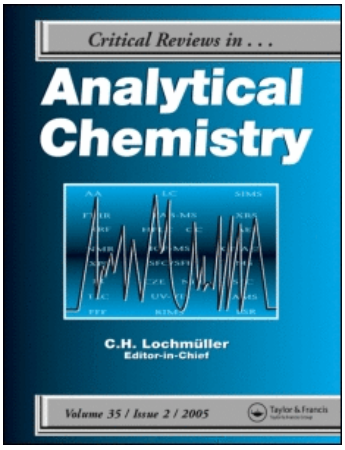

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Determination of Thiocyanate within Physiological Fluids and Environmental Samples: Current Practice and Future Trends

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Determination of Thiocyanate within Physiological Fluids and Environmental Samples: Current Practice and Future Trends

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As the toxicological and physiological importance of thiocyanate has become more and more evident during the past 20 years, there has been an increasing interest in this anion. Thiocyanate is a detoxication product of cyanide and its content in human saliva is considered as a biomarker for identification of nonsmokers and smokers. Chronically elevated levels of thiocyanate in body fluids are known to be toxic and its relation to local goiter, vertigo, or unconciousness has been pointed out. On the other hand, thiocyanate-containing waste discharged into rivers is harmful to aquatic life due to its degradation to cyanide in the presence of oxidants. Therefore, precise knowledge of the thiocyanate content in biological fluids and environmental samples is mandatory. An overview of the existing analytical methodologies for thiocyanate is presented and their advantages and limitations are highlighted. This review is intended not only to provide a comparison of the different methods employed, but also to make it possible for the interested reader to quickly find developments and relevant work that have transpired over the past 10 years.

Keywords anions, biological fluids, cyanide, environmental samples, thiocyanate analysis

The toxicological importance of thiocyanate has become evident during the past 20 years. Thiocyanate is hardly as toxic as cyanide and as such it has received attention from researchers in diverse fields such as medicine, food chemistry, and environmental sciences. Thiocyanate ion is naturally present in human body fluids and its concentration varies according to diet, among other factors. It is derived endogenously as a detoxification product of cyanide in the liver (1); this being the major route of biological cyanide degradation (2, 3). Inorganic cyanide can enter the body in several natural ways (4), yet the most important source is tobacco smoke. Renal elimination of low levels of CN^- from the body occurs by its conversion to SCN^-, reaction which is catalyzed by the mitochondrial enzyme rhodanese (produced in the liver and kidneys) (3, 5).

The determination of SCN^- is particularly important in saliva, urine, and blood serum because it is considered to be a biomarker in distinguishing smokers from nonsmokers. This is due to the fact that the low levels of thiocyanate present in body fluids (especially saliva) increase when there is an "exposure" to cyanide. Therefore, the concentration of SCN^- in the body of a smoker increases because it is a metabolic product of substances in tobacco smoke that contain cyanide (6–8).

While the saliva of nonsmokers contains concentrations between 0.5 and 2 mM (1, 9), in smokers concentrations as high as 6 mM can be found. Lower values are detected in urine and blood serum, but they are always higher in individuals who smoke (especially cigarette smokers) compared to nonsmokers (8–10). Some authors have determined that the SCN^- content decreases from saliva to blood to urine and that this implies thiocyanate is accumulated in blood from the mouth, while it becomes diluted when excreted (11). Yet in other papers, mention has been made of higher contents in urine than in blood (7, 10).

There are other several reasons why this anion is of medical interest. Among them, we can include:

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a. Chronically high levels of thiocyanate can inhibit the normal uptake of iodine in the thyroid gland, thus reducing the formation of thyroxine and is therefore possibly related to goiter (12–14).
b. Monitoring patients that have needed lengthy treatments of sodium nitroprusside, after heart surgery or malignant hypertension, is important since this hypotensive agent metabolizes into SCN^- (6).
c. SCN^- plays an important role in antimicrobial systems by acting as a substrate for peroxidases (1).
d. High concentrations may conduce to vertigo, nasal bleeding, or unconsciousness (15).
e. Saliva thiocyanate may have an antibacterial role thereby reducing the possibility of corrosion of amalgams or the danger of caries (13).
f. The negative effect of this anion on protein iodination has been studied (14).
g. It is used as a drug treatment in certain thyroid conditions and arterial hypertension (7, 10, 15).
h. Recently, its capacity to detoxify ingested carcinogens has also been investigated (1).

On the other hand, the normal concentrations of thiocyanate in the body originate fundamentally from food. The ingestion of vegetables of the genus *Brassica* (5), such as turnips, kale, and cabbage (7) that contain glucosinolates, or milk and cheese that actually contain inorganic thiocyanate (4) are the most important sources of this anion in the body.

SCN^- is formed as a degradation product of indol-3-ylmethyl-glucosinolates and other glucosinolates which give a carbonium ion and the thiocyanate ion in nonenzymatic and enzyme catalyzed reactions. This anion is thus considered to be important when the quality of certain vegetables is to be studied (12). Plasma thiocyanate is also important when studying populations that consume cyanogenic plant foods (6). Metabolism of vitamin B_{12} and foods that contain cyanogenic glucosides, such as nuts and almonds, increase saliva thiocyanate (1). Yet, only extreme eating habits can considerably elevate the levels of thiocyanate (7).

Another important sample where the determination of the thiocyanate anion is of interest is in water, especially wastewaters. This not only is so because of the content of SCN^- itself, but because when wastewaters that contain the anion are chlorinated highly toxic cyanogen chloride is formed (16). At low pH values, thiocyanate contained in wastewaters (17) which are discharged into natural waters converts into cyanide in the presence of oxidants and thereby causes profound damage to aquatic life (18). Its monitoring in plant outlets of certain industries (metallurgic and coal gasification) cannot be underestimated (19).

Areas of use of the anion include the application of thiocyanate salts (ammonium and alkali) in several fields such as agricultural chemicals (as weed killers), dyeing and printing of textiles, in paints (inhibiting rust), and in photography (18). Ammonium thiocyanate is an important starting material in the manufacture of thiourea (20), while sodium thiocyanate solution serves as a dispersive medium for copolymers obtained in the manufacture of acrylic fiber (21). Thiocyanate is also used as a petroleum tracer in oil fields to investigate the distribution of oil deposits and the stratigraphic structure as well as the effectiveness of water blocking (22).

