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Determination of thiocyanate, iodide, nitrate and nitrite in biological samples by micellar electrokinetic capillary chromatography

C. Bjerregaard, P. Møller, H. Sørensen*

Chemistry Department, Royal Veterinary and Agricultural University, 40 Thorvaldsensvej, DK-1871 Frederiksberg C, Denmark

Abstract

Micellar electrokinetic capillary chromatography (MECC) has been developed as an efficient method for the determination of the thiocyanate ion, iodide, nitrite and nitrate. The use of various alkyltrimethylammonium ions was found advantageous for MECC compared to negatively charged surfactants, as this resulted in low migration times for the anions and thereby fast analysis. Moreover, the MECC system, using positively charged surfactants (dodecyltrimethylammonium bromide, DTAB), was effective in separating the anions of interest from interfering organic anions present in extracts from biological samples such as milk and blood. Detection of the anions was performed by direct UV. The performance of the developed method was satisfying; however, a low number of theoretical plates for the thiocyanate ion indicated the need for some improvements in this respect. The sensitivity of the method increased with a factor two by using a 75 μm I.D. capillary instead of the 50 μm I.D. capillary, whereas use of a high-sensitivity optical cell assembly increased the sensitivity an additional nine times. Repeatability with the 50- μm capillary was satisfying with respect to migration time, relative migration time and normalised peak area (except for the thiocyanate ion) of the anions, with relative standard deviations varying between 0.24 and 0.29%, 0.07 and 0.16%, and 1.75 and 2.37%, respectively, whereas some improvements are still needed for the normalised and relative normalised peak areas for the thiocyanate ion (13.50 and 13.91%). Apart from the thiocyanate ion ($r^2 = 0.9748$), linearity studies gave correlation coefficients between 0.9962 and 0.9975 for the normalised peak areas.

1. Introduction

The thiocyanate ion is formed as a degradation product of indol-3-ylmethylglucosinolates and other glucosinolates which give a carbonium ion and the thiocyanate ion in non-enzymatic and enzyme-catalysed reactions [1]. When absorbed from digesta to the blood, the thiocyanate ion can be transferred to the milk [2–5] or transformed by liver enzymes. It may also competi-

tively inhibit the transfer of iodide to the thyroid hormones in the thyroid gland [6–10]. The thiocyanate ion and iodide are thus considered to be important in relation to studies of the quality of vegetables, e.g. antinutritional effects of glucosinolates [1] as well as processing effects on oilseed rape [11,12] and on cruciferous vegetables rich in indol-3-ylmethylglucosinolates [13]. For various other reasons nitrate and nitrite are considered to be important in relation to the quality of food and feed. It will therefore be of value to have a simple analytical method for the

* Corresponding author.

simultaneous determination of the thiocyanate ion, iodide, nitrate and nitrite in biological samples such as glucosinolate-containing vegetables [14], food, feed, blood and milk. An analytical technique which in addition can be used for other types of degradation products from glucosinolates would be preferable [1,15].

Recently, a most interesting method for the separation and detection of anions by high-performance capillary electrophoresis (HPCE) has been described [16]. This method and other pioneering work performed on various anions [17,18] use, however, a system with indirect photometric detection which is less efficient for the analysis of glucosinolates and its degradation products. This is also the case with a recently described HPCE method for the determination of nitrate and nitrite in plasma [19]. Micellar electrokinetic capillary chromatography (MECC) using alkyltrimethylammonium bromides as surfactants has formerly been developed for the determination of various types of natural products including glucosinolates [20] and different types of indolylic compounds from glucosinolate degradation [15]. This system was tested and found satisfying for the simultaneous determination of the thiocyanate ion, iodide, nitrate and nitrite, using detection by direct UV at 235 nm. In the present work, we describe the performance of this system with respect to separation efficiency, repeatability and linearity of the optimised MECC method as well as sensitivity, using a special Z-shaped high-sensitivity optical cell assembly. Determination of the anions in biological samples was also demonstrated.

