# Quantitative Analysis of Mercaptoundecahydrododecaborate by Fourier Transform Infrared Spectroscopy

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Mercaptoundecahydrododecaborate (BSH) is an important agent in boron neutron capture therapy (BNCT) of various cancers. A simple and rapid analytical method for the measurement of mercaptoundecahydrododecaborate in aqueous solution and in urine by Fourier transform infrared spectroscopy has been developed. A thin-pathlength sampling apparatus was used to minimize the strong absorption of water. The subtraction of water absorbance from sample spectra resolved a B–H band at 2493 $cm^{-1}$. The quantitative measurement of BSH concentration was carried out by integrating the B–H band above baseline in the range of 2534–2440 $cm^{-1}$. The lower limit of measuring the concentration of sodium BSH ($Na_2B_{12}H_{11}SH$) in our experiment was 10 μg/ml (about 5 ppm of boron). This method measures the hydroborate (B–H) concentration instead of total boron and, thus, may be utilized to measure the BSH concentration in *in vivo* samples for metabolic studies.

KEY WORDS: boron neutron capture therapy; mercaptoundecahydrododecaborate; Fourier transform infrared spectroscopy; quantitative analysis in aqueous media.

## INTRODUCTION

Boron neutron capture therapy (BNCT) of cancer is based on the neutron irradiation of tumor cells that are bound with $^{10}B$-containing agent. The nuclear reaction between the low-energy neutrons and the nonradioactive $^{10}B$ isotope produces high-energy and short-range alpha particles and $^{7}Li$. The high-energy beam is capable of destroying the tumor cell within a range of 10 μm from the site of the capture reaction. Mercaptoundecahydrododecaborate ($^{2-}$) (BSH, $B_{12}H_{11}SH^{2-}$) is known to be an effective $^{10}B$-containing agent for BNCT because of its capacity of binding with tumor cells (1–3). The structure of BSH is shown in Fig. 1 (4,5).

Several analytical methods have been used to determine boron levels in biological samples. The methods include colorimetric analysis (lower limit, 0.5 μg of boron per sample solution), inductively coupled plasma atomic emission spectrometry (ICP-AES) (lower limit, 0.05 ppm of boron), and isotachophoretic analysis (lower limit, 90 ppm of boron) (6–10). The methods are sensitive and accurate but require complicated procedures and long sample preparation times, especially for colorimetric analysis and ICP-AES.

Recently, we have been using Fourier transform infrared spectroscopy (FTIR) to determine BSH concentrations in aqueous solution and in urine. The studies have demonstrated the capabilities of FTIR in measuring low concentrations of BSH in aqueous solution and in urine. The advantage of the FTIR analysis is that it is a relatively rapid and simple method compared to other analytical methods. The analysis does not require complicated procedures and the aqueous BSH solution or the BSH/urine sample can be directly introduced into the sampling apparatus for measurement.

One difficulty for the FTIR measurement was to resolve the relatively weaker BSH absorption bands from the stronger water absorption bands. We have used a thin-pathlength (15-μm) sampling apparatus to minimize the water absorption. The capability of the FTIR software to subtract the water absorption bands makes it possible to resolve the B–H peak at 2493 $cm^{-1}$ for the quantitative measurement.

## MATERIALS AND METHODS

### Materials

Sodium mercaptoundecahydrododecaborate (Ben Venue Laboratories) was a generous gift from the Idaho National Engineering Laboratory's (INEL) BNCT Program. Urine samples were collected from adult male Sprague–Dawley rats. The rats were housed individually in metabolic cages and the urine was collected in a 20-hr period. Sufficient BSH was dissolved in distilled deionized water to produce a stock solution of 2.5 mg/ml. For the experiments of determining BSH level in aqueous solution, the stock BSH solution was diluted to make final concentrations of BSH ranging from 10 μg/ml to 2.5 mg/ml. For the experiments of determining BSH level in urine, the stock BSH solution was first diluted to appropriate concentrations. One volume of the diluted solution was then mixed with 3 vol of urine to make the BSH/urine sample. The final concentrations of BSH in urine were varied from 10 to 500 μg/ml. Each sample was injected into the FTIR sampling apparatus without further preparation.

