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Short communication

Determination of thiodiglycolic acid in urine by capillary electrophoresis

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Abstract

A sensitive capillary electrophoretic method was developed for the determination of thiodiglycolic acid (TDA) in urine which avoids the pretreatment of the urine sample. Several carrier electrolytes were examined. The most suitable carrier electrolyte system consisted of potassium hydrogen phthalate (5 m*M*), 2-(*N*-morpholino)ethanesulfonic acid (50 m*M*) and tetradecyltrimethylammonium bromide (0.5 m*M*), pH 5.2. Ten times diluted fresh midstream void urine was used for the determination. In this way, the concentrations of TDA between 5 and 50 mg/l in undiluted urine samples can be determined.

Keywords: Vinyl chloride; Thiodiglycolic acid

1. Introduction

Thiodiglycolic acid (TDA) is considered to be the most important metabolite of vinyl chloride in the human body. Vinyl chloride monomer (VCM) is the precursor of the widely used plastic, polyvinyl chloride (PVC). Owing to its polar properties, TDA is excreted in the urine. Monitoring of the urine TDA levels is of great importance in places with increased concentrations of VCM in air, i.e., mainly inside and outside PVC producing factories. The risk of exposure to VCM is due to its carcinogenic effects. Several methods have been developed for the determination of TDA in urine which make use of either gas chromatographic [1–5] or isotachophoretic [6] techniques. However, all separation methods, except the latter, require pretreatment of the urine samples.

Determination of the metabolites of xenobiotics in urine represents a difficult analytical task, because the metabolite concentrations are usually considerably lower than those of other urine components, e.g., various inorganic anions. Concentrations of TDA in the urine of exposed people have been found between 5 and 50 mg/l, while the undiluted urine contains about 6000 mg/l of chlorides, 1200 mg/l of phosphates and 1800 mg/l of sulfates [7]. Capillary electrophoresis (CE) has turned out to be a very useful method for the determination of many substances in complex mixtures, such as human serum

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[8], urine [7] and beverages [9]. It is extremely efficient in separating small organic molecules including organic acids, which are very often the metabolites of the toxic substances. The aim of this work has been to develop a sensitive CE method for the determination of TDA, while avoiding the pretreatment of the urine sample.

2. Experimental

2.1. Chemicals

All solutions (carrier electrolyte and standards) were prepared by using 18-MΩ water from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Analytical reagent-grade chemicals, potassium hydrogenphthalate, sodium tetraborate, and potassium chromate, MES (2-(*N*-morpholino)ethanesulfonic acid) and the electroosmotic flow modifier TTAB (tetradecyltrimethylammonium bromide), all purchased from Sigma (St. Louis, MO, USA), were used for the preparation of the carrier electrolytes. The final concentrations of the carrier electrolytes are given in Section 3.

2.2. Instrumentation

Two CE instruments were employed for the determination of thiodiglycolic acid, i.e., a SpectraPhoresis 1000 (Thermo Quest, San Jose, CA, USA) equipped with PC 1000 System Software, and a Unicam CE (ATI Unicam, Boston, MA, USA) equipped with 4880 Software. When using the former instrument, samples were introduced in the hydrodynamic injection mode set to a pressure of 100 mbar with an injection time selected between 15 and 30 s. Separations took place in an untreated fused-silica capillary, 70 cm×75 μm I.D. The measurements were carried out in the anionic mode. When using the latter instrument, the experimental conditions were similar, with some modifications comprising a constant injection time of 18 s, an injection pressure between 10 and 30 mbar, and a constant applied voltage of 20 kV. All measurements were carried out at a temperature of 25°C.

3. Results and discussion

3.1. Optimalization of conditions for the determination of TDA

Three running buffer systems were examined: 20 m*M* sodium tetraborate buffer with 1 m*M* TTAB (pH 9.2), 5 m*M* potassium chromate with 0.5 m*M* TTAB (pH 6.0), and 2.5–20 m*M* potassium phthalate with 0.25–0.5 m*M* TTAB (pH 5.2). In all these systems, TDA can be determined by using the direct or indirect UV detection. Calibration graphs were measured for aqueous samples containing TDA at concentrations expected in urine. Then the possibility of interference by phosphates, sulfates and chlorides was investigated for aqueous samples containing TDA and the interfering anion in excess.