The thiocyanate anion is a reductor and is very stable at any pH value (23). It forms colored complexes with several cations, the best known being the red ferric ion complex which is used for the identification of Fe(III) (24–26) as well as for SCN^- as will be seen in the present article. This anion is also employed for the qualitative determination of iodate and the cuprous ion (27, 28).

Its analytical application not only is in qualitative analysis since it is mostly used as reagent in several quantitative determinations. For example, it forms numerous ion pair systems (ternary complex or ion-associate complex) which are used for extraction or precipitation purposes (11, 13, 29–32) in the quantitative analysis of different inorganic as well as organic components by several instrumental methods. In the chloride ion spectrophotometric determination, mercury thiocyanate is employed as the color reagent (33, 34).

The anion is also the basis of the indirect determination of various species. Sulfide, for example, is determined indirectly through the SCN^- formed when S^{2-} reacts with CN^- and I_2.; thiocyanate is then measured spectrophotometrically with Fe(III) (23). The same basic procedure is employed in the determination of rhodanese enzyme activity, where thiosulfate in the presence of cyanide produces the thiocyanate anion which is then monitored using capillary zone electrophoresis; the rate of the enzymatic reaction depends on rhodanese concentration (2).

Many analytical methods have been used for thiocyanate determination, beginning with the traditional gravimetric and volumetric methods used for larger quantities of the anion to the costly and sophisticated gas chromatography/mass spectrometry (GC/MS) employed for the analysis of traces (35). The major difficulty in its quantitation in most methods is the potential interference of cyanide which usually accompanies thiocyanate in real samples (36).

In the past 10 years or so three types of instrumental techniques stand out: spectroscopy (especially molecular methods), electrochemical sensors (ion selective electrodes [ISEs]), and chromatography (in several varieties). The scope of this review is to provide a tighter focus on these techniques for thiocyanate determination with a particular emphasis on their applicability.

SPECTROSCOPY

Molecular (spectrophotometry and spectrofluorimetry) as well as atomic (atomic absorption) spectroscopic methods have been used for the determination of thiocyanate, the first being the most applied.

The spectrophotometric method most widely employed for thiocyanate determination is the one based on the red complex ($\lambda = 480$ nm) formed with ferric ion, $Fe(SCN)^{2+}$, in acid

TABLE 1
Application of the Fe(III)-SCN^- Spectrophotometric Method

Analyte	Sample	Comments	Ref.
SCN^-	Saliva	Comparison between smokers and nonsmokers	(1)
SCN^- and CN^-	Extract of bitter almonds (only CN^-)	After retention on a melanine-formaldehyde resin, SCN^- is eluted and then determined	(38)
SCN^-	Industrial wastewaters and process solutions	Optimization of a sequential injection system (SIA)	(19)
SCN^-	Biological reactor in a steel plant	Flow injection system (FIA) and continuous flow system (CFA) are optimized and a miniature spectrophotometer is constructed	(39)
SCN^-	Saliva, urine, and serum	The spectrophotometric method is used as reference	(11)
SCN^-	Saliva and serum	The spectrophotometric method is used as reference	(14)
Cl^- (indirect)	Cigarettes	The spectrophotometric method is used to monitor the SCN^-, after reaction of Cl^- with mercury thiocyanate	(34)
S^{2-} (indirect)	Natural waters	The spectrophotometric method is used to monitor the SCN^- formed in the reaction of sulfide	(23)

medium (37). The method is simple, straightforward, nontoxic, and cyanide does not interfere, yet it has some disadvantages. Several species can be formed between Fe(III) and SCN^-, therefore ferric ion must be in excess; there is a wide range of interference and color stability depends on the acid used and its concentration (19).

Although this method dates back a long time (20) and despite its disadvantages, it is still valid and has extensive use not only for the quantitation of the anion in diverse samples, but also for its detection in the indirect determination of other species where thiocyanate is formed. It also has been employed by many authors as a reference method against which the results of their proposed methods were compared. It is the recommended method for the analysis of drinking water and wastewaters (16).

Some examples of these applications are shown in Table 1. Of those used for determining SCN^- as such, the most interesting ones are those in which some sort of flow system is employed instead of the traditional batch analysis.

In the recent sequential injection system (SIA) automated determination (19), the physical (flow rates, loop volumes, coil lengths) and chemical (reagent concentrations) parameters are optimized. The system is totally computerized and sample throughput is 24 h^{-1}. The advantages of the SIA technique overcome one of the disadvantages of the spectrophotometric determination, the color instability, and therefore a great number of samples can be analyzed on-line without any lack of reproducibility.

Analytical characteristics are determined and interferences are studied; the species commonly interfering in the Fe(III) method do so here, but most are not present in the type of samples investigated or are below the interfering level. Several samples are analyzed and results are in good agreement with those obtained with the same spectrophotometric method but in a batch approach and using a diode array spectrophotometer.

Díaz-García et al. (39) optimized three different flow systems (physical as well as chemical parameters) for the determination of SCN^- using the Fe(III) spectrophotometric method: flow injection system (FIA), continuous flow system (CFA), and (CFA) with coupled fiber optics (CFA-FO). The first two were applied to the analysis of the anion at the entrance and exit of a biological reactor installed in a coke plant of a steel industry for the treatment of wastewaters. The results obtained were in good agreement with the official manual method while the rate of sampling and the analytical characteristics were superior. For the CFA-FO system, a flow cell was designed and constructed in the lab; the complete system is shown in Figure 1. This configuration was employed in the continuous monitoring of the biological degradation process of SCN^- in a minireactor.

The same authors (39) went even further in the application of the systems studied, designing and constructing a miniature flow spectrophotometer for the in situ control of the anion using the ferric ion method and the CFA. The small dimensions of the detector and flow cell make the system ideal for field measurements. A linear calibration curve was obtained when employed, and present work is being done in the application to real samples.

Many other spectrophotometric reagents have been employed for the determination of thiocyanate. Some of those developed within the past decade or so are listed in Table 2.

One of the best known, and oldest, reactions for the determination of cyanide is that which occurs, after halogenation with bromine, with a mixture of pyridine and benzidine to form a strongly colored dye (44). This method, first described by Aldridge and based on the modified König (45) reaction, has suffered modifications due to replacement by less toxic reagents (46), and for a faster reaction and a more highly colored product (40). Yet, whatever the combination of reagents, thiocyanate interferes positively since it also reacts after halogenation. Because of this, the method is used by many authors for the spectrophotometric simultaneous determination of both anions.

