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# A Colorimetric Determination of Boron in Biological Materials

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To measure sodium mercaptoundecahydrododecaborate (I), $Na_2B_{12}H_{11}SH$, used for boron neutron-capture therapy, a colorimetric method with curcumine for determining boron in biological materials was developed.

First, biological samples such as the tumor, surrounding tissues, and blood plasma were ashed gently using the radiofrequency combustion system with excited oxygen plasma. Urine samples were not ashed but usually required dilution with water. After oxidative degradation with potassium permanganate, boron in the degradation products was extracted with chloroform containing 2-ethyl-1,3-hexanediol. Boron in the chloroform extract was converted into the boron-curcumine complex by adding an acetic acid solution of curcumine, followed by concentrated sulfuric acid. After dilution with 95% (v/v) ethanol, the absorbancy of the solution at 554 nm based on the complex was measured by spectrophotometry. The linear dynamic range for the assay was from 0.5 to 20 μg of boron per sample solution.

Compared with the 1,1′-dianthrimide method the present method is simple and safe, and is sensitive enough to be useful for the determination of boron in biological materials.

**Keywords**——colorimetry; biological materials; mercaptoundecahydrododecaborate; boron-curcumine complex; boron neutron-capture therapy

A markedly successful clinical use on brain tumor has suggested the practical application of boron neutron-capture therapy using sodium mercaptoundecahydrododecaborate (I), $Na_2B_{12}H_{11}SH$, in the near future. I is the only drug used in practice for boron neutron-capture therapy,[2] which is based on the destruction of tumor cells by α-particles with high energy produced by nuclear reaction of $^{10}B$ selectively incorporated into the malignant tissue. In basic studies on boron neutron-capture therapy, boron uptake by the tumor and its distribution in the tissue must be determined. Several methods for measuring boron in biological materials have been described. A colorimetric method with 1,1′-dianthrimide for the determination of boron in tissue has been established,[3] but it uses dangerous 90% hydroperoxide and is tedious. Mair and Day[4] reported a sensitive method for the determination of boron with curcumine. An α-radioautographic method such as physicochemical assay for the evaluation of boron uptake by a tumor, has also been reported.[5]

We have developed a sensitive and accurate method for measurement of boron based on I in biological materials. This report describes a modified curcumine method which is simple, safe, and sensitive enough to be useful for determining boron levels in biological samples.

## Experimental

**Apparatus**——Combustion of biological materials was made with a Yanagimoto LT-2S low-temperature asher.

Spectrophotometric measurements were made using a Hitachi 323 spectrophotometer with a 1 cm quartz cell.

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[1] Location: *Sagisu, Fukushima-ku, Osaka 553, Japan.*
[2] H. Hatanaka and K. Sano, *Z. Neurol.*, 204, 309 (1973).
[3] J.R. Messer and C.E. Peirce, *Anal. Chem.*, 43, 271 (1971).
[4] J.W. Mair and H.G. Day, *Anal. Chem.*, 44, 2015 (1972).
[5] K. Amano and W.H. Sweet, *Nippon Igaku Hoshasen Gakkai Zasshi*, 33, 268 (1973).

The quartz boats (6×25×4 mm), quartz tubes (diameter, outside 10 mm, inside 8 mm; length 150 mm), and a quartz air-cooler (diameter, outside 6 mm, inside 4.5 mm; length 220 mm) were used for the oxidative reaction, extraction, and complex formation, respectively. They effectively prevented contamination when used with 50 ml polypropyrene volumetric flasks.

**Materials**——Chemicals: Sodium mercaptoundecahydrododecaborate (I), $Na_2B_{12}H_{11}SH$, is so hygroscopic that it absorbs water during weighing and is also liable to produce its disulfide in air. Thus, nonhygroscopic cesium mercaptoundecahydrododecaborate monohydrate (II), $Cs_2B_{12}H_{11}SH \cdot H_2O$, and cesium bis-(tioundecahydrododecaborate) (III), $Cs_4B_{24}H_{22}S_2$, were used instead of I and its disulfide.

