Ion Chromatographic Determination of SCN^- in Soils

Paul D. Brown and Matthew J. Morra*

Division of Soil Science, University of Idaho, Moscow, Idaho 83843

An ion chromatographic (IC) procedure designed to avoid interferences from both plant tissues and soluble soil organic materials was developed to measure SCN^- in soil extracts. Ionic thiocyanate (SCN^-) was extracted from 4 g of soil with 10 mL of 0.01 M $CaCl_2$ and the concentration determined with suppressed IC in combination with conductivity detection. Extraction efficiency from six SCN^--amended soils averaged 94% for SCN^- concentrations of 1.25–12.5 μg of SCN^-/g of soil. Colorimetric determination of SCN^- in soil extracts was comparable to IC at higher amendment rates but averaged only 67% of the amount added with spikes of 1.25 μg of SCN^-/g of soil. The IC method is compatible with infrared analysis of isothiocyanates when soil is extracted simultaneously with $CaCl_2$ and CCl_4. Various anions including S^{2-}, $S_2O_4^{2-}$, SO_3^{2-}, SO_4^{2-}, $S_2O_3^{2-}$, and $S_4O_6^{2-}$ do not interfere with SCN^- quantification.

Ionic thiocyanate (SCN^-) is a major metabolite of cyanide in biological systems (Wood, 1975). Thiocyanate in soils is a byproduct of industrial processes or a degradative product from natural sources. As a specific example of the latter, it has been proposed that indole and *p*-hydroxybenzyl glucosinolates contained in *Brassica* spp. tissues are hydrolyzed by the enzyme thioglucosidase (E.C. 3.2.3.1) to produce unstable intermediates which spontaneously form SCN^- (Van Etten and Tookey, 1979).

Numerous methods of SCN^- analysis have been reported (Ashworth, 1972), but many of these are time-consuming, impractical for use on a routine basis, or unsuitable for use with soil extracts. Tabatabai and Singh (1976) described a method for determining rhodanese (E.C. 2.8.1.1) activity in soils based on the formation of SCN^-. A similar method was later applied to thiosulfate and tetrathionate determination in soil (Nor and Tabatabai, 1976). This relatively simple procedure involves colorimetric determination of a Fe–SCN complex formed under acidic conditions. However, water-soluble soil organic matter included in the extract produces a background absorbance at the wavelength used for Fe–SCN measurement. Subtraction of a reagent blank absorbance from the treatment absorbance is therefore required. A similar method used for the measurement of SCN^- in plant tissues also suffers from interferences. The amount of background absorbance caused by phenolic compounds is determined by destruction of the Fe–SCN complex using $HgCl_2$ (Johnston and Jones, 1966; Josefsson, 1968). In addition, both color formation and fading are dependent on analysis time and composition of the sample (Hughes, 1975; Ashworth, 1975). The color is reported to be stable for 1 h in rhodanese determinations (Tabatabai and Singh, 1976), while strict observance of a 15-min time period is recommended for evaluation of SCN^- in extracts from low glucosinolate containing seed meal of *Brassica* spp. (McGregor, 1990).

Quantification of SCN^- in body fluids such as saliva (Matsushita et al., 1983) and serum (Michigami et al., 1988) has been performed by ion chromatography (IC). Dick and Tabatabai (1979) showed that SO_4^{2-} and other S-containing anions could be determined in soil extracts using suppressed IC, but SCN^- was not eluted by using the specified column and eluent combination. Our objective was to develop an IC procedure to measure SCN^- in soil extracts to avoid matrix interferences occurring with colorimetric methods. Our specific need is to quantify SCN^- in glucosinolate-amended soils and thus eliminate potential interferences caused by both soil and plant tissues. This method must also be applicable when used in combination with the analysis of other glucosinolate degradation products, such as isothiocyanates.

EXPERIMENTAL PROCEDURES

Instrumentation. A Dionex 4000i ion chromatograph equipped with an AS5 column, anion micromembrane suppressor, and conductivity detector was used in combination with a Spectra Physics integrator (4270) for SCN^- quantification. The eluent, which contained 4.3 mM $NaHCO_3$, 3.4 mM Na_2CO_3, and 0.8 mM 4-cyanophenol, was filtered through a 0.45-μm membrane filter, degassed with He for approximately 15 min, and pumped at a flow rate of 2 mL/min. The suppressor reservoir contained 0.025 M H_2SO_4 and was pressurized at 0.04 MPa. Background conductivity of the suppressed eluent averaged 26 μS. A detector output setting of 3 μS was used for soil extract analysis.

Chemicals. Chemicals used for SCN^- extraction and IC were of reagent grade. 4-Cyanophenol used in the eluent preparation was obtained from Aldrich Chemical, Milwaukee, WI. Stock SCN^- solutions of 100 μg of SCN^-/mL were prepared from KSCN (J. T. Baker) in double-deionized water. Calibration curves to determine the linear relationship between concentration and peak area were performed with triplicate dilutions of solutions containing 0.02, 0.05, 0.1, 0.5, 1.0, 3.0, 5.0, and 10.0 μg of SCN^-/mL.