2. Experimental

2.1. Apparatus

The apparatus used was an ABI Model 270 A-HT capillary electrophoresis system (Applied Biosystems, Foster City, CA, USA) with a 760×0.05 mm I.D. fused-silica capillary, unless stated otherwise. Detection was performed by on-column measurement of the UV absorption at a position 530 mm from the injection end of the

capillary. The performance of a high-sensitivity optical cell assembly (Applied Biosystems), consisting of a fused-silica capillary (1000×0.075 mm I.D.) with an optical cell (Z-cell) placed 780 mm from the injection end, was tested. The path length of the optical cell is specified to 3 mm. Data processing was carried out by use of an IBM-compatible 486 DX, 50 MHz personal computer with Turbochrom 3.3 (PE Nelson, Perkin-Elmer, Beaconsfield, UK).

2.2. Samples and reagents

Sodium nitrite, sodium nitrate, potassium iodide, potassium thiocyanate, dodecyltrimethylammonium bromide (DTAB) and cetyltrimethylammonium bromide (CTAB) were purchased from Sigma (St. Louis, MO, USA). All chemicals were of analytical-reagent grade.

2.3. Procedure

The separation buffer consisted of 50 mM surfactant, 18 mM sodium tetraborate, 30 mM disodium hydrogenphosphate and 10% 2-propanol. The pH was adjusted to 7.0 with 1 M hydrochloric acid. Buffers were filtrated through a $0.20\text{-}\mu\text{m}$ membrane filter prior to use. Washing of the capillary was performed with 1.0 M NaOH for 2 min and with buffer for 5 min before each analysis. Buffers were changed by auto buffer-vial change after varying numbers of analyses. The separation parameters used were: voltage of 20 kV and temperature of 60°C. Detection was performed at 235 nm. Injection by vacuum was performed from the negative end of the capillary for 1 s.

2.4. Calculations

Relative migration times (RMT), normalised peak areas (NA), relative normalised peak areas (RNA) and resolution (R_s) were calculated as described elsewhere [21]. Calculations of RMT and RNA were performed relative to nitrite. The number of theoretical plates (N) was calculated by the Foley–Dorsey approximation (PE Nelson, Perkin-Elmer, Beaconsfield, UK), assuming an

exponentially modified Gaussian distribution as the skewed peak model. The equation for N is

$$N = \frac{41.7(MT/w_{0.1})^2}{B/A + 1.25}$$

where MT is the migration time for the peak, $w_{0.1}$ is the peak width at 10% of peak height, and B/A is an empirical asymmetry ratio.

The linearity of the method was determined from linear regression analysis based on least-squares estimates. Repeatabilities were estimated from the means and relative standard deviations (R.S.D.)

3. Results and discussion

The separation of anions in the positive micellar system tested is based on simple differences in mass/charge ratio combined with variations in ionic interaction between the analytes and the micelles. This MECC system was found to give a more efficient separation of the inorganic anions from various organic anions and impurities in biological samples [20] than free-zone capillary electrophoresis. It was also found to be preferable to systems based on negative micellar systems such as sodium dodecylsulfate (SDS) and cholate [20]. The MECC system was optimised with respect to buffer composition, temperature and voltage by the procedure described for other anions [20,21]. The migration order obtained for anions in a test mixture using the optimised MECC-DTAB system is illustrated in Fig. 1, showing nitrite as the fastest migrating compound followed by nitrate, iodide and finally the thiocyanate ion. The total time of analysis can be held below 15 min, including wash of the capillary, which makes the developed method suitable for screening of large numbers of samples. Both DTAB and CTAB could be used as surfactants; however, generally DTAB was chosen, as it gave the best results for the degradation products of the indolylglucosinolates [15], and because the thiocyanate ion was better separated from the solvent peak.

The performance of the method varied with

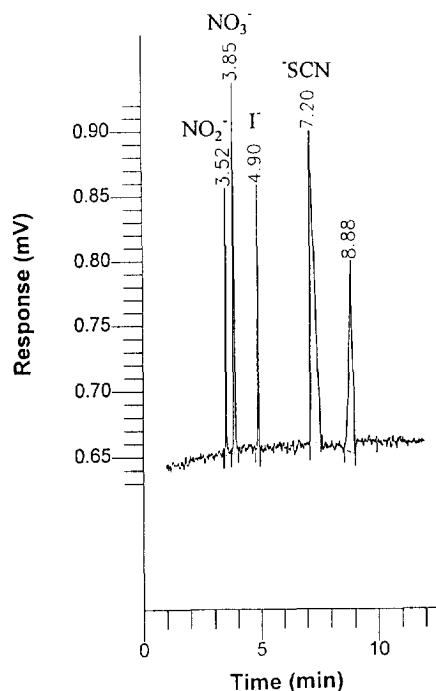


Fig. 1. Migration order of anions in the test mixture analysed in the MECC-DTAB system. Separation conditions were as described in the Experimental section.