### Method

A Perkin Elmer 1760 FTIR spectrometer with a deuterated triglycine sulfate (DTGS) detector was employed for FTIR measurement. Perkin Elmer IR Data Manager (IRDM v.3.30) software was used for handling infrared data. Fourier transformation of the interferogram was performed using the normal Norton–Beer apodization function. The spectra were acquired using a demountable pathlength liquid sampling cell with $CaF_2$ windows and a 15-μm Teflon spacer (Spectra Tech Inc.). For each determination, 300 scans were coadded at a 4-$cm^{-1}$ resolution. Four experiments were carried out for each concentration. During the experiment, a single-beam spectrum of the empty cell was recorded as the background. The water or the test sample (BSH in aqueous solution or in urine) was then injected into the cell and a single-beam spectrum was recorded. The spectrum of water or the test sample was ratioed against the background spectrum. After conversion of the resultant transmittance spectrum into absorbance units, the spectrum of water was digitally

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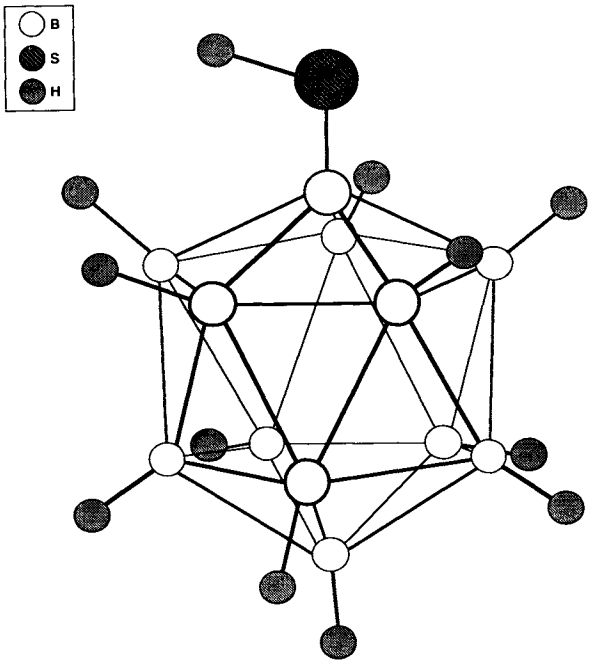


Fig. 1. The structure of mercaptoundecahydrododecaborate(2$^-$) (BSH, $B_{12}H_{11}SH^{2-}$).

subtracted from the spectrum of the test sample. The subtraction factor was automatically adjusted by the FTIR software according to a wavenumber range of 2300–1900 cm$^{-1}$. Each spectrum was baseline-corrected before quantitative measurement. Quantitative measurements of BSH in aqueous solution and in urine were conducted by integrating the B–H band above baseline from 2534 to 2440 cm$^{-1}$.

## RESULTS AND DISCUSSION

The spectra of BSH/urine (500 μg/ml BSH), BSH/water (500 μg/ml BSH), and water are shown in Figs. 2A, B, and C, respectively. The figures indicate the typical problem encountered in FTIR analysis of aqueous samples. The water absorption was strong and the magnitude of the water absorption was about 1.5 absorbance unit even when a thin-pathlength (15-μm) sampling apparatus was used in the experiment. The strong water absorption made the measurement of BSH difficult since the absorbance of the B–H band was only about 0.2% of the water absorbance. In order to resolve the relatively weaker B–H absorption band from the much stronger water absorption bands, the latter had to be subtracted from the spectra of the BSH/urine and BSH/water samples. It can be seen that all the spectra were dominated by the strong O–H band (1640 cm$^{-1}$) due to water absorption. However, it was difficult to carry out the subtraction procedure using the O–H band (1640 cm$^{-1}$) as the reference, because the presence of the amide I peak (around 1650 cm$^{-1}$) of proteins in urine interfered with the subtraction. Therefore, a smaller band in the wavenumber range of 2300–1900 cm$^{-1}$ was used as the reference band for the subtraction of water absorbance from the spectra of the BSH/urine and BSH/water samples. The FTIR software calculated the subtraction factor based on the relative integration

