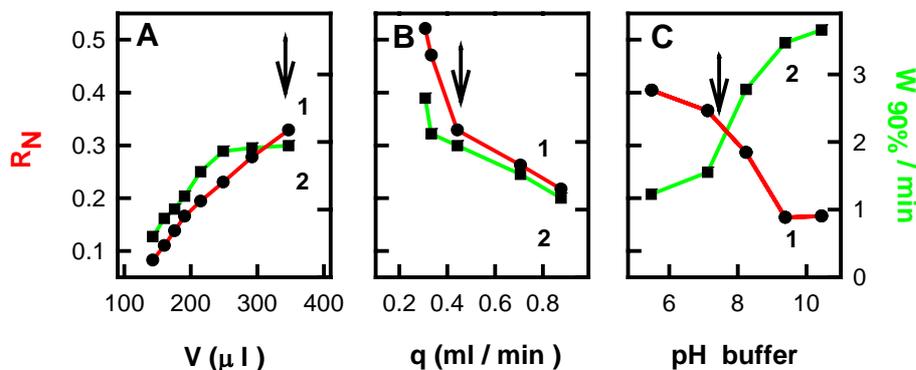


Figure 1. Effect of sample volume (A); flow rate (B) and pH (C), on the normalized reflected radiation intensity (curves 1) and on the peak width at 90% ($W_{90\%}$) (curves 2). Sample injected: 1×10^{-3} M SCN^- solution.

An eluent that can also be used as carrier is best for this type of sensor since this simplifies the FI procedure. The best results were obtained using 10^{-2} M NaOH solution as carrier. This also permitted a short membrane regeneration time, defined as the time taken for the sensor to reach the base line signal after the maximum reflectance has been reached, which was found to be dependent on the SCN^- concentration.

The lifetime of the membrane optode was at least one month in continuous operation.

2.3 Features of the flow injection method.

The reflectance response of the optode versus time under the selected experimental conditions for different SCN^- concentrations in the range $0 - 5 \times 10^{-2}$ M is shown in Fig. 2(A). The reflectance base line was previously adjusted to arbitrary units in the detector. When the SCN^- plug reached the cell, the reflectance increased rapidly and continued increasing as the H^+SCN^- pair zone passed through the cell, due to protonation of the pH indicator in the membrane. Maximum reflectance was obtained at the very end of the sample zone, after which the 10^{-2} M NaOH solution contained in the carrier quickly eluted the H^+SCN^- ion pair of the optode, leaving the membrane ready for a new sample.

The corresponding calibration graph obtained by plotting the normalized reflected radiation intensity R_N vs. $\log \text{SCN}^-$ concentration is shown in Fig. 2(B). The experimental data obtained were fitted to a sigmoidal curve of the type: $R_N = a + b / (1 + \exp(-\log[\text{SCN}^-] - c) / d)$. The values obtained for the parameters a, b, c, d and the correlation coefficient were -7.7×10^{-4} , 0.70, 2.92, 0.47 and 0.999, respectively. The dynamic range of the response toward thiocyanate was $1 \times 10^{-5} - 5 \times 10^{-2}$ M SCN^- . The detection limit, calculated as the concentration of SCN^- which provided a signal equal to the blank signal plus three times standard deviation of the blank [29], was estimated to be 2.0×10^{-6} M.

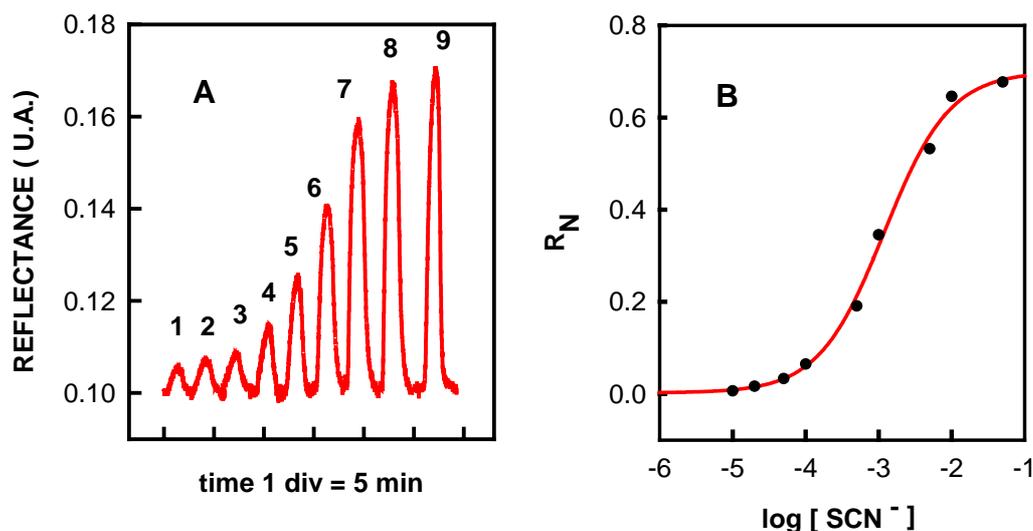


Figure 2. (A) Response to different SCN^- concentrations: (1) 0, (2) 1×10^{-5} , (3) 5×10^{-5} , (4) 1×10^{-1} , (5) 5×10^{-4} , (6) 1×10^{-3} , (7) 5×10^{-3} ; (8) 1×10^{-2} , (9) 5×10^{-2} M. (B) Calibration graph.

