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Isotachophoretic Analysis of Mercaptoundecahydrododecaborate Anion in Human Serum and Urine

ISAO IKEUCHI* and TAMEYUKI AMANO

Shionogi Research Laboratory, Shionogi & Co., Ltd., Sagisu 5–12–4, Fukushima-ku, Osaka 553, Japan

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An isotachophoretic assay method was developed for sodium mercaptoundecahydrododecaborate (**1**), $Na_2{}^{10}B_{12}H_{11}SH$, which is used for boron neutron-capture therapy. The method is simple, specific and suitable for the determination of **1** as an anion in human serum and urine.

Compound **1** was separated from serum and urine constituents with 0.01 M hydrochloric acid and β-alanine (leading electrolyte, pH 3.70) and 0.01 M *n*-caproic acid (terminating electrolyte). The recoveries of **1** from serum and urine were about 85 and 101%, respectively. The detection limit was 7×10^{-7} mol/ml in serum and urine. The procedure could also detect the oxidation product, sodium di(thioundecahydrododecaborate), at 5×10^{-7} mol/ml or more in urine.

The present method was used to determine the levels of **1** in urine samples of patients with malignant brain tumor being treated by boron neutron-capture therapy.

Keywords——isotachophoresis; sodium mercaptoundecahydrododecaborate; boron neutron-capture therapy; patients' urine; sodium di(thioundecahydrododecaborate)

Boron neutron-capture therapy of malignant brain tumors has recently been developing rapidly and has attracted the attention of many investigators. At present, sodium mercaptoundecahydrododecaborate (**1**), $Na_2{}^{10}B_{12}H_{11}SH$, is the best agent for boron neutron-capture therapy, which requires the selective accumulation of ^{10}B at high concentration in the malignant tissue.[1] In a previous paper,[2] we reported a colorimetric method with curcumine for determining boron levels in biological samples.

In this paper, we describe a simple and specific isotachophoretic method for the determination of **1** in human serum and urine.

Experimental

Apparatus and Conditions——Isotachophoretic analysis was performed with a Shimadzu IP-1B isotachophoretic analyzer equipped with a PG-1 potential detector. The separation was run in a Teflon capillary tube (length 20 or 40 cm, i.d. 0.5 mm) maintained at 20 °C. The driving current was stabilized at 100 μA. Chart speed was 20 mm/min. The leading electrolyte was 0.01 M HCl adjusted to pH 3.70 by adding β-alanine; 1.5% Triton X-100 was also added to prevent diffusion of the electrophoretic zone. The terminating electrolyte was 0.01 M *n*-caproic acid.

Materials——Compound **1** is highly so hygroscopic and also liable to be transformed to its disulfide in air. Therefore, nonhygroscopic cesium mercaptoundecahydrododecaborate monohydrate (**2**), $Cs_2B_{12}H_{11}SH \cdot H_2O$, and cesium di(thioundecahydrododecaborate) (**3**), $Cs_4B_{24}H_{22}S_2$, were used instead of **1** and its disulfide as standard samples, respectively. Other chemicals were of reagent grade.

As human serum, Hyland Control Serum (Travenol Laboratories) and Moni-trol (American Hospital Supply Co.) were used. The urine samples from patients with malignant brain tumor were provided by Teikyo University Hospital.

Standard Solutions——A series of standard solutions for obtaining calibration curves were prepared by dissolving about 15 mg of **2** or **3** in 10 ml of distilled water, followed by dilution to obtain the desired concentrations. These standard solutions of **2** and **3** were freshly prepared each time.

Procedure——A 0.1-ml serum sample in a 2-ml centrifuge tube was mixed well with 0.2 ml of ethanol. After centrifugation of the mixture for 5 min, 5 μl of the supernatant was introduced into the apparatus.

A urine sample was diluted ten-fold with distilled water and 10 μl of the sample solution was directly introduced into the apparatus.

Results and Discussion

Compound **2** is easily converted in alkaline solution to its disulfide **3**. Therefore, the analytical conditions for the determination of **2** were examined with respect to potential unit (*PU*) value[3] using 0.01 M hydrochloric acid and β-alanine (pH 3.0—4.2), 0.01 M hydrochloric acid and histidine (pH 5.6) and 0.01 M hydrochloric acid and ammediol (pH 6.8) as leading electrolytes and 0.01 M *n*-caproic acid and 0.01 M glutamic acid as terminating electrolytes. A combination of 0.01 M hydrochloric acid and β-alanine (pH 3.70) and 0.01 M *n*-caproic acid was the most appropriate for detecting **2** and **3** without interference from serum and urine constituents based on comparison of the *PU* value. Under the analytical conditions, compound **2** was distinctly separated from **3** and the *PU* value was determined to be 0.09 for **2** and 0.05 for **3**. Addition of Triton X-100 improved the sharpness of the zone boundary between **2** and the serum or urine constituents.

The standard curves for **2** and **3** between the zone length and the injection amount were linear in the ranges of 2.5—48×10^{-9} and 2.5—20×10^{-9} mol, respectively. As no oxidized anions from **2** were formed in standard solutions, we concluded that oxidation of **2** did not occur during analysis.

Ethanol was selected as a deproteinizing agent for serum samples because it was easy to handle and did not interfere with the detection of **2** under the analytical conditions described above (Fig. 1). The recovery of **2** which had been added to human serum was about 85% in the range of 1.0 to 10.1×10^{-6} mol/ml upon addition of two volumes of ethanol. About 15% of the added **2** may have bound to the serum protein. It was possible to detect 7×10^{-7} mol/ml of **2** in the serum.