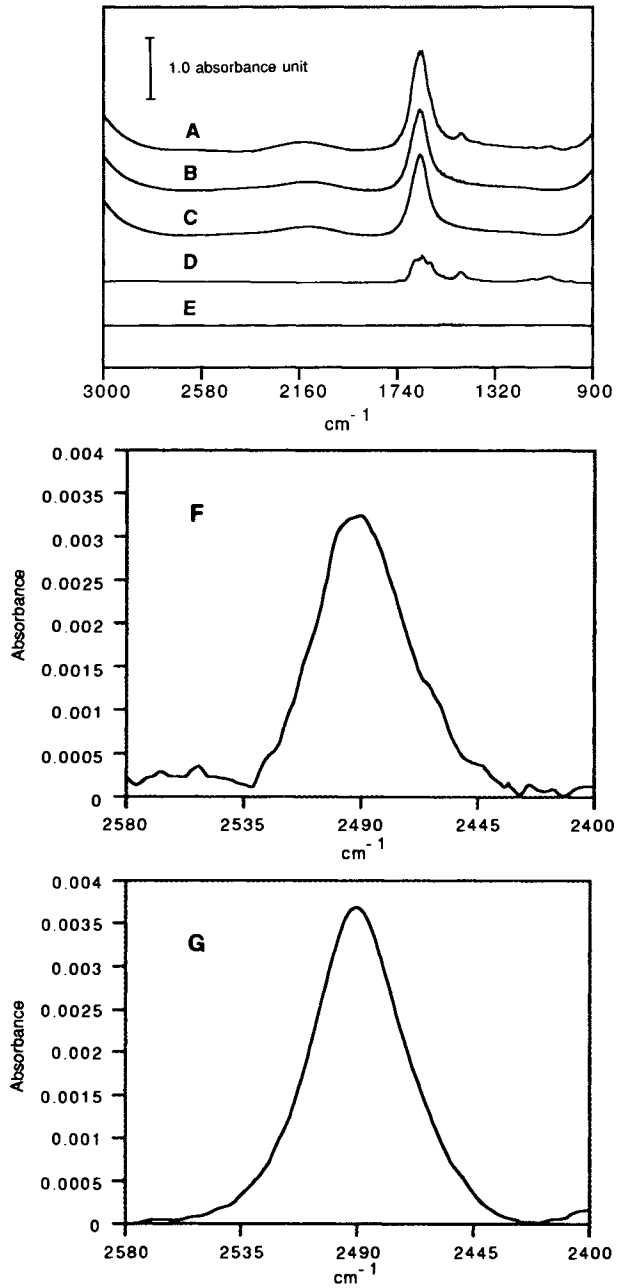


Fig. 2. Subtraction of water spectrum from the spectra of BSH/urine and BSH/water samples. (A) BSH/urine spectrum; (B) BSH/water spectrum; (C) water spectrum; (D) water-subtracted BSH/urine spectrum; (E) water-subtracted BSH/water spectrum; (F) B–H band in spectrum of D with 500× expansion in *Y* axis; (G) B–H band in spectrum of E with 500× expansion in *Y* axis. All the spectra in A through E are on the same absorbance scale as indicated by the scale bar.

area in the spectra of the test samples and water within the specified wavenumber range.

The water-subtracted spectra from the spectra of BSH/urine and BSH/water samples are shown in Figs. 2D and E, respectively. All the spectra in Figs. 2A–E are in the same scale (2.0 absorbance units on the *Y* axis). The amide I, II, and III bands of the proteins in urine are shown in Fig. 2D at the wavenumbers 1650, 1540, and 1250 cm$^{-1}$, respectively

(11). To display the B–H band in the water-subtracted spectra, the region of 2580–2400 $cm^{-1}$ was selected and the absorbance scale was expanded about 500 times. The expanded spectra for the BSH/urine and BSH/water samples are shown in Figs. 2F and G, respectively. The expanded spectra clearly indicate the presence of the B–H band at 2493 $cm^{-1}$. Urine samples alone (without adding BSH) were examined during the experiment and no peak was found at 2493 $cm^{-1}$. By comparing the spectra in Figs. 2F and G, it can be seen that the background noise of the BSH/urine sample is higher than that of the BSH/water sample. The noise is, however, much smaller than the magnitude of the B–H peak and it appears feasible to measure the BSH concentration in urine quantitatively without sample separation.

Quantitative measurement of BSH was performed by integration of the B–H band above baseline in the wavenumber range of 2534–2440 $cm^{-1}$. Baseline correction was made in the experiments since it appeared helpful in the lower limit range. Figures 3 and 4 show the relationships between the integrations of the B–H band and various concentrations of BSH in aqueous solution and in urine, respectively. The BSH concentrations range from 10 to 500 μg/ml in both figures. The integration of the B–H band (above the baseline) correlates linearly with the BSH concentration in aqueous solution ($r^2$ = 0.991) and in urine ($r^2$ = 0.996). Furthermore, the two plots (Figs. 3 and 4) are very similar, suggesting that the presence of urine in the sample does not interfere with the quantitative measurement of BSH. A range of high concentrations of BSH in water was also examined and the results are listed in Table 1. The results indicate that the linear correlation between the integration of the B–H band and the BSH concentration extends to the higher concentration range.