Masking or separation of one of these anions is necessary if simultaneous determination is to be made when using some

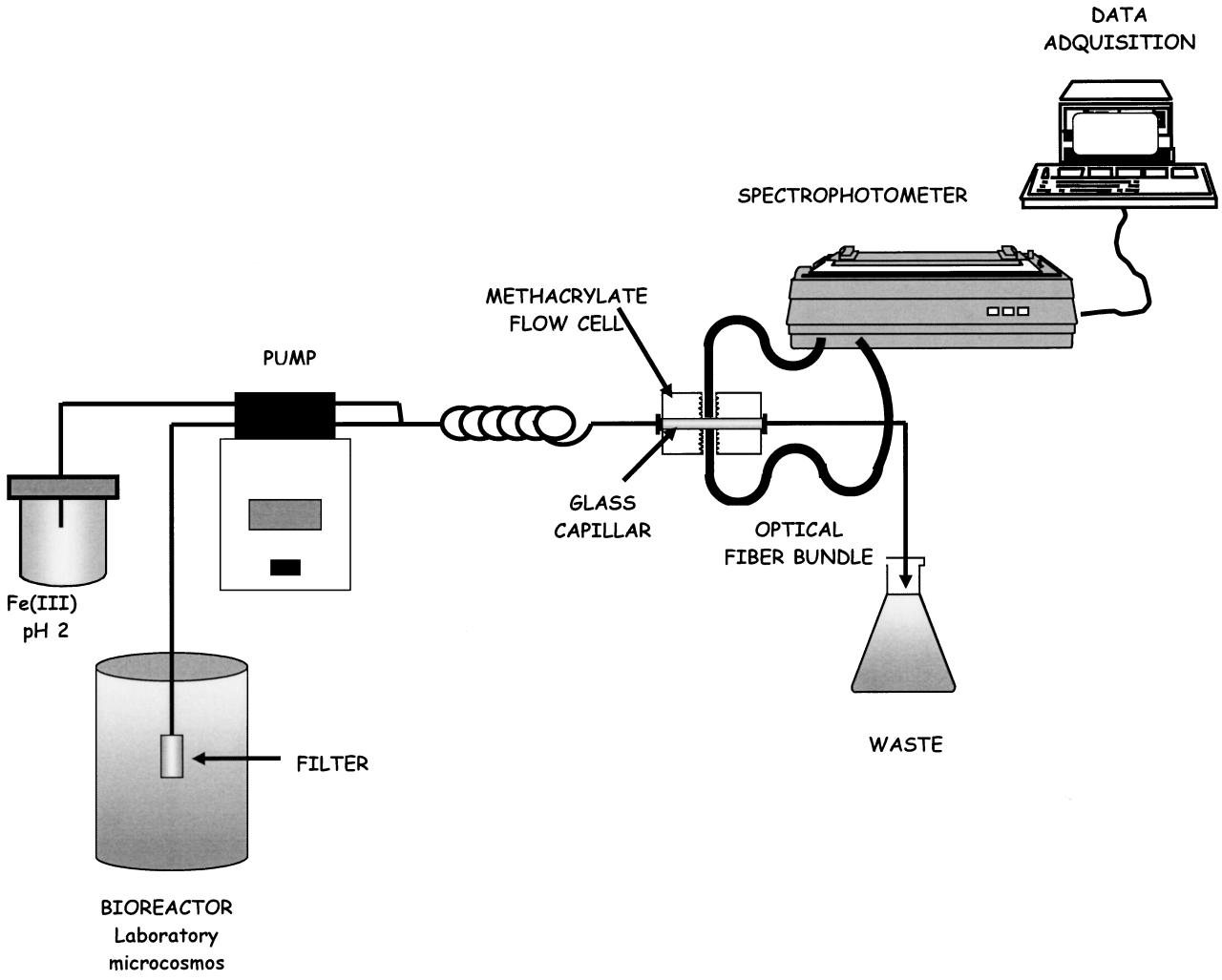

FIG. 1. Schematic diagram of a flow system for continuous monitoring thiocyanate degradation in a simulated bioreactor using an optical fiber setup.

modification of Aldridge's method. Sweileh (41), for example, masked cyanide with a nickel salt, while Tanaka et al. (47) made use of the different diffusion rates of each ion through a microporous membrane and combined this with their different reactivities toward chloramine T to differentiate them. The method recommended (16) is cyanide masking with formaldehyde, a technique employed by Meeussen (40). This author found that for effective masking of 250 ng/mL of CN^-, the sample must be heated at 50° for at least 15 min or left overnight at room temperature. When masking by formaldehyde was adapted to a stopped flow setup, less effectivity was found (48).

Since Ruzicka and Hansen (49) introduced flow injection methods in analytical chemistry, the advantages of flow techniques, combined fundamentally with spectrophotometry, over traditional manual methods, have encouraged their use by many researchers for the quantitation of a wide variety of analytes, thiocyanate included.

A flow injection configuration was proposed by Bendtsen and Hansen (7) for the sole determination of thiocyanate in saliva, requiring only centrifugation and dilution of the sample, previous to determination. Detection is made using a three-channel system where each reagent, besides the carrier, is mixed with the sample producing an intensely colored product ($\lambda = 570$ nm). Reaction kinetics and conditions avoid cyanide interference. The method has an ostensible disadvantage: a large background signal.

A complex continuous flow configuration was used by Meeussen et al. (40) where all the necessary steps (sample decomposition under ultraviolet [UV] light, distillation, chlorination, cyanide masking, and color reaction) occur on-line. This complexity is due to the fact that both cyanide and thiocyanate as well as iron-cyanide complexes are determined. In spite of the system employed, sampling rate is reported as 22 h^{-1}.