On the other hand, compound **3** was scarcely recovered from the serum deproteinized with ethanol. This result probably reflects the occurrence of coprecipitation with the serum protein.

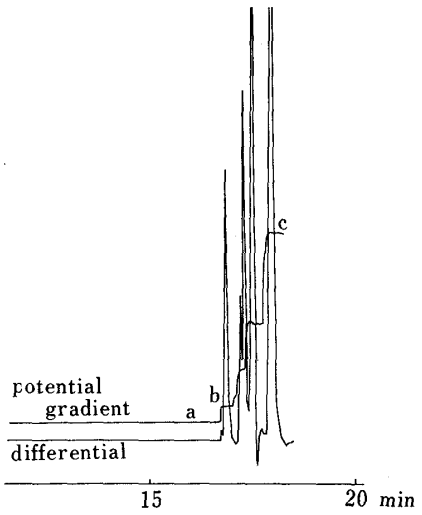

Fig. 1. Isotachophoretic Separation of **2** in Human Serum

A serum containing 4.03×10^{-6} mol/ml of **2** was treated as described in the text.
a, Cl^-; b, $B_{12}H_{11}SH^{2-}$ (**2**, *PU* value 0.09); c, *n*-caproic acid.

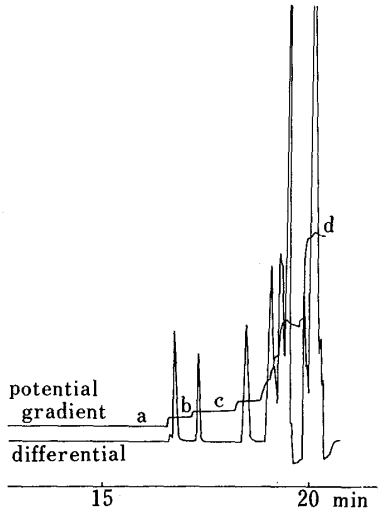

Fig. 2. Isotachophoretic Separation of **2** and **3** in Human Urine

Urine containing 1.83×10^{-5} mol/ml of **2** and 6.16×10^{-6} mol/ml of **3** was treated as described in the text.
a, Cl^-; b, $(B_{12}H_{11}S)_2^{4-}$ (**3**, *PU* value 0.05); c, $B_{12}H_{11}SH^{2-}$ (**2**, *PU* value 0.09); d, *n*-caproic acid.

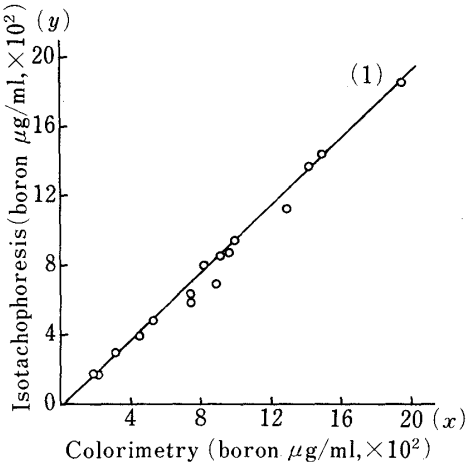

Fig. 3. Correlation of Boron Levels in Urine Samples of Patients Determined by the Colorimetric and the Present Methods

For equation (1): $y=0.962x-41.4$, $s=52.7$ $c.v.=6.64\%$, $n=16$.

Figure 2 shows a typical isotachopherogram for the separation of **2** and **3** in human urine. Compounds **2** and **3** were separated completely from each other and were well resolved from urine constituents under the analytical conditions.

Regression analysis for the determination of **2** in human urine was examined in the concentration range of 1.5×10^{-6} to 3.1×10^{-5} mol/ml using mixed urine samples in which **3** was present in 0.3- to 10-fold molar excess over **2**. The relationship between the added (x) and found (y) values of **2** was linear; $y=1.013x-0.17$, standard deviation (s) $=0.28$, coefficient of variation ($c.v.$) $=1.79\%$, $n=16$. The regression equation indicated that the present method is accurate to within about 2% coefficient of variation.

In addition, regression analysis for the determination of **3** detected simultaneously was examined in the concentration range of 1.6×10^{-5} to 7.7×10^{-7} mol/ml. The relationship between the added (x) and found (y) values of **3** was also linear; $y=1.009x-0.04$, $s=0.25$, $c.v.=3.16\%$, $n=16$. The regression equation indicates that the present method could determine **3** with about 3.2% coefficient of variation.

These results confirmed that the present method could be used for simultaneous determination of **2** and **3** in urine. The detection limit was 7×10^{-7} mol/ml for **2** and 5×10^{-7} mol/ml for **3**.

The present method was applied to the determination of **1** in urine samples from patients. Compound **1** was administered to patients with malignant brain tumor at 50 mg of boron per kg of body weight by intra-arterial infusion. Urine samples from five patients were analyzed by the present method and the colorimetric method using curcumine.[2] Compound **1** was clearly detected in all urine samples collected within 17 h after administration while the disulfide was scarcely detectable. The urinary concentrations of **1** ranged from 1.41×10^{-6} to 1.43×10^{-5} mol/ml (183—1855 μg B/ml). The values found by the present method were compared with those from the colorimetric method, and as shown in Fig. 3, a close correlation was found (correlation coefficient, $r=0.9942$).

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