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FT–IR measurement of mercaptoundecahydrododecaborate in human plasma

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Abstract: A simple and rapid method for the quantitative measurement of mercaptoundecahydrododecarborate (BSH), (which presently is one of the most useful agents for Boron Neutron Capture Therapy) in human plasma was developed by using Fourier transform infrared spectroscopy. Different spacer thicknesses of the liquid sampling cell were examined and the optimal results were obtained by the 0.05 mm spacer. The subtraction of water absorbance from sample spectra resolved a B–H band at 2493 cm^{-1}. The quantitative measurement of BSH was carried out by integration of the B–H band in the wavenumber range of 2534–2440 cm^{-1}. However, at the lower BSH concentration range, a visual inspection of the spectrum to determine the wavenumber range was necessary so as to avoid any negative areas to be integrated. The lower limit of detection of BSH in aqueous solution and human plasma was 5 μg ml^{-1} (about 2.5 ppm of boron).

Keywords: *Boron neutron capture therapy; human plasma; spacer thickness; mercaptoundecahydrododecaborate; Fourier transform infrared spectroscopy; quantitative analysis in aqueous media.*

Introduction

The boron-10 enriched compound, sodium mercaptoundecahydrododecaborate ($Na_2B_{12}H_{11}SH$, BSH), is a useful agent for boron neutron capture therapy (BNCT) [1, 2]. BNCT is a potential treatment for various cancers which is based on the destruction of cancer cells resulting from the boron–neutron interactions at the intracellular sites. The successful treatment requires the selective accumulation of boron compounds in the malignant tissue/cells followed by adequate neutron irradiation. The interactions between boron and neutron produce short-range α particles, which destroy the cells within the 10 μm diameter range [3]. Figure 1 illustrates the boron–neutron nuclear reaction at the capture site.

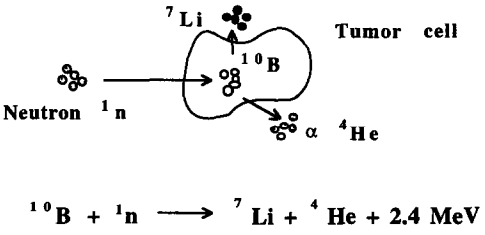

Figure 1
Boron neutron capture reaction for cancer treatment.

Though several analytical techniques are being employed for boron determination in biological samples, most of these require expensive instrumentation and/or involve complicated and time consuming procedures [4, 5]. As an integral part of the BNCT program, relatively simple and rapid but reliable methods to determine the boron concentrations in biological samples are of paramount importance. Recently, a Fourier transform infrared (FT–IR) spectroscopy method has proven to be a simple and rapid technique suitable for the quantitation of BSH in aqueous solution and urine [1].

To further examine the applicability of FT–IR spectroscopy in the determination of BSH concentration in aqueous biological samples, we have optimized the FT–IR method to achieve a higher measurement sensitivity by changing the spacer thickness of the liquid sample cell. The spacers with thicknesses of 0.015, 0.05, 0.1 and 0.2 mm (commercially available from Spectra Tech, CT) were examined. The quantitative measurement of

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BSH in human plasma was then carried out with the improved technique.

Materials and Methods

Materials

Sodium mercaptoundecahydrododecaborate was manufactured by Centronic Ltd, UK and was a generous gift from Neutron Technology Company. Since the chemical is very hygroscopic, special care was taken with the storage and handling of the chemical during the experiments. Human plasma samples were obtained from American Red Cross Society (Atlanta Center, GA). The human blood was tested to be negative for human immunodeficiency virus (HIV) and hepatitis viruses and centrifuged at 2900 rpm for 3.5 min to obtain plasma which was then shipped to our laboratory. The plasma sample was kept at −70°C before use. Other chemicals were purchased from The Sigma Chemical Co. (St Louis, MO).

Sample preparation

In the experiments to determine the optimal spacer thickness, sufficient BSH was dissolved in phosphate buffered saline (PBS, pH 7.2) to make a stock solution of 1 mg ml^{-1}. Serial dilutions of the stock solution in PBS were made to obtain the BSH concentrations ranging from 5–500 μg ml^{-1}. In the experiments to measure the BSH concentration in human plasma samples, a stock solution of 5 mg ml^{-1} BSH in PBS was made. The plasma was centrifuged at 6000 rpm for 45 min in a CU-5000 centrifuge (Damon/IEC division) and filtered through a sterile 0.8 μm filter unit (Millex-PF, Millipore, MA). One volume of the stock solution (diluted to appropriate concentrations) was mixed with 9 volumes of plasma to obtain concentrations ranging from 5–500 μg ml^{-1}. Each sample was injected into the FT–IR sampling apparatus without further preparation.