Themelis and Tzanavaras (13) also used an FIA for the on-line extractive-photometric determination of thiocyanate plus cyanide. Simultaneous extraction with chloroform occurs after the anions react with DPQH (Table 2) forming ternary complexes ($\lambda = 540$ nm); therefore, for the individual

TABLE 2
Spectrophotometric Reagents Used for SCN^- Determination

Reagent	Sample	Characteristics	Ref.
Chloramine T and 1,3-dimethyl-barbituric acid + isonicotinic acid	Water	Modification of Aldridge method Simultaneous determination where cyanide is masked with formaldehyde Continuous flow system	(40)
Sodium isonicotinate and sodium barbiturate	—	Flow injection method Two-step procedure for simultaneous determination where CN^- is masked with Ni(II)	(41)
2-(5-Br-2-pyridylazo)-5-diethyl aminophenol and dichromate	Saliva	Turned around procedure formally used for bromate determination Cyanide does not interfere Flow injection system	(7)
—	Saliva	Sample is hydrolyzed in concentrated acid medium and volatile octadecyl silica (OCS) is generated Cryogenic preconcentration system The vapor is determined directly in the ultraviolet (UV) using a continuous flow cell	(10)
I_2–azide	Saliva	Iodine concentration changes are monitored using starch solution Stopped-flow kinetic method where reaction is induced by thiocyanate	(42)
Cu(II) and 2,2′-dipyridyl-2-quinolyl hydrazone (DPQH)	Saliva and pralidoxime solutions	Ternary complexes are extracted into chloroform using a flow injection system CN^- is masked on-line with formaldehyde; simultaneous determination is possible	(13)
NaOCl and 1,3-dimethyl-barbituric acid + isonicotinic acid	Plasma and urine	Modification of Aldridge method SCN^- is isolated from alkaline solution of sample on an ion exchange resin and eluted with $NaClO_4$ Sodium nitroprusside (SNP) interferes	(43)
NaOCl and 1,3-dimethyl-barbituric acid + isonicotinic acid	Plasma	Sample treatment with 5% trichloroacetic acid SNP shows no inhibitory effect	(6)

determination of SCN^-, cyanide is masked with formaldehyde. In this case masking was successful because a binary inlet static mixer, that allowed efficient mixing, was used. Cyanide was determined by difference after total analyte concentration was determined in another run in which no masking agent was present. A 60 h^{-1} sample throughput was reported.

A spectrophotometer equipped with a stopped-flow module (42) was used for the kinetic determination of thiocfanate and thiosulfate monitoring the decrease of the absorbance of the iodine-starch complex near zero time reaction. This initial rate of decrease is found to be related to the anion concentration. The proposed method is reported to be faster, more economic, accurate, precise, and sensitive than the corresponding batch method where measurement is made after reaction is completed.

Other molecular spectroscopic methods used for thiocyanate determination are based on fluorescence measurements. Tanaka et al. (50) studied several combinations of reagents, all based on the modified König reaction, for the spectrofluorimetric determination of thiocyanate, since the polymethine dye formed was found to be fluorescent. The best reagent mixture was the combination of isonicotinic acid and barbituric acid and a four-channel flow injection procedure was proposed. Cyanide can also be determined by the same method, and therefore it interferes in thiocyanate analysis. All analytical parameters were optimized, but various practical problems were encountered for real sample application: several on-line operations require different pH values, while heating (at 60°C) and then cooling in an ice bath are necessary operations.

Some reported spectrofluorimetric methods for thiocyanate are not based on the fluorescent product formed when SCN^- reacts with adequate reagents, but on its inhibitory effects on a reaction where fluorescence is quenched, thereby increasing the signal as thiocyanate concentration increases. In this line Zhang et al. (15) reported the thiocyanate determination in saliva and urine without pretreatment of the samples, based on the inhibition produced by the anion on the bromate oxidation of the

fluorescent dye Rhodamine 6G (the molecular structure of the dye is destroyed). A kinetic approach was used and excellent results were obtained for traces of the anion; the method proved to be simple, accurate, highly sensitive, and selective.

Gong (14) also used a similar technique to determine SCN^- in saliva and serum. In this case, the reaction in which iodine and the strongly fluorescent 2′,7′-dichlorofluorescein molecule form a weakly fluorescent compound was hampered, due to the iodine consumption:

$$SCN^- + 4I_2 + 4H_2O \rightarrow ICN + SO_4^{2-} + 8H^+ + 7I^-$$

Therefore, fluorescence intensity increased with increasing thiocyanate concentration. For the reaction to proceed, heating in a boiling water bath for 3 min was necessary. Cyanide interference must be separated by distillation. Good agreement between the proposed method and reference method was obtained when samples were analyzed.

Fluorimetry has also been applied in optical sensing, although the use of this type of sensor for thiocyanate determination is not as widespread as electrochemical sensing (ISEs) as will be seen below.

A fluorimetric optical sensing approach comprising a fluorophore host based on a crown ether (PIP) has been developed for monitoring thiocyanate in aqueous environments (51). The PIP acted as a logical AND chemosensor for the recognition of SCN^-, being its fluorescence quenched only in the presence of both an alkali earth metal AND the thiocyanate anion. In a previous work (52), we have reviewed this type of chemosensing action.

Another fluorescent chemical sensor for SCN^- was described by Kopelman et al. (53). An optode based on a ruthenium(II)-porphyrin ionophore combined with a universal pH chromoionophore responds to log $[a_{H+}a_{SCN-}]$. As will be seen later, this organometallic compound is mainly employed in thiocyanate determination when using ISEs.

Atomic absorption spectroscopy is also used for the indirect determination of thiocyanate employing several metal complexes (binary or ternary) extracted into different solvents. The anion is then determined through the absorbance signal of the corresponding metal ion. The indirect atomic absorption technique for thiocyanate determination does not seem to have much acceptance by authors as in the past decade there has been a dearth of papers using this approach. Good results were obtained by Chattaraj and Das (11) when analyzing thiocyanate in several biological fluids using the [Cu2-benzoylpyridine thiosemicarbazone–SCN]$^+$ complex extracted into isoamyl alcohol. The organic phase, after two extractions, was directly aspirated into the air-acetylene flame and copper was determined. The method is straightforward and analytical figures of merit are satisfactory.

ION SELECTIVE ELECTRODES

ISEs have become one of the most important types of chemical sensors due to several advantages: relatively rapid response, low cost, simplicity, and wide linear range. In the past decades polymeric membrane-based ISEs have reached outstanding development. The key component in the sensor is the ionophore (or ion carrier) contained in the membrane, which is capable of interacting selectively with the analyte in the sample solution.

Much has been done in the search (or design, including biomimetic strategies) (54, 55) of selective ionophores for the determination of cations and anions using ISEs and, although some of these electrodes are now commercially available, there is a limited number of ion carriers that are really useful for anions. It seems that the synthesis of selective anion-carriers is more complex, due to some inherent characteristics of anions (ionic radii, hydration in protic solvents, shapes, and so forth) (56, 57). Therefore, the demand for selectivity in the field of anion ISEs is very high; thiocyanate is not an exception.

Besides the polymer matrix (usually PVC) and the plasticizer (membrane solvent), the ionophore-impregnated membrane also contains ionic additives in order to increase selectivity (58–61). We recommend readers interested in the subject view papers by Bakker, Buhlmann, and Pretsch (59, 62) on the general characteristics of carrier-based ISEs where considerations are made about the requirements of the different membrane components, all of which influence the selectivity of these electrodes.