Soils. Soils were selected to obtain a range in organic C, pH, and particle size distribution (Table I). Surface soil samples (0–15 cm) were collected, air-dried, and sieved (2 mm). Analyses were conducted according to the following methods: pH by glass electrode (1:1 soil to water), organic C by the Walkley–Black method (Nelson and Sommers, 1982), and total C and N by Dumas combustion (LECO CHN-600 determinator, St. Joseph, MI). Particle size distributions for Nyssaton, Palouse, Latahco, Feltham, and the unnamed sandy clay soils were determined by the hydrometer method and for the unnamed silt loam soil by the pipet method (Gee and Bauder, 1986).

Extraction Procedures. Air-dried soil (4 g) was placed in a 50-mL Erlenmeyer flask, amended with 10 mL of deionized water containing 0.0, 0.5, 1.0, 3.0, or 5.0 μg of SCN^-/mL, and shaken at room temperature for 1.5 h. Thiocyanate was extracted from the soils by shaking for 1–2 min with 10 mL of 0.01 M $CaCl_2$. The resulting soil extract was filtered through Whatman No. 40 or 42 filter paper and stored until IC analysis. Approximately 5 mL of the filtered extract was refiltered through a 0.45-μm membrane syringe filter (Gelman, Supor-450) immediately prior to IC injection. All analyses were performed with a minimum of three replicates. Calibration curves for SCN^- were constructed for each group of soil extracts.

We have adapted the method of Ashley and Leigh (1963) for measuring methyl isothiocyanate in soils to the determination

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Table I. Characteristics of Soils Used in SCN^- Recovery Tests

soil							
series	subgroup	pH	organic C, g/kg	total C, g/kg	total N, g/kg	clay, g/kg	sand, g/kg
Feltham ls	Xeric Torriorthent	6.3	6.3	8.2	0.8	40	830
Nyssaton sil	Xerollic Calciorthid	6.9	8.5	20.2	0.8	71	263
Palouse sil	Pachic Ultic Haploxeroll	5.7	19.2	19.5	1.6	211	88
Latahco sil	Argiaquic Xeric Argialboll	6.1	41.0	41.0	3.9	159	122
unnamed scl	Ultic Paleustalf	4.9	1.5	2.1	0.5	422	548
unnamed sil	Typic Vitrandept	5.4	20.0	20.5	1.8	117	190

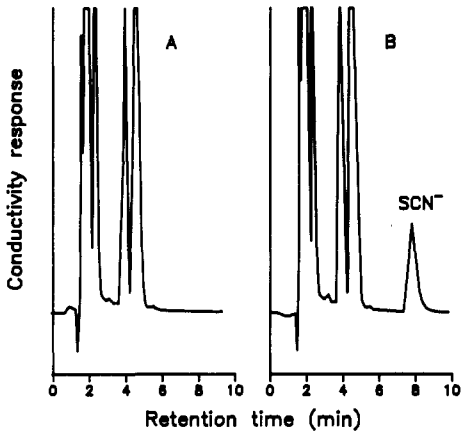

Figure 1. Chromatograms of $CaCl_2$ extracts from unamended (A) and SCN^--amended (B) soils.

of isothiocyanates produced as a result of glucosinolate degradation. A similar extraction procedure to that detailed above for SCN^- was therefore used in combination with CCl_4 extraction of isothiocyanates from the Palouse soil. This optional procedure involved the amendment of 5 mL of SCN^- solution to 4 g of soil and a 1.5-h incubation, followed by the addition of 10 mL of CCl_4 and 5 mL of the $CaCl_2$ extractant. The mixture was shaken vigorously for 1–2 min and centrifuged at 630*g* for 10–12 min. The aqueous layer was removed with a syringe and analyzed for SCN^- as previously described.

Colorimetric Method. Comparison of IC analysis to a colorimetric method was performed by using three of the six soils (Nyssaton, Latahco, and the unnamed sandy clay). Soils were amended with SCN^-, incubated, and filtered in the same manner as reported previously for IC analysis. The procedure of Tabatabai (1982) was followed for reagent preparation and color development.

Potential Interferences. The retention times of various anions suspected to interfere with SCN^- analysis were determined. Anions were selected to represent common soil constituents as well as S-containing ions not normally found in unamended soils. All solutions of the potential interfering anions were prepared in concentrations of 100 μg/mL.

RESULTS AND DISCUSSION

Calibration curves using standard solutions were linear over the entire concentration range 0.02–10.0 μg of SCN^-/mL ($R^2 > 0.98$). The coefficient of variability was less than 5.5% for all solutions with 0.1 μg of SCN^-/mL or greater but increased to 29% for the 0.05 μg/mL standard. Peak heights for 0.02 μg/mL SCN^- solutions were approximately 2.5 times background noise, bordering on the limit of detection for the instrument.