Repeatability was evaluated by performing ten determinations with the same 10^{-3} M standard solution of SCN^- . The variation coefficient of the SCN^- concentration was 1.0 %. The reproducibility of the response between days ($n = 5$) and between membranes ($n = 3$) was obtained by determining 10^{-3} M SCN^- injected in triplicate. The CV was 1.8 % and 2.2 %, respectively.

2.4 Selectivity

The selectivity coefficients for the different anions with respect to thiocyanate were calculated for the optode using the mixed solutions method [30] with samples containing 1×10^{-3} M SCN^- and a certain concentration of each foreign ion that produced a significant change in the R_N with respect to the value corresponding to 1×10^{-3} M SCN^- alone. The calibration graph, Fig. 2(B), was used to calculate the concentration of thiocyanate, $[SCN^-]_{equiv}$, that gives the same normalized reflected radiation intensity R_N as the mixed sample containing 1×10^{-3} M SCN^- and the ion assayed. The selectivity coefficient, K_{SCN^-, X^-} , was calculated from:

$$K_{SCN^-, X^-} = ([SCN^-]_{equiv} - [SCN^-]_{sample}) / [X^-]_{sample}$$

where $[SCN^-]_{sample}$ and $[X^-]_{sample}$ are the concentration of thiocyanate and the foreign anion present in the sample, respectively. The resulting selectivity coefficients are shown in Table 1. SO_4^{2-} , HCO_3^- , Cl^- , PO_4^{3-} , Ac^- , Br^- did not produce any change in the signal up to 1×10^{-1} M. When the concentration of these anions was substantially increased, the signal R_N decreased with respect to that of the solution containing SCN^- alone (i.e. it provided negative interference). We attribute this to the great increase in the ionic strength and the corresponding decrease in the thiocyanate activity and therefore the selectivity coefficients values were not calculated for these ions.

Table 1. Anion selectivity coefficients.

Anion	log K_{SCN^-, X^-}	$[X^-] / M$ assayed
SO ₄ ²⁻ , HCO ₃ ⁻ , Cl ⁻ , PO ₄ ³⁻ , Ac ⁻ , Br ⁻	No interference up to 1×10^{-1}	
NO ₃ ⁻	-2.5	1×10^{-1}
I ⁻	-2.1	1×10^{-2}
ClO ₄ ⁻	0.1	1×10^{-3}

2.5 Applications

The flow injection method proposed was satisfactorily applied to the determination of thiocyanate in water and human saliva.

For the determination of thiocyanate in water, known amounts of thiocyanate were added to water samples of different sources and the proposed FI and reference colorimetric [31] methods were applied. The results obtained are summarized Table 2. Good recoveries were obtained in all cases.

Table 2. Determination of anion thiocyanate in water.

Sample	SCN ⁻ content / mg l ⁻¹			
	Added	Proposed Method *	Recovery %	Reference Method *
Spring water	29.0	28.8 ± 0.4	99.3	29.2 ± 0.1
	58.1	58.3 ± 0.8	100.3	58.7 ± 0.2
Tap water	29.0	29.1 ± 0.1	100.3	29.4 ± 0.1
	58.1	59.3 ± 0.4	102.1	58.7 ± 0.2
Reservoir water	29.0	29.1 ± 0.1	100.3	29.4 ± 0.1
	58.1	60.4 ± 0.9	104.0	57.0 ± 0.2

* Mean ± standard deviation (n=3).

For the determination of thiocyanate in human saliva the proposed FI and reference colorimetric methods were applied to saliva samples of smokers and non-smokers. The results obtained are summarized in Table 3. Good recoveries were obtained in all cases and are in agreement with clinical studies that have shown that the saliva thiocyanate concentration is higher in smokers than in non-smokers.

In the two applications, the results obtained by the two methods were compared by applying the F-test and t-test at the 95% confidence level. The calculated F and t values did not exceed the theoretical values, which indicates that there is no significant difference between the two methods with respect to precision and accuracy in the determination of thiocyanate.

Table 3 Determination of anion thiocyanate in human saliva.