the compound of interest, nitrite and iodide having the highest separation efficiency (100 000 and 125 000 N/m , respectively). The thiocyanate ion, giving problems because of its asymmetric peak shape (vide infra), gave a very low N/m . The resolutions for nitrite–nitrate, nitrate–iodide and iodide–thiocyanate ion were found to be 4.3, 12.6 and 9.2, respectively.

3.1. Linearity and detection limits

Linear regression analysis by the least-squares method was performed for the test mixture (100%; $[NO_2^-] = 18.12$ mM, $[NO_3^-] = 139.7$ mM, $[I^-] = 1.19$ mM, $[SCN^-] = 76.54$ mM), using stepwise dilution (down to 0.5%, $n = 18$). The correlation coefficients (r^2) obtained with NA as function of concentration were in general high, being 0.9964 for nitrite, 0.9975 for nitrate and 0.9962 for iodide. The thiocyanate ion showed

an unsatisfactory low correlation coefficient ($r^2 = 0.9308$) when concentrations higher than 38 mM were used, which can probably be explained by the asymmetric peak shape for the thiocyanate ion at high concentrations ($> 30\% = 22.96$ mM). Calculation of the correlation coefficient for the dilution series 0.5–30% improved the r^2 value to 0.9748.

The detection limits (signal-to-noise ratio of 2:1) were determined from the results from the linearity study, using the standard conditions of a 1-s sample injection into a 760-mm long capillary with an internal diameter of 50 μ m. Under the stated conditions, nitrite, nitrate and iodide could be detected at 0.27, 0.9 and 0.02 mM levels, respectively, whereas the detection limit for the thiocyanate ion was about 0.15 mM (further dilution of the 0.5% test mixture). With an injection volume calculated to be 4.28 nl [22,23], this corresponds to detection limits for nitrite, nitrate and the thiocyanate ion of ca. 1.2, 3.9 and 0.6 pmol, respectively, whereas the detection limit for iodide was about a factor 1000 lower (0.9 fmol). The detection limits in weight amounts were 80 pg, 327 pg, 143 fg and 58 pg for nitrite, nitrate, iodide and the thiocyanate ion, respectively. It was possible to improve the detection limits considerably by use of a high-sensitivity optical cell assembly (Z-cell). The sensitivity, based on NA found for the various anions, was thus increased by a factor of 18, changing the 50- μ m capillary to the 75- μ m capillary with a Z-cell. Compared to the 50- μ m capillary, an ordinary 75- μ m capillary improved sensitivity about 2-fold; however, this increase in response is due to an increased injection volume and does not affect the molar concentration detection limit. Theoretically, the increase in path length, obtained using the high-sensitivity optical cell assembly, should change the NA values found by a factor of 40 compared to the NA values found for the 75- μ m capillary. This was, however, not the case. The reason for this is explainable from the concave/convex shape of the detection windows and for the Z-cell with a circular part included. However, the changed detection system had no negative effects on the resolution of the ions.

3.2. Repeatability

Repeatability of the MECC method for anions was tested, changing the buffer at the inlet side between each analysis and at the outlet side after five analyses. In general, repeatability of the migration time (absolute as well as relative) was very satisfactory, whereas NA and RNA varied between consecutive analyses, leading to high relative standard deviations (Table 1). This was especially pronounced for the thiocyanate ion, which showed a tendency for asymmetric peaks even for the diluted test mixture (30%; $[\text{SCN}^-] = 22.96$ mM) used in the repeatability study. Further optimisation of the MECC method with respect to peak profile for the thiocyanate ion should be performed prior to approaching quantitative analysis of this anion.