Most of the work done in the past 10 years or so, dealing with SCN^- sensors, is dedicated to the study of ionophores (usually organometallic compounds) that strongly interact with the anion and interact only weakly with all others. If this interaction is purely electrostatic, selectivity is governed only by the lipophilicity of the anions (the well-known Hofmeister pattern) (63–65). Although SCN^- occupies one of the first places among the inorganic anions in this series, "real" selectivity (sometimes referred to as anti-Hofmeister behavior) can be found only when the anion and the ionophore interact by some sort of chemical binding (generally, metal-ligand interaction where the ligand is the anion and the metal is part of the host molecule).

It has been demonstrated by several authors (57, 58, 60, 66–69) that the stronger or weaker metal-ligand interaction, and therefore selectivity and sensitivity, depends fundamentally on the structure of the ionophore (also considering the nature of the substituents and their position in the molecule, and the nature of the central metal) as well as on the possibility of thiocyanate to act as an axial ligand to the metal center of the carrier molecule,thus forming a mixed ligand complex. The stability constant (or the association constant at the fifth or sixth coordination site) of the so-formed compound should determine the order of selectivity (70, 71). Overall, qualitative and quantitative composition of the ISE determines its analytical performance toward the ion of interest; the role played by coordination chemistry has been proven without doubt (64, 72).

Since 1990, and even before, many papers can be found in the field of carrier-based membrane anion selective electrodes that include thiocyanate as part of the anions studied in order to establish a selectivity series (56, 58, 68, 73–76). We will make reference to only those that center their research on thiocyanate

TABLE 3
Characteristics of Carrier-Based Membrane Ion Selective Electrodes (ISEs) for Thiocyanate

Ionophores	(−) Slope (mV/decade)	Linear range (M)	pH	DL (M)	Resp. time (sec)	Life time	Ref.
Mn(III)-porphyrins	59	$\sim 10^{-5}$– $10^{-1.8}$	5.5	—	—	—	(71)
Co(II)-Tetrakis (o-amino phenyl) porphyrin	43	$\sim 10^{-5}$–$10^{-2.5}$	6	5×10^{-7}	<25	At least 2 m	(77)
Benzyltin-dichlorides	52.8	From 5.3×10^{-5}	7	7.3×10^{-6}	—	—	(69)
Au(III)-Triisobutylphosphine sulfide	∼55	10^{-4}–10^{-1}	6.6	—	∼5.8–7	12 days	(70)
μ-Oxotetraphenyl porphyrinatoiron	53	10^{-6}–10^{-1}	∼3–5	3.9×10^{-7}	—	—	(64)
Mn(III)-porphyrins	∼50	10^{-4}–10^{-1}	6.5	3.2×10^{-5}	—	—	(72)
Mn(II)-N,N′-bis-(4-phenylazosalicylidene) o-phenylene diamine	57.3	7.0×10^{-6}–10^{-1}	5.38	—	<10	At least 2 m	(66)
Ni(II)-phthalocyanine chloride	58.4	5×10^{-7}–10^{-1}	3–10	5×10^{-7}	2	At least 2 m	(60)
BenzoN_4 nickel(II) macrocyclic complexes	59.8	1.4×10^{-7}–10^{-1}	3.5–10.5	1.4×10^{-7}	5	At least 3 m	(57)
1,8-dibenzyl-1,3,6,8,10,13-hexaazacyclotetradecane Ni(II) perchlorate	58.4	3.3×10^{-6}–10^{-1}	4.0–9.2	3×10^{-6}	15	At least 2 m	(67)

selective electrodes. Table 3 lists a number of the more recent SCN^- electrochemical sensors of the type mentioned. In those cases where the information that appears in the table has been calculated from the data given by the authors, the numbers are preceded by an approximation symbol (∼).

Most of these papers put their main attention on the study of the different parameters that can influence the ISE's analytical performance (in order to obtain the optimum conditions), as well as on the determination of the possible mechanism of response, according to the carrier used. Some also dedicate part of the research to the synthesis of the ionophore. However, few of the ISEs developed are applied to real thiocyanate-containing samples, probably because selectivity is not always high or because application of the electrode was not an objective of the work. Since real life is what determines the functionality or not of a sensor, we will discuss fundamentally those that have been applied to SCN^- determination in some type of sample.

Gao et al. (64) selected μ-oxotetraphenyl porphyrinatoiron among several metalloporphyrins as thiocyanate carrier for the preparation of an ISE because better potentiometric characteristics were expected due to the stability of the binuclear compound and because it has two coordination sites, as well as higher lipophilicity in comparison with mononuclear metalloporphyrins. Effect of pH, as well as influence of solvent mediators, on response characteristics were studied. The authors confirm the interaction mechanism by obtaining UV/Vis spectra which indicate that two thiocyanates (through the N atoms) were coordinated with the two central iron atoms. Smokers' and nonsmokers' urine samples, after 10-fold dilution, were analyzed at pH 3 using the proposed ISE; results correlated very satisfactorily with the traditional colorimetric Fe(III)-SCN^- method.

Transitional metal chelates of Schiff bases have also been used as neutral carriers for SCN^- by Li and coworkers (66). The base, as well as different metal chelates, were synthesized and four electrodes (varying in central metal, additive, and so forth) were prepared and studied. In this paper, the mechanism of response was also investigated and conclusions similar to Gao et al. (64) (coordination of the anion to the metal of the host molecule) were obtained. The selectivity of the electrodes was tested and the Mn(II)-chelate electrode was applied to thiocyanate determination in wastewaters. The results were compared with a high-performance liquid chromatography (HPLC) method and good accordance was obtained. Unfortunately, the authors did not report the detection limits (DL).

A similar work was done by Amini et al. (60) with thiocyanate selective electrodes based on nickel and iron(III) phthalocyanines, but the carriers were not synthesized. Several electrodes were prepared and their performance studied. After conditions were optimized, the nickel carrier electrode proved to have somewhat better sensitivity and selectivity for SCN^- when compared to the iron compound. These authors also prepared similar electrodes with both carriers but containing different additives (cationic and anionic), and demonstrated how the nature of the additive can change the selectivity pattern. In this case, the anionic additive seemed to improve selectivity of one of the carriers, but not of the other. Cationic additives only worsened the responses.