A stock solution of boron was prepared by dissolving 38 mg of analytical grade boric acid in 100 ml of distilled water. Further dilutions were made to obtain standard solutions containing 0.3—13.3 μg of boron per ml.

Curcumine was recrystallized once from EtOH and dissolved in glacial AcOH [0.375% (w/v)]. 2-Ethyl-1,3-hexanediol in $CHCl_3$, 10% (v/v), was used. All other chemicals were of analytical grade.

Biological Materials: Brain (wet weight, 0.4 g), muscle (wet wt., 0.2—0.3 g), and blood plasma (pooled, 0.1 ml) were obtained from adult C57BL/6 mice. Urine was obtained from healthy human. These materials were frozen until use. Blood plasma and urine were centrifuged at room temperature and the supernatants were used.

**Procedure**——Sample Preparation: The sample, such as the brain, muscle, blood plasma, malignant tissues, and other biological materials, was placed on a quartz boat and allowed to stand overnight in an evacuated desiccator containing $P_2O_5$. The boat with the dried sample was inserted into a chamber for radiofrequency combustion and ashed for 17 hr at 50 W, with $O_2$ flow of 5 ml/min and a pressure of 3—4 Torr. With the plasma-ashing system, 10—12 samples can be ashed easily at the same time. After the combustion, the ash was transferred to a quartz tube together with the boat, and 1.5 ml of 2% (w/v) aqueous solution of $KMnO_4$ was added and the quartz air-cooler was set up. Oxidative degradation of polyhedral boranes with permanganate was performed by heating for 1 hr at about 95° in a water bath. After cooling to room temperature, 2.0 ml of 10% (v/v) 2-ethyl-1,3-hexanediol in $CHCl_3$ was added. The material in the tube was shaken for 3 min and then centrifuged for 2 min. The aqueous phase containing precipitated $MnO_2$ was removed by aspiration.

For the urine, a 0.5 ml sample in a quartz tube was mixed well with 1.0 ml of 3% (w/v) aqueous solution of $KMnO_4$. All subsequent operations for oxidative degradation and extraction with $CHCl_3$ containing 2-ethyl-1,3-hexanediol were the same as above. If the boron concentration in urine sample was too high, the sample was diluted with water to keep boron not more than about 40 μg/ml.

Assay by Colorimetry: To 0.5 ml of $CHCl_3$ extract in a 50 ml polypropyrene volumetric flask, 1.0 ml of AcOH solution of curcumine and 0.3 ml of $H_2SO_4$ were added. The mixture was allowed to stand for 15 min at room temperature, then made up to 50 ml with 95% (v/v) EtOH. The color of this solution ranging from orange-yellow to red, shows the amount of boron present. The absorbancy at 554 nm was read *vs.* a blank solution which had been treated similarly.

The boron content in the sample solution was found from the calibration curve. The boron content in the tissue and urine was calculated using the following equations.

For the tumor, muscle, brain, blood plasma, and other biological materials: Boron (μg/g or ml) = [boron found (μg) × factor (1000/869)]/sample size (g or ml). For the urine sample: Boron (μg/ml) = [boron found (μg) × factor (1000/945)] × 2 × dilution ratio.

## Results and Discussion

Boron in aqueous solution reacts with 2-ethyl-1,3-hexanediol to form a weak complex which is extractable with chloroform.[6] Furthermore, boron in chloroform is converted into a highly absorbing complex, rosocyanine, upon addition of acetic acid solution of curcumine followed by concentrated sulfuric acid as described by Mair and Day.[4] These characteristic reactions were used in our method.

Under our assay conditions, almost quantitative recovery of boron was possible by extraction with 10% 2-ethyl-1,3-hexanediol-chloroform. The boron-curcumine complex showed an absorption peak at 554 nm in the visible region and was stable for at least 1 hr. The calibration curve was linear from 0.5 to 20 μg of boron in the standard solutions.