The crystal CE system was used to find the proper conditions for the TDA determination in the tetraborate and chromate running buffers, with the direct detection at 190 nm and the indirect detection at 254 nm, respectively. The linear calibration graphs were obtained in the concentration range from 1 to 25 mg/l. A relative standard deviation (R.S.D.) of 3.5% was calculated for the peak area and of 2% for peak area over migration time. The concentrations of phosphates, sulfates or chlorides added to the aqueous sample were 100 mg/l. In both buffer systems, phosphates could not be separated from thiodiglycolic acid.

The phthalate running buffer was examined on the SpectraPhoresis 1000 system. While the indirect UV detection of TDA at 230 nm was not very sensitive, the indirect UV detection based on the strong phthalate absorption at 205 nm provided satisfactory results [10]. By varying concentrations of phthalate and TTAB, the optimum composition of the system was found to be 5 m*M* potassium phthalate and 0.5 m*M* TTAB (pH 5.2). This was further modified by adding 50 m*M* MES buffer, which was expected to ensure a better pH control, a more stable baseline and a symmetrical peak shape [9]. In order to decrease the noise and to increase the sensitivity, the applied voltage was decreased from 20 kV (current about 20 μA) to 5 kV (current about 6 μA). An injection time of 30 s was found to provide the best results. The calibration graph for the TDA peak area y including six data points was linear in the range of

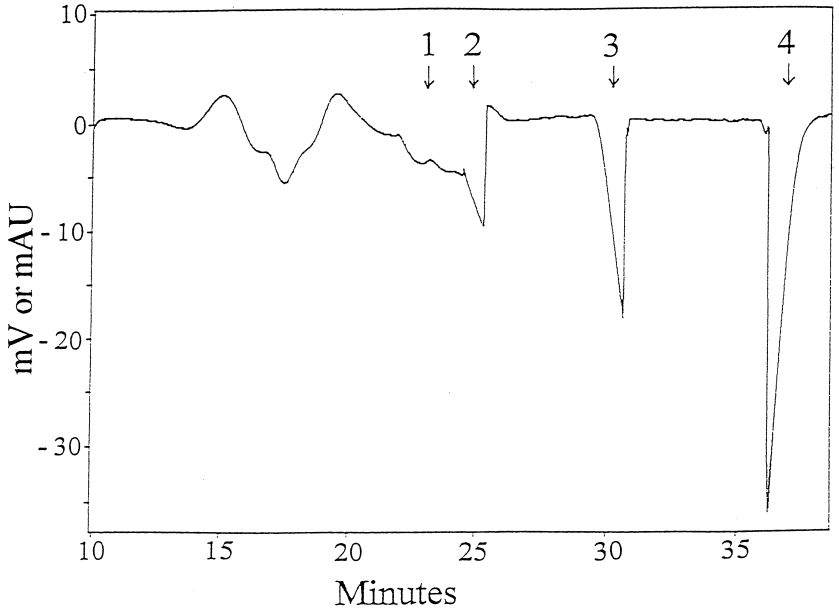

Fig. 1. Electropherogram of the aqueous samples containing 25 mg/l chloride (1), sulfate (2), thiodiglycolate (3) or phosphate (4). Buffer: 5 m*M* potassium phthalate+0.5 m*M* TTAB and 50 m*M* MES. Hydrodynamic injection, 100 mbar for 30 s. Capillary (effective length 62 cm) 70 cm×75 μm I.D. Applied voltage, −5 kV. Indirect detection at 205 nm.