These results are opposite to that obtained by the previous authors (66), which proved only that the ionophores involved

were in one case neutral (66), and in the other (60) charged. Two electrodes (one with each metal compound and no additives) were used to evaluate thiocyanate concentration in urine (60). Again, results were compared with the spectrophotometric method and agreed.

In the most recent papers by Ardakani (67) and by Abbaspour (57), two different nickel-based compounds were employed as ionophores. In both cases, the authors synthesized the ionophores, prepared several electrodes of different composition (changing the structure of the ionophore slightly, the plasticizer, or the percentage of the components), and studied the electrodes' response characteristics in order to optimize the composition and select the best one.

Abbaspour (57) also investigated the coordination of the central metal with the analyte anion and concluded that the unique saddle structure of the group of unsymmetrical benzo N_4 nickel(II) macrocyclic complexes favored axial coordination, and therefore selectivity of the electrode. Both Abbaspour (57) and Ardakani (67) reported that their electrode was more selective to SCN^- than previous ones and, as can be seen in Table 3, the macrocyclic compound showed the best performance. Thiocyanate concentration was determined by applying the proposed electrodes in both papers to clinical chemistry, urine, and saliva samples of smokers' and nonsmokers. The two authors compared their results with the standard colorimetric method; good correlation was obtained in the comparison. In both cases the urine samples were pretreated in the same manner (1:10 dilution and pH adjusted to 5 with phosphoric acid and potassium hydroxide), but the results obtained were not always similar, probably due to the difference in the population studied.

Although in their work Florido et al. (70) did not apply the ISE developed, there are so few of these electrodes combined with flow injection methods that attention must be taken to this work. The ionophore, Au(III)-triisobutylphosphine sulfide, was synthesized and electrodes with two different compositions were prepared. The electrodes were first characterized in the conventional configuration, but also in a flow-through tubular system. Although optimized flow parameters were given the actual configuration did not appear in the paper, but is referred to a previous work (78). In order to stabilize the baseline, the carrier solution consisted of a pH 6.6 buffer containing a small concentration of SCN^-. Changes in analyte concentration were measured as changes in potential relative to the baseline. The analytical characteristics of the flow-through electrode seemed satisfactory and so was the sample throughput (80 h^{-1}); yet, the lifetime of the sensor was shorter due to stripping of the ionophore by the carrier stream.

Another flow injection determination of thiocyanate was made by Cookeas and Efstathiou (79), but in this case the electrode was a cobalt phthalocyanine modified carbon paste electrode (CoPC-CPE) and the detection was not potentiometric but amperometric. The preparation of the electrodes was quite simple and the flow system was a FIAstar analyzer coupled to a wall-jet–type amperometric flow detector developed by the authors; all the system was automated. The reported figures of merit were quite good (e.g., the DL 0.78 μM, the linear range 1 to 50 μM, and slope of calibration graph 121 nA/μM). Thiocyanate determination in saliva samples of smokers and nonsmokers (after 100-fold dilution and at pH 2) using a standard addition procedure compared well with the conventional spectrophotometric method.

ION CHROMATOGRAPHY

Since Small et al. (80) introduced ion chromatography (IC), the two-column system using conductimetric detection has developed into a simple single-column system employing several types of detectors: direct or indirect photometry, conductimetry, or amperometry. Also, stationary phases as well as eluents can be greatly varied according to the nature of the species to be determined and the accompanying components of the sample.

The literature discussed previously in this review shows that thiocyanate determination is of most interest when biological fluids, such as saliva, urine, and blood or serum, are concerned. According to Singh (81), IC is the method of choice for the analysis of certain anions (SCN^- included) and cations in these types of samples. Evidently this seems to be the case when the simultaneous determination of several of these inorganic species is desired due to the possibility not only of the separation of the analytes but also of their quantitation, whether or not employing derivatization and/or postcolumn reactions (PCR) and different detectors. Because of UV absorption of thiocyanate around 210 nm, this type of detector is satisfactory for its IC determination; usually lower detection limits are obtained when compared with conductimetric detection (82–84). On the other hand, new sorbents are constantly being synthesized and studied for the ion chromatographic determination of anions (among them thiocyanate) (85).

Although SCN^- is quite hydrophobic and therefore strongly retained on most columns, several IC methods have been successful in its elution in short periods of time and with rather sharp peaks. Table 4 shows the general characteristics of some of these. Only in those papers related to biological samples has thiocyanate been specifically determined. In the cases where the sample matrix was water (whether lake, ground, or spring), it was part of the anions studied but its analysis was not undertaken. The units for the DL reported by the authors have been respected.

Chinaka et al. (3) and Michigami et al. (84) have determined trace thiocyanate in human serum and blood using simple pretreatments for the samples. In blood, extraction using water and methanol was sufficient while the serum was deproteinized using acetonitrile. In both cases conditions affecting retention times and separation factors (choice of eluent, pH, μ), as well as wavelength of the detection, were studied. Samples of smokers and nonsmokers were analyzed and SCN^- recoveries were over 95%. Chinaka simultaneously determined cyanide in the blood samples of fire victims using prior derivatization and fluorescence detection.