3.3. Anions in biological samples

The detection of anions in biological samples is rendered difficult by the high concentrations of interfering compounds present, and some degree of isolation/purification is generally required. We used a fast and simple ion-exchange technique for isolating the anions in blood and milk. A 400- μ l volume of a sample (200 μ l plasma/milk and 200 μ l 100% test mixture) was transferred to a 1-ml pipette tip with 0.5 ml Sephadex A-25 (AcO^-), and washed four times with 1 ml water. Here 4×4 ml of 2 M NH_4OH was used as

Table 1
Repeatability of the MECC method for determination of nitrite, nitrate, iodide and the thiocyanate ion

Anion	Relative standard deviation (%) ^a			
	MT	RMT ^b	NA	RNA ^b
Nitrite	0.24	–	2.37	–
Nitrate	0.29	0.14	1.75	3.27
Iodide	0.26	0.07	2.18	2.85
Thiocyanate ion	0.26	0.16	13.50	13.91

^a $n = 9$.

^b Relative to nitrite.

Separation conditions were as described in the Experimental section.

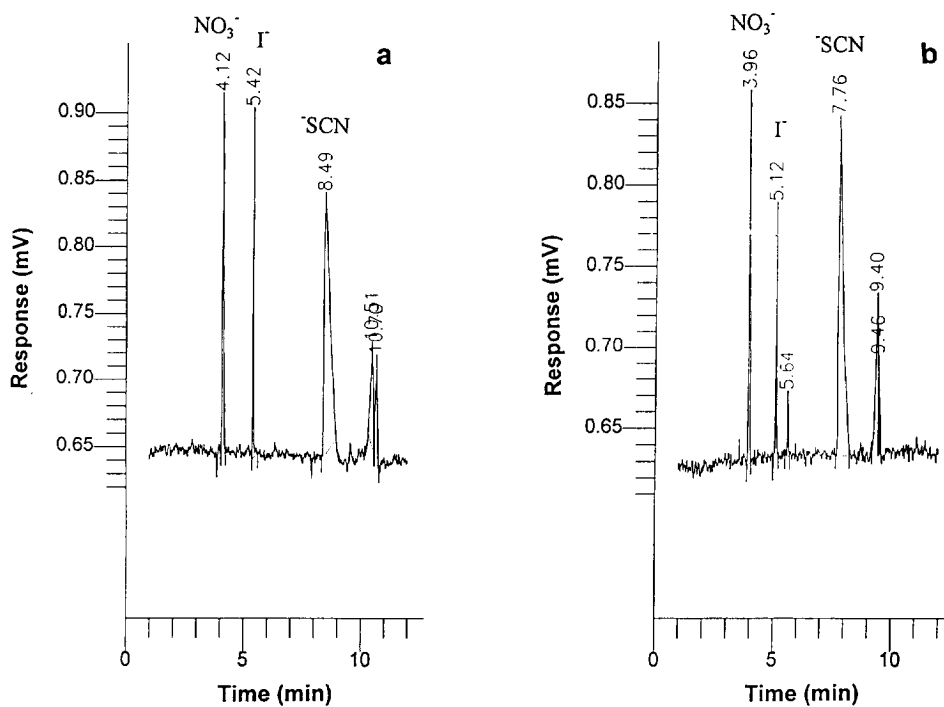


Fig. 2. Examples of detection of anions in (a) blood plasma and (b) milk. The samples were prepared as described in the Results and discussion section. Separation conditions were as described in the Experimental section.

eluent, and the 16 ml obtained was evaporated to dryness, redissolved in 600 μl water, and then analysed by MECC. An example of the determination of anions in blood and milk is given in Fig. 2. As seen, the purification method used was successful, eliminating most of the interfering compounds.

4. Conclusion

MECC based on DTAB has been developed for the determination of nitrite, nitrate, iodide and the thiocyanate ion. The four anions separated well within 10 min, with good repeatability as well as linearity of the method. Analysis of the anions in biological samples such as blood and milk was rendered possible with a very simple ion-exchange procedure, separating the compounds of interest from interfering substances such as proteins, etc. Moreover, the high-sensitivity optical cell assembly increased

the sensitivity of the method considerably, resulting in improved detection of anions at low concentration levels.

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