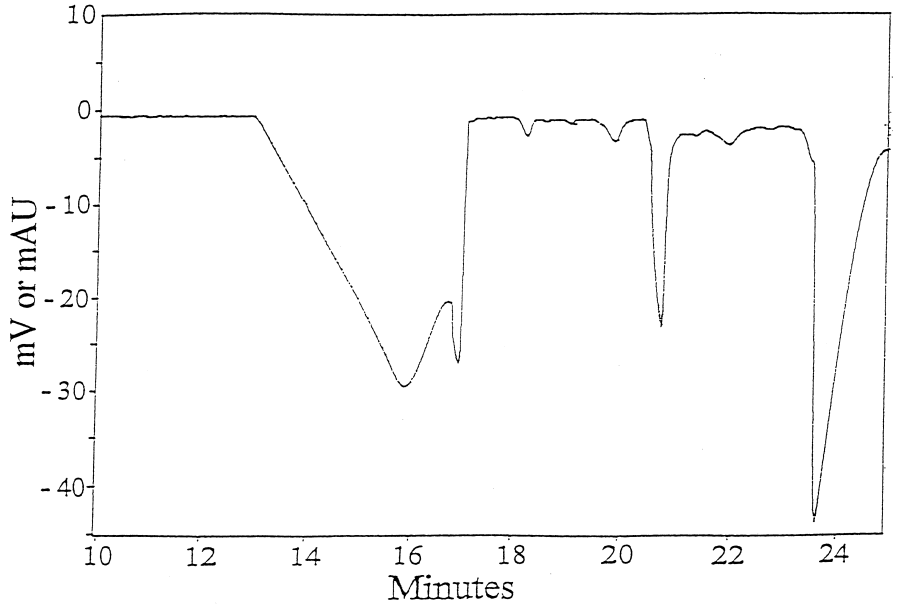

Fig. 2. Electropherogram of a 10-fold diluted urine sample. Experimental conditions the same as for Fig. 1.

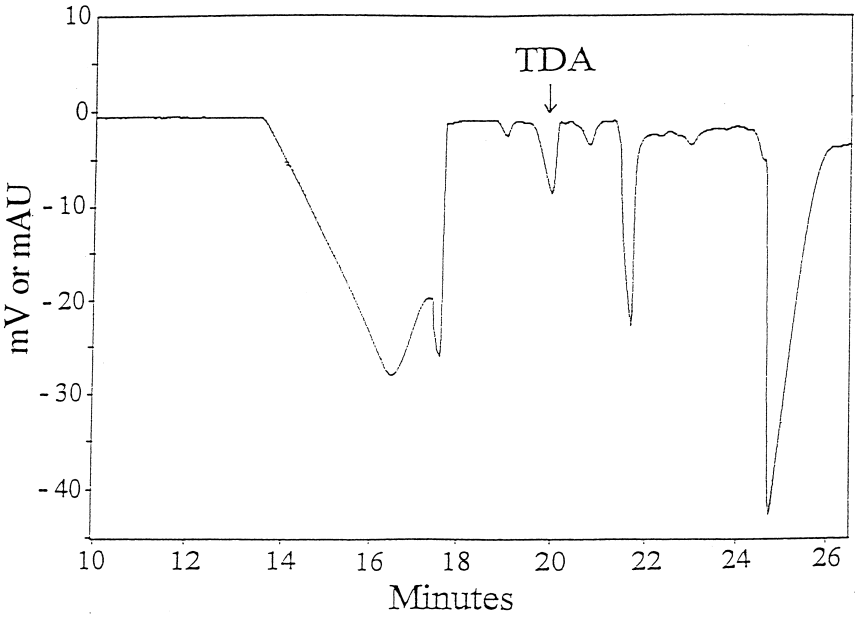

Fig. 3. Electropherogram of a 10-fold diluted urine sample after addition of TDA to a concentration of 5 mg/l. Experimental conditions the same as for Fig. 1.

concentrations c from 0.5 to 5.0 mg/l. Data fit to the regression line $y=-702.1+22022c$ with a correlation coefficient of 0.998, and standard deviation (S.D.) of 2568.3. The electropherogram of the aqueous solution containing TDA, phosphate, sulfate and chloride anions, all at a concentration of 25 mg/l, is shown in Fig. 1. This indicates that phosphates, sulfates or chlorides do not interfere with the TDA determination.