Boron recovery in the oxidative degradation with permanganate under the conditions described is shown in Table I. To evaluate the boron recovery, boric acid was run under the same conditions as II and III. In the concentration range of 0.5 to 20 μg of boron, the re-

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[6] E.J. Agazzi, *Anal. Chem.*, 39, 233 (1967); B. Egneus and L. Uppström, *Anal. Chim. Acta*, 66, 211 (1973).

covery from II and III was calculated to be 95.0% and 93.8%, respectively. These values were estimated to be equal to those of boric acid, as can be seen in Table I. The good agreement of boron recovery between II and III, between polyhedral boranes and boric acid, and between the reaction temperatures for boric acid indicated that the oxidative reaction of II and III with permanganate had occurred perfectly. Loss of boron during the oxidative reaction, corresponding to about 5% of the boron recovered in each compound, was probably due to adsorption of boron into the manganese dioxide precipitate formed from permanganate. At concentrations higher than 20 μg of boron, recovery from II and III agreed well, although the values decreased with increasing concentration. It is assumed that the lower boron recovery was due to insufficiency of permanganate in the reaction mixture.

Table I. Oxidative Degradation of II and III with Permanganate[a]

| Boron in 0.5 ml of test solution (μg) | Recovery of boron | | | | | |
|---|---|---|---|---|---|---|
| | Boric acid | | II | | III | |
| | (%) | (*n*) | (%) | (*n*) | (%) | (*n*) |
| 0.5—20 | 94.5 | 7 | 95.0 | 5 | 93.8 | 5 |
| | 95.3[b] | 7 | — | | — | |
| 50 | — | | 86.0 | 1 | 87.4 | 1 |
| 100 | — | | 69.6 | 1 | 72.2 | 1 |

Reaction conditions: *a*) 95°, 1 hr; *b*) room temp., 1 hr.

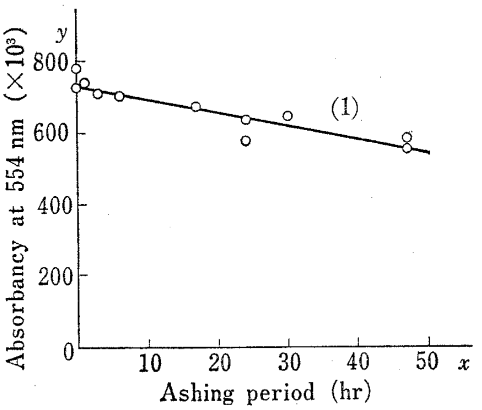

Fig. 1. Effect of Ashing Time on II

II corresponding to 9.51 μg of boron was treated by the present procedure with varying ashing period. For equation (1): $y=-3.896x+735$, $s=31.0$, $c.v.=4.66\%$, $n=11$.

As an ashing method for biological materials, the radiofrequency combustion system with excited oxygen plasma was used. Biological materials such as the brain, muscle, and blood plasma could be ashed completely with this simple and relatively low-temperature system. Wet ashing method using hot inorganic acid and dry ashing method using high-temperature furnaces are dangerous and troublesome.

Fig. 1 shows the effect of varying the ashing period on II under the conditions of our method. Absorbancy at 554 nm based on the boron-curcumine complex decreased with ashing time and this relationship is represented by Eq. (1). From Eq. (1), degree of the loss of boron in II due to ashing period was calculated to be 0.5%/hr. Therefore, recovery of boron from the radiofrequency combustion process in this method was estimated to be 91.5%.

From these results, total boron recovery based on II and III in the sample requiring oxygen plasma ashing was evaluated to be 86.9%. In the case of urine sample, which does not require oxygen plasma ashing, total recovery of boron was estimated to be 94.5%.

Table II shows the overall recovery of boron in mixed samples. Each mixed sample of the brain, muscle, or blood plasma was prepared by adding II or III in 0.5 to 20 μg of boron per sample. Urine samples contained II 1 to 10000 μg/ml of boron. Total recovery of boron based on II or III in the brain, muscle, and blood plasma was in the range of 81.7 to 88.2%, and the average value was calculated to be 85.1%. These values agree well with the expected value of 86.9% within an experimental error. In urine samples, total recovery of boron was estimated to be 94.3% and evaluated to be equal to 94.5% of the expected value. Such a good agreement between expected and experimental values suggested no interaction of metallic and other components in the ash and the urine.