The modern Fourier transform infrared spectrometer is a powerful analytical tool that has a high signal-to-noise ratio, excellent resolution, and good stability (12). The instrument is often equipped with extensive data-handling computer software. The capability of the instrument has been improved so that important information from various substances, including proteins, in aqueous environment can be obtained (11,13). Using the water-subtraction technique, the resultant B–H peak can be conveniently measured for determination of the BSH concentration. The sensitivity of the FTIR method is high. The lower limit of determining the concentration of sodium BSH in our experiments was 10 μg/ml (about 5 ppm of boron). The sensitivity of the present method is adequate for the range of BSH concentrations that are likely to be encountered in the INEL BNCT project. The method is relatively simple. Urine samples can be directly injected into the demountable pathlength liquid sampling cell without further preparation. Moreover, the method is relatively rapid compared to other existing methods. It takes about 20 min for analyzing one sample (with 300 scans at a 4-$cm^{-1}$ resolution).

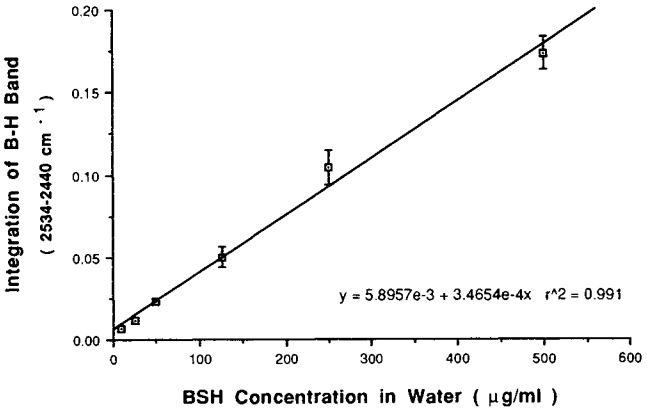


Fig. 3. The relationship between the integrations of the absorbance (2534–2440 $cm^{-1}$) and various concentrations of BSH in aqueous solution. The standard errors of the means are shown by the error bars.

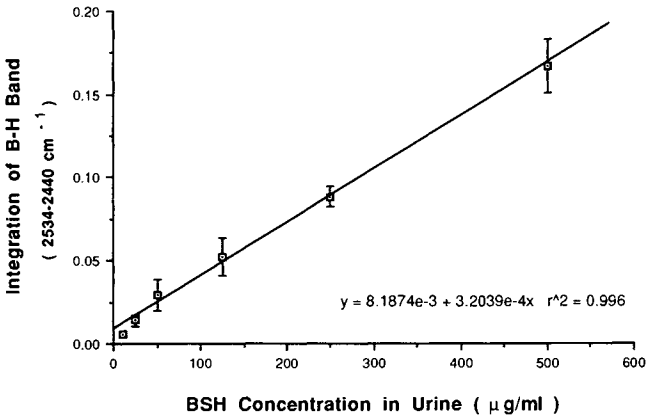


Fig. 4. The relationship between the integrations of the absorbance (2534–2440 $cm^{-1}$) and various concentrations of BSH in urine. The standard errors of the means are shown by the error bars.

The rapid quantitative measurement of BSH levels in biological fluids is important in the BNCT treatment since the real-time adjustment of the neutron dose according to the BSH level in the body may be necessary. The existing analytical methods require longer time for sample preparation and are, thus, unsuitable for monitoring the body levels of BSH during the neutron dose adjustment. This FTIR method provides a rapid and accurate way to measure the BSH concentration in urine and may be useful for the neutron dose adjustment. It should also be noted that the FTIR method measures the hydroborate (B–H) concentration, while other methods measure total boron concentration. Therefore, the FTIR method may be utilized to measure the BSH concentration (instead of total boron) in *in vivo* samples for metabolic studies.

Table I. Results from the BSH/Water Experiments in a Higher BSH Concentration Range[a]

| BSH concentration (μg/ml) | Integration of B–H band (2534–2440 $cm^{-1}$) |
|---|---|
| 1,000 | 0.471 |
| 1,500 | 0.692 |
| 2,500 | 0.846 |
| 3,640 | 1.174 |
| 7,280 | 2.389 |
| 14,550 | 4.592 |

[a] Combined with the data in Fig. 3, the overall $r^2$ = 0.997.

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