Soil extracts contained no anions in concentrations that interfered with SCN^- analysis, allowing quantification in 8 min (Figure 1). Concentrations of SO_4^{2-} (the nearest eluting anion in soil extracts) high enough to mask the SCN^- peak would not be expected to occur in soils unless such soils had been artificially altered or amended.

Extraction efficiency of SCN^- from the amended samples exceeded 83% and averaged 94% for all concentrations and soils (Table II). In general, the average percent recovery was directly proportional to the amount of SCN^- added. Extraction of SCN^- was equally effective from the tested soils with no obvious effect of soil organic C or particle size (Table I) on efficiency. The unnamed silt loam soil was included in the extraction efficiency test because of its high volcanic ash content. Although volcanic ash soils have relatively large exchange capacities for some anions (Wada, 1989), SCN^- recoveries from this soil ranged from 83.1 to 95.9%. Further investigations of extraction efficiency are warranted if low pH soils high in volcanic ash or hydrous oxides of aluminum and iron are used.

Table II. Average Percent Recovery of SCN^- from Spiked Soil Samples[a]

soil	soil spike, μg of SCN^-/g of soil			
	1.25	2.5	7.5	12.5
Feltham	89.3	93.8	100.9	101.5
Nyssaton	89.4 (60.8)	94.5 (94.0)	97.1 (99.7)	103.7
Palouse	86.0	93.6	94.8	96.8
Latahco	95.6 (67.9)	96.4 (92.3)	96.4 (99.1)	105.3
unnamed scl	84.7	88.3	99.3	96.8
unnamed sil	83.6 (71.4)	83.1 (97.6)	95.9 (99.1)	94.7

[a] Recovery values in parentheses were determined by using the colorimetric method.

Recoveries determined with the colorimetric method compared favorably to those determined with the IC method at the higher SCN^- amendment rates but dropped significantly with SCN^- additions of 1.25 μg/g of soil (Table II). An apparent decrease in recovery with the colorimetric method resulted from the necessity of subtracting background absorbance of a blank sample from actual absorbance of the Fe–SCN complex. At lower SCN^- concentrations, background absorbance contributes a greater proportion of the total absorbance, making low concentrations of the Fe–SCN complex difficult to accurately measure. Alternatively, the extract matrix may alter the rate of Fe–SCN formation or color fading to a greater extent at lower SCN^- concentrations. The IC determination of SCN^- does not suffer from a similar background problem and, therefore, has important advantages at low SCN^- concentrations and in working with high organic matter soils.

Although the soil sample size reported here was 4 g, we have found this extraction method to yield similar recoveries with soil samples from 3 to 8 g. Water was as efficient as $CaCl_2$ in extracting SCN^- from soil, but such extracts resulted in much longer filtration times because of the presence of dispersed clay-sized materials. Filtered extracts could be stored under refrigeration for at least 48 h with no change in SCN^-.

Aliphatic and aromatic (i.e., indole and *p*-hydroxybenzyl) glucosinolates are usually present in combination in *Brassica* spp. plant tissues. Isothiocyanates are major degradative products of aliphatic glucosinolates, whereas SCN^- is the major product of aromatic glucosinolates. A combined method to measure both isothiocyanates and SCN^- in soil amended with glucosinolate-containing plant tissues would therefore be advantageous. A method for the indirect determination of allyl isothiocyanate in soil

Table III. Retention Times of Potential Interfering Anions Relative to That of SCN^-

anion	rel retention time	anion	rel retention time
NO_2^-	0.25	SO_3^{2-}	0.54
NO_3^-	0.30	SO_4^{2-}	0.56
S^{2-}	0.47 (1.63)[a]	SCN^-	1.00
PO_4^{3-}	0.48	$S_2O_3^{2-}$	1.96
$S_2O_4^{2-}$	0.51 (1.63)[a]	$S_4O_6^{2-}$	RT[b]

[a]Multiple peaks occurred even with fresh solutions as a result of the formation of oxidation products. [b]Compound retained by column as defined by lack of elution 25 min following injection.

has been suggested (Chae and Tabatabai, 1983). However, this method has not been tested with soil extracts or plant tissues. We have instead chosen to use an infrared method originally proposed by Ashley and Leigh (1963) for the measurement of methyl isothiocyanate produced in dithiocarbamate-amended soils. We have successfully adapted this method to monitor isothiocyanate production in soils amended with defatted seed meal from *Brassica* spp. (unpublished data). The mixed-solvent extraction procedure in which CCl_4 is added prior to $CaCl_2$ resulted in SCN^- recoveries of nearly 100% (data not shown).

A potential problem with the IC method is coelution of another ion at the same retention time as SCN^-. In addition to the common anions found in soil extracts, other anions, particularly those containing S, may be produced from specific substrates. None of the tested anions was found to interfere with SCN^- analysis (Table III). Because of the relatively large separation between the elutions of the potential interfering anions and SCN^-, concentrations of these ions would have to exceed the SCN^- concentration by several orders of magnitude to cause an interference. As a result of the lack of interferences and high sensitivity, the IC determination of SCN^- in soils has wide applicability.

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