Instrumentation

Infrared spectra were acquired using a Nicolet 510 P FT–IR spectrometer (Nicolet Analytical Instruments, Madison, WI), equipped with a deuterated triglycine sulphate (DTGS) detector. The spectra were obtained using a demountable pathlength liquid sampling cell with CaF_2 windows and 0.015–0.2 mm polyethylene spacers (Spectra Tech). The spectra were collected at a resolution of 4 cm^{-1}. Five hundred interferogram scans were collected and coadded to enhance the signal to noise ratio. At least three experiments were carried out for each BSH concentration. PCIR software (Nicolet) was used for data processing and calculation of the area under the B–H peak. Fourier transformation of the interferogram was performed using the normal Happ-Genzel apodization function. During each experiment, a single-beam spectrum of the empty cell was recorded as the background spectrum. The blank sample (e.g. phosphate buffered saline) or the test sample (BSH in phosphate buffered saline for studies on the spacer thickness or BSH in plasma) was then injected into the cell and a single-beam spectrum was recorded. The spectrum of PBS or the test sample was normalized against the background spectrum. The transmittance spectra were converted into absorbance units and the spectrum of water (PBS) was digitally subtracted from the spectrum of the test sample in the wavenumber range of 3000–1000 cm^{-1}. The subtraction factor was calculated using the Autosubtract Mode of the PCIR software based on the wavenumber range of 2300–1900 cm^{-1} [1]. Quantitative measurements of BSH in PBS and plasma were carried out by integrations of the B–H band from 2534–2440 cm^{-1} [1].

Results and Discussion

Optimal spacer thickness of the sampling apparatus

To determine the optimal thickness of the spacer to be used in the sampling apparatus, different spacers ranging from 0.015 to 0.2 mm thickness were examined in the aqueous environment. The sensitivity of each of the spacers is listed in Table 1.

The results indicated that the lower limit of detection of BSH in an aqueous environment using the 0.05 mm thick spacer was the best. The spacers with 0.015, 0.1 and 0.2 mm thick-

Table 1
Results of the measurement sensitivity with different spacers in the sampling apparatus

Spacer thickness (mm)	Sensitivity (μg ml^{-1})
0.015	10
0.05	5
0.1	10
0.2	25

ness could also be used for the measurement of BSH in aqueous environment if the situation demands, but the detection became less sensitive. Among the spacers examined, the spacers with 0.015 and 0.1 mm thickness gave similar results and the detection limit using the spacer with 0.2 mm thickness was 25 μg ml^{-1} (which is undesirable for the measurement of BSH in the BNCT project).

For quantitative analysis in aqueous environment using FT–IR, the major difficulty to be overcome is the strong absorption of water and the necessity to resolve the relatively weaker sample absorption band from the much stronger water bands [6, 7]. Although the modern FT–IR instrument is highly sensitive and is equipped with a powerful software capable of resolving the weaker bands from the stronger bands by spectral subtraction technique, the water absorbance generally is too strong to be subtracted out. In our previous studies [1], the thin-pathlength sampling apparatus with the spacer thickness of 0.015 mm was used to minimize the water absorption. The water absorption bands obtained using the 0.015 mm thickness sampling apparatus could be easily subtracted out to generate the BSH band for quantitative measurement. However, the sensitivity of the measurement may suffer because relatively smaller amounts of the drug molecules are present in the pathway of IR light when a thinner spacer is employed in the sampling cell. A thicker spacer may be desirable to allow for more drug molecules to be exposed to the IR light but again water interference may be too strong to be totally subtracted out. Therefore, one aim of this study was to investigate the effect of spacer thickness on the sensitivity of FT–IR measurement of BSH in an aqueous environment, and to determine the optimal spacer thickness for this measurement. The previous studies showed that the sensitivity of the assay in both buffer and urine samples was 10 μg ml^{-1} using the 0.015 mm spacer. It appears that among the spacers commercially available, the one with 0.05 mm thickness should be used as a higher sensitivity is achieved with this spacer thickness (5 μg ml^{-1} in buffer solution).