Saliva	SCN ⁻ content / mg l ⁻¹	
	Proposed Method *	Reference Method *
Non-smoker 1	29.3 ± 0.1	29.4 ± 0.1
Non-smoker 2	32.4 ± 1.2	31.9 ± 0.3
Smoker 1	193.4 ± 2.1	197.2 ± 2.3
Smoker 2	252.0 ± 1.5	252.6 ± 2.5

* Mean ± standard deviation (n=3)

3. Experimental Section

3.1 Apparatus.

Spectroscopic measurements were made using an Oriel modular spectrophotometer described in [24]. A Gilson Minipuls 3 peristaltic pump and Omnifit injection valve were used. A home made flow-through cell designed by the authors and described previously [24] was used and is shown in Fig. 3.

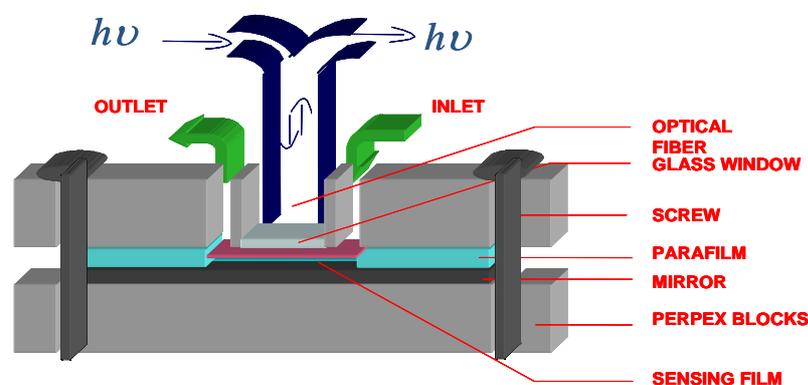


Figure 3. Schematic representation of the flow-through cell.

3.2 Materials and Reagents.

Poly (vinyl chloride) PVC high molecular mass, 2-nitrophenyl octyl ether (NPOE), tetrahydrofuran (THF), octadecanoyloxy-2- (4-nitrophenylazo) phenol (ETH 2412) and methyltridodecylammonium chloride (MTDDACl) were Selectophore products from Fluka. Filter paper 235 (Albet).

The buffer solutions of pH 7.5 were 2.5×10^{-1} M and 5×10^{-2} M TRIS (tris-(hydroxymethyl)-aminomethane)/ H₂SO₄. A 10^{-2} M NaOH solution.

The stock standard thiocyanate solution 1×10^{-1} M was prepared by dissolving 0.8107 g of NaSCN (Merck) in 100 ml of water. Working standard solutions of lower concentrations were prepared by suitable dilution of the stock solution with TRIS buffer of pH 7.5.

3.3 Optode membrane preparation.

The optode membrane preparation was described in [27].

3.4 Manifold and Calibration Procedure.

The single channel flow injection manifold selected, Fig. 3, and the calibration procedure were similar to these described in [27].

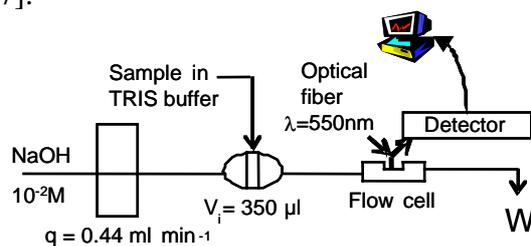


Figure 4. Flow-injection configuration.

3.5 Procedure for determination of thiocyanate in water.

No prior treatment of the samples was necessary for the determination of thiocyanate in water. Water samples of 15.0 ml were spiked with 75 and 150 μl of 10^{-1} M SCN^- solution and 5 ml of 2.5×10^{-1} M TRIS buffer of pH 7.5 were added, diluting the mixture with distilled water to 25 ml. The thiocyanate concentration was determined by applying the recommended FI procedure.

3.6 Procedure for determination of thiocyanate in saliva.

Saliva samples were obtained from volunteer smokers and non-smokers. About 5 ml of samples were centrifuged at 3000 rpm for 10 minutes. Aliquots of 2.0 ml of the supernatants were taken, 1.0 ml of 2.5×10^{-1} M TRIS buffer of pH 7.5 was added and the mixture was diluted with distilled water to 5 ml. The thiocyanate concentration was determined by the recommended FI procedure.

4. Conclusions

The optode membrane developed for the spectrophotometric determination of thiocyanate is easily prepared and incorporated in a flow injection system using a flow-through cell. The flow-through bulk optode membrane described provides a simple and rapid method for the determination of thiocyanate. The sensor is regenerated readily with the same carrier solution and has a long lifetime. The response of the optode is reproducible and the sensor presented good selectivity toward thiocyanate. The method permits the determination of thiocyanate in water from different sources and in human saliva, which allows differentiation between smokers and non-smokers

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