TABLE 4
Determination of Thiocyanate by Ion Chromatography

Stationary phase	Eluent	Other anions	Sample matrix	Retention time (min)	DL	Detector	Ref.
TSK-gel IC anion-SW (35°C)	4 mM KH_2PO_4/2 mM K_2HPO_4 (pH 6.5)	Cl^-	Serum	10	5 ng mL^{-1}	Ultraviolet (UV) (195 nm)	(84)
IC-PAK anion exchange	0.01 mM sodium naphthalene trisulfonate/10% CH_3CN	NO_2^-, Br^-, I^-, NO_3^-, SO_4^{2-}	—	17	0.02 μg/mL^{-1}	Indirect UV (280 nm)	(86)
Octadecyl silica (ODS) column coated with cetyldimethylamine (35°C)	1 mM citrate (pH 7.0)	—	Saliva, urine	16	0.02 μg/mL^{-1}	UV (210 nm)	(83)
Shim-Pack CLC-C_8	15:85 CH_3OH/60 mM phosphate pH 5.5 buffer containing 0.1 mM EDTA and 3 mM tetrabutyl ammonium hydroxide	NO_2^-, $S_2O_3^{2-}$, IO_3^-, S^{2-}	Lakewater	7	104 ng/mL^{-1}	Amperometry with glassy carbon indicator electrode	(87)
Bovine serum albumin immobilized on ODS	0.15 mM tartaric acid (pH 3.6)	Br^-, I^-, NO_3^-, IO_3^-	Saliva	~9.5	82 μM	UV (210 nm)	(88)
TSK-gel IC anion-PW	0.006 M carbonate/15% CH_3CN	S^{2-}, $S_2O_3^{2-}$, SO_3^{2-}	Hot springwater	21.5	0.05 ng in 50 μL	Postcolumn reactions (PCR) (350 nm)	(89)
TSK-gel IC anion-SW	10 μM phosphate buffer (pH 6.1)/50% CH_3OH	CN^-	Blood	~8	86 pmol/mL^{-1}	UV (210 nm)	(3)
ODS	20:80 CH_3CN/H_2O (pH 5) with 6 mM tetrapropyl ammonium salt	$S_2O_3^{2-}$, polythionates	Springwater	7.4	40 nM	UV (230 nm)	(90)
Dionex ion pac AS 16	Electrolytically generated KOH (in gradient mode)	$S_2O_3^{2-}$, ClO_4^-, AsO_4^{3-}	Groundwater	28	—	Suppressed conductivity	(91)
Phenomenex hypersil (ODS) column (45°C)	10 mM tetrabutyl ammonium phosphate in 20% CH_3OH	NO_2^-, NO_3^-	Urine	4.4	0.02 mg/L^{-1}	UV (230 nm)	(4)
Coated Zwitterionic-charged develosil ODS-5	Water	I^-	Saliva	~5	1.2 μM	UV (230 nm)	(92)
Coated Zwitterionic-charged develosil ODS-5	20 mM NaH_2PO_4/Na_2HPO_4 (pH 6.8)	NO_2^-, I^-, NO_3^-	Saliva	~13	—	UV (230 nm)	(93)

Michigami (83) also analyzed thiocyanate in human saliva and urine, but in this case the stationary phase was an octadecyl silica (ODS) column dynamically coated with cetyldimethylamine. Here again various experimental conditions were optimized: choice of reagent, eluent concentration, and pH, as well as λ. Saliva and urine samples were simply diluted before injection; the recovery experiments in both samples were over 95%. The results of SCN^- analysis in smokers and nonsmokers by the proposed IC method compared well with those of the Fe(III)-SCN^- spectrophotometric method.

Another short C_{18} column was used (4) at an elevated temperature (45°C) and with a mobile phase of tetrabutyl ammonium phosphate in order to obtain fast ion interaction and low retention times for thiocyanate (<5 min). Chromatographic conditions (selection of ion pair reagent, of organic modifier, concentration and pH of the eluent, as well as temperature and flow rate) were optimized. In this method, however, the urine samples were first pushed through an SPE C_{18} cartridge for the removal of organic interferents. A standard addition technique was employed to quantify SCN^- in the samples of smokers and nonsmokers.

Again a bovine serum albumin (BSA) modified ODS column was used by Zein et al. (88) for the separation of inorganic UV-absorbing anions. Proteins have amphoteric behavior and therefore can be used as potential stationary phases for both anion and cation exchange, all depending on the pH of the mobile phase. Under acidic conditions (sulfuric, aspartic, and tartaric acids were studied), several anions were eluted from the column. Tartaric acid provided the best separation in a reasonable period of time. The authors applied the BSA column, after optimizing experimental conditions, to the determination of nitrate, iodide, and thiocyanate in saliva. Yet, no comparison with other methods of analysis or recovery studies were done.

Hu et al. (92, 93) developed electrostatic ion chromatography (EIC) for the separation and detection of thiocyanate and other anions in human saliva. For this, they used Zwitterionic surfactants above the critical micellar concentration and passed the corresponding solution through an ODS column for 30 min at a flow rate of 2.8 μL/min. The positive and negatively charged stationary phase separated the anions using the simultaneous electrostatic attraction and repulsion reactions (Figure 2). The mobile phase in the earlier paper (92) was simply water, and in the later (93) a phosphate buffer. Resolution of nitrate and nitrite peaks was also achieved although the retention times increased. In another paper (94) these authors studied EIC, but from a more theoretical point of view (partitioning behaviors of cations, anions, and their corresponding ion pair-like forms).

Umemura et al. (95) also studied EIC by evaluating the analytical performance of dynamically coated ODS columns with three kinds of sulfobetaine-type surfactants, using water as eluent and a UV absorption detector for thiocyanate. These authors studied the stability of the stationary phases prepared (more than 3 mon) and the different variables that affected the separation of a great number of anions and cations. Among them were nature (ionic functional groups), hydrophobicity, and amount of the adsorbed Zwitterionic compound, as well as surface charge density.

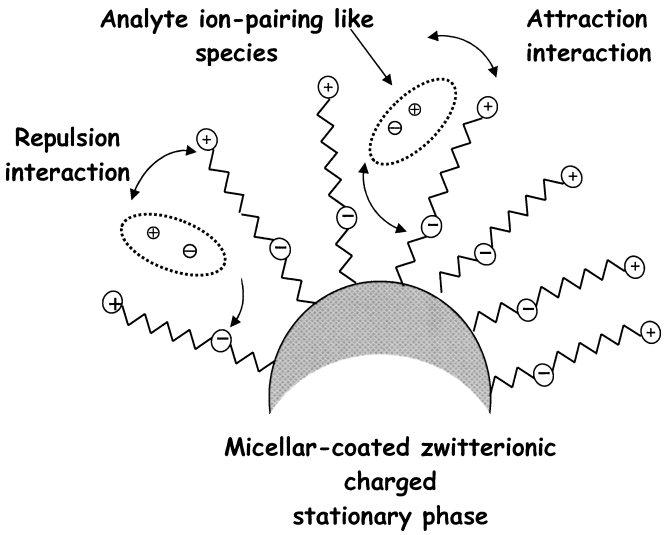

FIG. 2. Analyte stationary phase interactions concurring in electrostatic ion chromatography.

The authors reached interesting conclusions on the retention mechanism and found that the elution order of the anions responded to Hofmeister's series, being SCN^- next to last (ClO_4^-) among the anions studied. Therefore, they suggested that the hydration energies of the ions were responsible for their separation. The analytical performance of the ion chromatographic system was evaluated only by measuring the elution times of the many species investigated, but no figures of merit were determined.

OTHER METHODS

Table 5 lists several other methods used for thiocyanate determination which were not included above since the number of papers dedicated to the quantification of this anion by the particular method is rather limited. Of these, capillary electrophoresis (CE) seems to be the most important. According to some authors (96) CE will continue to be displaced by IC for the determination of ions found in water and other liquid matrices, while others (97) have stated that IC may have met its match in CE. This deserves some comment on those papers where CE is used to determine SCN^- in real samples.