In all subsequent experiments, we have used the 0.05 mm spacer to obtain the BSH spectra for quantitative measurement. The quantitative measurements of BSH in human plasma were performed by integration of the B–H band above the baseline in the wavenumber range of 2534–2440 cm^{-1}. The wavenumber range selected was based on the results of our previous studies using the 0.015 mm spacer and it appeared to be well suited to the experiments using the 0.05 mm spacer. As mentioned in the previous studies for urine samples [1], the BSH band at the wavenumber range of 2534–2440 cm^{-1} was well separated from the interference peaks such as amide I, II and III bands of the proteins or peptides which are at wavenumbers 1650, 1540 and 1250 cm^{-1}, respectively. The water-subtracted spectra (without baseline correction) of blank plasma and BSH/plasma (500 μg ml^{-1}) are shown in Fig. 2(A) and (B), respectively. Both the spectra in Fig. 2(A) and (B) are in the same scale (0.09 absorbance units on the *y*-axis). The figure clearly indicates the B–H band of BSH and suggests that the wavenumber range 2534–2440 cm^{-1} is adequate for the integration of the B–H band for quantitative measurement of BSH. No interference from the blank plasma sample can be seen.

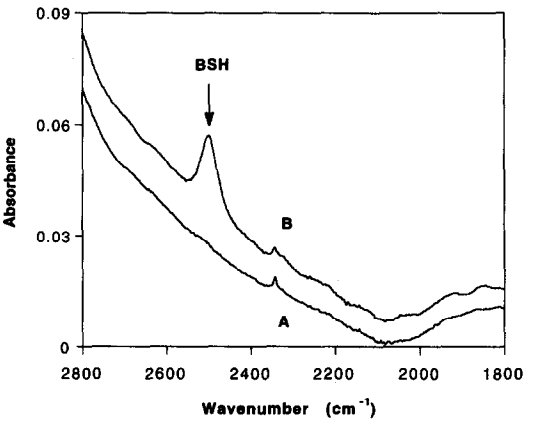

Figure 2
Water subtracted spectra (at wavelength 2800–1800 cm^{-1}) showing (A) blank human plasma (B) human plasma spiked with 500 μg ml^{-1} of BSH. No baseline correction was performed.

It should be emphasized, however, that at lower BSH concentrations, the wavenumber range needs to be reselected to avoid including any negative values (relative to the baseline) during the integration. The solvent subtracted spectra at two different concentrations are shown in Figs 3 and 4 to illustrate the selection of the wavenumber range for integration of the B–H band. At the lower BSH concentration (25 μg ml^{-1}, Fig. 3) the integration from 2534 to 2440 cm^{-1} would include some of the

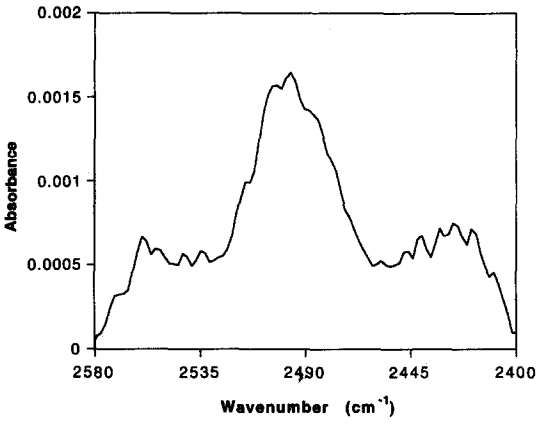

Figure 3
B–H band in the water subtracted spectrum of BSH/PBS sample at a concentration of 25 μg ml^{-1}. This illustrates the importance of visual examination for the selection of the wavenumber range for integration at low BSH concentration.