Table II. Total Recovery of Boron Based on II and III

| Mixed sample | Total recovery Found (%) | (*c.v.*) | (*n*) | Expected (%) |
|---|---|---|---|---|
| (1) Brain (C57BL/6 mice)+II | 84.7 | 1.9 | 7 | 86.9 |
| (2) Brain (C57BL/6 mice)+III | 85.6 | 12.2 | 7 | |
| (3) Muscle (C57BL/6 mice)+II | 88.2 | 2.4 | 7 | |
| (4) Blood plasma (C57BL/6 mice)+II | 81.7 | 7.2 | 7 | |
| av. | 85.1 | | | |
| (5) Urine (human)+II | 94.0 | 2.7 | 9 | 94.5 |
| (6) Urine (human)+II | 94.5 | 1.6 | 9 | |
| av. | 94.3 | | | |

The results of regression analysis for the boron assay based on II and III in the brain, muscle, blood plasma, and urine are shown in Fig. 2 and 3, respectively. In the regression analysis, the factor 1000/869 was used for the brain, muscle, and blood plasma, and that of 1000/945 for urine, based on total recovery of boron. Regression analysis proved statistically that these boron assay methods were accurate to within 10%.

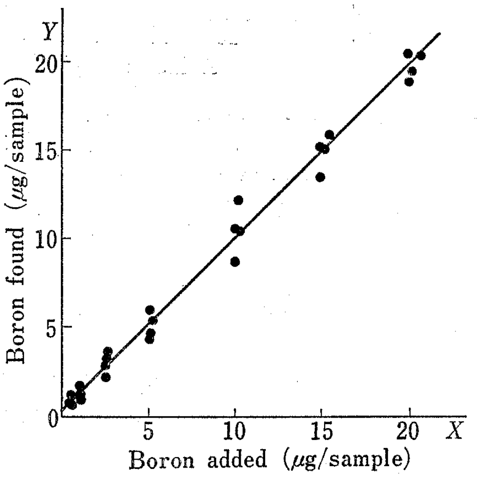

Fig. 2. Regression Analysis for Boron Assay in Mouse Brain, Muscle, and Blood Plasma

For regression equation:
$Y=0.9795X+0.308$, $s=0.77$, $c.v.=9.80\%$, $n=28$

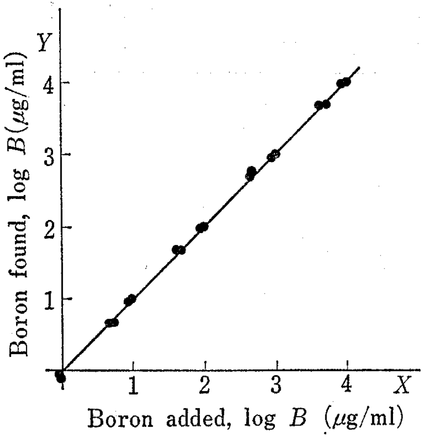

Fig. 3. Regression Analysis for Boron Assay in Human Urine

For regression equation:
$Y=1.0034X-0.027$, $s=0.137$, $c.v.=6.62\%$, $n=18$

The lower limit of analytical utility of present method was estimated to be 0.2 μg boron per biological sample.

In basic studies on boron neutron-capture therapy using I, a colorimetric method with 1,1′-dianthrimide has been applied for the determination of boron uptake in the tumor and the surrounding normal tissues. For 43 samples such as mammary gland tumor, normal mammary gland, skin, and blood plasma of the dogs administered I, the boron content in each sample was determined by the present method and 1,1′-dianthrimide method.[3] The boron content obtained by the two methods was found to be 0.3 to 60 μg/g or ml in those samples, and the values by the present method were found to be closely correlated with those by the 1,1′-dianthrimide method (correlation coefficient, $r=0.8925^{**}$, $r(41, 0.01)=0.3883$).

Compared with the 1,1′-dianthrimide method, the present method is simple and safe, and is sensitive enough to be useful for the determination of boron in biological materials for the boron neutron-capture therapy.

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