The petroleum tracers, nitrate and thiocyanate, are determined by capillary ion electrophoresis in subterranean waters in spite of the high content of salts present. The authors (21) examined the effect of NaCl in large concentrations in order to simulate the possible difficulties they could find when salty samples were to be analyzed. Their results showed that variations in the salt content produced changes in the peak heights or widths, but not on peak area. By using 100 mM sodium chloride with 2.0 mM cetyltrimethylammonium chloride (electroosmotic flow modifier) as carrier electrolyte solution, both tracers could be determined in samples containing up to 200 mM of the salt.

TABLE 5
Other Methods for Thiocyanate Determination

Method	Characteristics	Other anions	Sample	DL	Linear range	Ref.
Gas chromatography (GC) and GC/mass spectrometry (MS)	Use of an extractive alkylation technique	CN^-	Blood	0.003 μmol mL^{-1}	0.02–1.0μmol mL^{-1}	(35)
High-performance liquid chromatography (HPLC)	Derivatization to a fluorogenic compound	—	Saliva and plasma	165 fmol mL^{-1}	0.5–10 nmol mL^{-1}	(99)
HPLC	Ultraviolet (UV) detection after postcolumn derivatization	CN^- and metallocyanides	Gold cyanidation leachates	—	—	(100)
Thin-layer chromatography	Use of an Fe(III) chromogenic reagent for detection	Several	Spiked river water, seawater, and wastewaters	—	—	(18)
Ion-exchange chromatography	Using amperometric or potentiometric detection	Halides, NO_2^-, $S_2O_3^{2-}$	—	1 μM	—	(101)
Potentiometric titration	Using $AgNO_3$ with a Ag indicator electrode and multivariate calibration	Halides	—	—	—	(102)
Capillary electrophoresis	Use of different electrolytes in nonaqueous media and amperometric detection	I^-, NO_2^-, N_3^-, Cl^-	—	4×10^{-9} M	5×10^{-8} to 1×10^{-6} M	(103)
Micellar electrokinetic capillary chromatography	Alkyltrimethyl ammonium bromides were used as surfactants in the separation buffer: UV detection	I^-, NO_2^-, NO_3^-	Blood, plasma, and milk	0.15 mM	Up to $\sim$ 38 mM	(11)
Capillary ion electrophoresis	Carrier electrolyte solution was NaCl with cetyltrimethyl-ammonium chloride: UV detection	I^-, NO_2^-, NO_3^-, Br^-	Subterranean water	6286 ng/mL^{-1}	—	(22)
Capillary electrophoresis	Suppressed electroosmotic flow is used, as well as UV detection	Halides, oxyhalides, and metal oxoacids	—	0.9 mg/L^{-1}	10 to 200 mg/L^{-1}	(104)
Capillary electrophoresis	Potentiometric detection with coated-wire ion selective electrodes (ISEs)	Several	—	—	—	(105)
Capillary electrophoresis	Electrokinetic sample injection and conductivity detection	SO_3^{2-}, SO_4^{2-}, S^{2-}, $S_2O_3^{2-}$	Open-pit mining lake water	0.012 mg/L^{-1}	—	(98)
Capillary zone electrophoresis (CZE)	Enzymatic reaction monitored through SCN^- formation, which is followed by CZE using UV detection	—	—	2.5 μM	50 μM to 5 mM	(2)
CZE	Application of the previous paper to SCN^- analysis	Br^-, NO_3^-	Serum, urine, and saliva	0.7–1.5 μM (depending on sample)	25 to 500 μM	(5)

Migration time for thiocyanate was around 12 min, good linearity was found and recoveries in the samples were over 91%.

Another paper centered on the analysis of environmental samples by CE was that of Hissner et al. (98). A method was developed for the determination of various sulfur-containing anions and applied to the analysis of water from an open-pit mining lake that had been contaminated with humic substances. Separation conditions were determined (electrophoretic buffer), and comparison was made of hydrodynamic versus electrokinetic injection of sample. A standard addition procedure was employed to correct for strong matrix influence. Thiocyanate analysis using CE has also been applied in biological samples. Glatz et al. (2) developed an indirect method of determining rhodanese activity by monitoring through CE the SCN^- produced in the following reaction, which is catalyzed by the enzyme:

$$S_2O_3^{2-} + CN^- \rightarrow SCN^- + SO_3^{2-}$$

In the paper (2), the authors stated that the method developed also has great potential for thiocyanate determination as such. Therefore, in a later paper (5) it was applied to the analysis of the anion in human serum (after deproteinization with acetonitrile), saliva, and urine samples. Bromide was added as an internal standard (it migrates closely before the SCN^- peak). In this way, correction for changes in sample composition was made. No flow modifier was needed. Background electrolyte was 0.1 M β-alanine with HCl and the electroosmotic flow resulted to be minimal.

Bjergegaard et al. (12) also analyzed biological samples, blood plasma, and milk. The micellar electrokinetic capillary chromatographic method using positively charged surfactants (particularly dodecyltrimethylammonium bromide), was effective in the separation of several anions. According to the authors, in the case of thiocyanate, a low number of theoretical plates indicates that improvements had to be made since this ion showed a tendency for asymmetric peaks. Interfering compounds in the samples were eliminated by a previous ion exchange technique and evaporating the eluate to dryness in order to reduce volume.

FINAL REMARKS

A number of methods are available for thiocyanate determination. This review illustrates the most commonly applied analytical and chromatographic methods for quantitating thiocyanate ion in both clinical and environmental samples, and highlights their strengths and weaknesses. The UV/Vis spectrophotometric based on the $FeSCN^{2+}$ complex and that based on the König reaction are the most common methods used. Ion chromatography is a powerful separation technique which offers low limits of detection (if adequate detectors are used), and more specific and complementary information about accompanying anions (such as cyanide). This technique is useful for clinical and environmental analysis. For process and on-line analysis electrochemical sensors are probably well suited, although their limited selectivity and lifetime are important drawbacks. It is likely that the most adaptable techniques for process analysis are the spectrophotometric ones (absorbance, fluorescence). The potentials of techniques such as CE, GC/MS, and micellar electrokinetic chromatography (MEC) may not yet be fully realized for thiocyanate determination. There remain many opportunities to develop and adapt methods that will be faster, automated, and rugged especially when considering the need to analyze thiocyanate in complex matrices (e.g., food science, wastewaters, body and plant fluids).

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