3.2. Determination of TDA in urine

Concentrations of TDA expected in urine of exposed persons are in the range 5–50 mg/l. However, owing to the high concentrations of inorganic anions present and to the technical problems associated with column blocking, the analysis of the undiluted urine is practically impossible. Therefore, it was necessary to increase the sensitivity of the TDA determination, so as to allow the dilution of the urine samples. By following the procedure described above, we were able to determine 0.5–5 mg/l TDA in 10-fold diluted urine, which corresponds to the expected TDA concentration range.

The relevant amount of TDA was added to the fresh midstream void urine samples after their dilution. Electropherograms of the diluted urine in the absence and presence of TDA are shown in Fig. 2 and Fig. 3, respectively. No interference with other species present in urine was observed. Fig. 4 illustrates the change in the peak of TDA when its concentration is varied. The calibration graph for the TDA peak area y versus the TDA concentration c (0.5–5.0 mg/l) including six data points was linear. Data fit to the regression line $y=-971+26681c$, with a correlation coefficient of 0.999 and S.D. of 2393.9.

Table 1 shows the measured peak area and the corresponding TDA concentrations together with

Table 1
Measured peak area and the corresponding TDA concentrations together with calculated y values from the regression

TDA concentration (mg/l)	Peak area	y
0.5	13 605	12 370
1.0	21 184	25 710
2.0	53 896	52 391
3.0	80 257	79 072
4.0	106 561	105 753
5.0	131 260	132 434

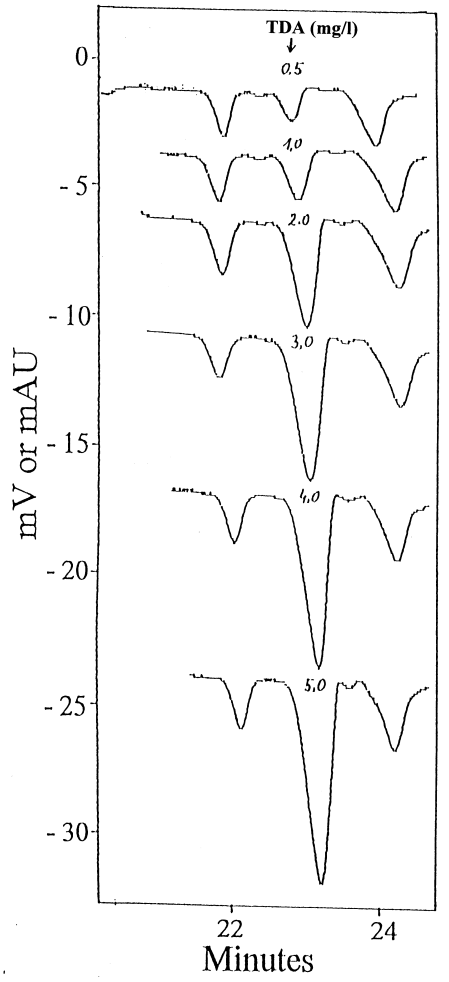

Fig. 4. Electropherograms of the 10-fold diluted urine sample containing TDA at concentrations of 0.5, 1.0, 2.0, 3.0, 4.0 or 5.0 mg/l. Experimental conditions the same as for Fig. 1.

calculated y values from the regression. The reproducibility was estimated from six independent determinations of 2 mg/l TDA. The R.S.D. value of the peak area was found to be 6.5%, and that of the peak area over the migration time was found to be 8.2%.

4. Conclusions

CE can be used for the determination of 0.5–5 mg/l thiodiglycolic acid in 10-fold diluted urine samples without the necessity of their pretreatment. This allows to monitor the exposure of people to vinylchloride, the main metabolite of which is thiodiglycolic acid, which is excreted by urine at concentrations typically between 5 and 50 mg/l. High concentrations of chlorides, phosphates and sulfates in urine do not interfere the determination.

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