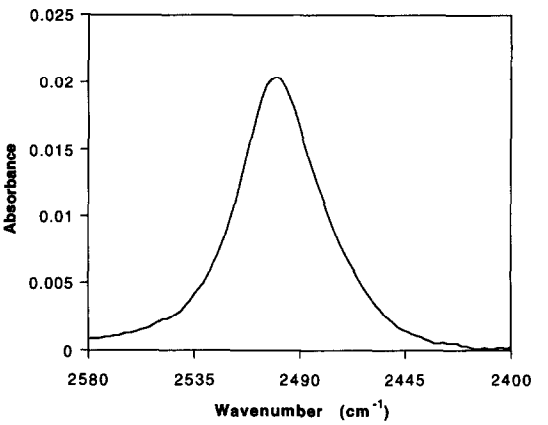

Figure 4
At higher concentrations the peaks are well shaped. Therefore, integration can be performed between 2534 and 2440 cm^{-1} according to our earlier studies [1].

Table 2
Data obtained from the standard curves for the determination of BSH in aqueous solution and in human plasma using 0.05 mm spacer

BSH	Range (μg ml^{-1})	Slope	Intercept	SD_{Slope}	$SD_{Intercept}$	r
In PBS	5–500	1.3287^{-3}	1.2609^{-2}	2.1898^{-5}	6.8997^{-3}	1.0000
In human plasma	5–500	1.2966^{-3}	8.4659^{-3}	2.7589^{-5}	4.8076^{-3}	0.9945

negative values since part of the peak was below the baseline which was established based on the peak heights at wavenumbers 2534 and 2440 cm^{-1}, respectively. To avoid integrating the negative values, the baseline was established based on the heights at wavenumbers of 2534 and 2459 cm^{-1} and the integration was carried out at this wavenumber range. The spectrum is shown (Fig. 3) for BSH in aqueous solution after baseline correction. For the higher concentration range (500 μg ml^{-1}, Fig. 4) the peak between the wavenumbers 2534 and 2440 cm^{-1} was well shaped and the integration of the B–H band was performed using these wavenumbers for establishing the baseline. The problems appear consistent with our studies. At higher concentrations of BSH, the peak within 2534–2440 cm^{-1} was sharp and no negative area was integrated between the wavenumbers 2534 and 2440 cm^{-1}. As the concentration of BSH was decreased, the B–H band became narrower and a visual inspection of the spectrum to determine the wavenumber range to avoid integrating any negative values became important.

The performance data for the determination of BSH in aqueous solution (PBS) and in human plasma using the 0.05 mm spacer are presented in Tables 2 and 3. The data have been calculated based on the plots of integration of the B–H band vs various concentrations of BSH in aqueous solutions and in human plasma, respectively. The lower limit of detection in both solutions was 5 μg ml^{-1} (which is approximately equivalent to 2.5 μg ml^{-1} of boron). There was a linear correlation

Table 3
Statistics of the quantitation of BSH in aqueous solution and human plasma

Concentration (μg ml^{-1})	RSD (aqueous solution)	RSD (human plasma)
5	0.2497	0.1381
10	0.1399	0.0523
25	0.1855	0.1969
50	0.0939	0.1745
125	0.0385	0.1139
250	0.0160	0.0183
500	0.0097	0.0298

Note: At least $n = 3$ measurements were made at each concentration.

between the integration of the B–H band and concentration of BSH in aqueous solution (r = 1.00) or in plasma (r = 0.9944). Furthermore, the data suggests that the presence of plasma in the sample does not interfere with the quantitative measurement of BSH. Table 2 shows the relative standard deviation (RSD) for BSH in aqueous solution and in human plasma samples at each concentration range. The concentration range employed in our studies covers the concentration range most likely to be encountered in the BNCT studies.

The Fourier transform infrared (FT–IR) spectroscopy has proven to be a rapid, sensitive and reproducible technique for the quantitation of the BSH. Since computer-aided data handling is routinely available on modern instrumentation, water subtraction has become less problematic. In our studies, the FT–IR method was found to be relatively simple and rapid compared to other existing methods for quantitating BSH levels. Each sample can be analysed in about 20 min (500 scans, resolution 4 cm^{-1}). Regression analysis of the data shows good linear sample response in aqueous solution and in human plasma.

Conclusions

Our results have indicated that the FT–IR method is an adequate method for quantitative measurement of BSH in aqueous solutions and in human plasma. The method is simple and rapid. The lower limit of detection is about 2.5 $\mu g\ ml^{-1}$ of boron which is comparable to the lower detection limits of other methods. As this method requires relatively less time for sample preparation and analysis, it could be of use in the BNCT program for monitoring BSH levels in the body during the neutron irradiation.

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