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Original Papers

Quantitative Analysis of Amino Acids in Dietary Supplements Using Terahertz Time-domain Spectroscopy

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We successfully analyzed the concentrations of five amino acids in commercially available dietary amino acid supplements by using terahertz time-domain spectroscopy (THz-TDS) with an error of ±12% for the best reproduced components. We also succeeded in analyzing tablets of the supplements wrapped in paper, and thus showed the merit of using THz waves for the nondestructive quantitative analysis of packaged samples by employing the fact that THz waves are capable of passing through several types of packaging material. The ability of THz waves to pass through the paper made it possible to perform a quantitative analysis using the same standard spectra as those used for an unwrapped sample, and the accuracy of a direct quantitative analysis of a packaged sample was almost the same as that of an unwrapped sample.

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Introduction

Terahertz time-domain spectroscopy (THz-TDS) is a promising tool in physics and chemistry for characterizing far-infrared vibrational modes, such as rotational, torsional, phonon, inter- and intramolecular modes. Those modes exist in the 0.3 – 3.0 THz (10 – 100 cm^{-1}) range, which corresponds to the lower end of the far-infrared region.[1] One of the attractive properties of THz waves is their ability to pass through a wide variety of materials,[2] thus enabling us to see through many types of packaging material, including paper, plastics, leather and wood. This property allows nondestructive inspections of such items as drugs in mail envelopes at post offices.[3] The high transparency applies to several molecules that form few inter- and intramolecular modes, such as hydrogen bonds. Therefore, it becomes possible to achieve the selective detection of certain molecules in mixed samples, which form strong inter- and intramolecular modes in a sample mixed with impurities. Moreover, THz waves cause almost no damage to materials, and so they can be used to detect fragile biological samples[4-9] and to characterize pharmaceutical materials.[10-13] Thus, THz techniques are attracting increasing interest not only in basic research, but also for practical applications in a variety of fields.

Recently, the usefulness of the quantitative analysis of chemicals which have characteristic absorption in the THz region, such as amino acids, by using THz-TDS has been demonstrated;[14-17] however, the analysis of amino acids in actual foods or drugs has not yet been tested. In this paper, we describe the quantitative analysis of five amino acids in an actual dietary amino acid supplement by using THz-TDS. The supplement contains other ingredients, such as sugars and vitamins, and thus is a suitable sample for testing the selective detection of an amino acid using THz-TDS. We also analyzed tablets of a commercially available dietary amino acid supplement wrapped in paper, and compared the accuracy of the direct quantitative analysis of the packaged samples with that of unwrapped samples. Since one characteristic of THz waves is their ability pass through commonly used packaging materials, such as paper and plastics, the accuracy with which they can provide a nondestructive quantitative analysis of packaged samples is of interest. The merit of using THz-TDS for the direct quantitative analysis of packaged samples was also shown by comparing it with Raman spectroscopy.

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Experimental

Sample preparation

Commercial dietary amino acid supplements were purchased from two different makers. The samples of the two different kinds of supplements are denoted as Supplements A and B here. The concentrations of the amino acids and other ingredients were calculated based on nutrition fact sheets and are summarized in Table 1. The supplement granules were ground into powder, and about 80 mg of this powder was compressed into a tablet 10 mm in diameter. The mechanically determined tablet thicknesses were 0.7 and 0.8 mm for Supplements A and B, respectively. In order to eliminate from the spectra the effect of the multiple reflections that occur between the two surfaces of a sample tablet (etalon artifacts), we removed the signal of the multiple reflections prior to Fourier transformation of the time-domain signal to obtain the frequency-domain spectrum. These thickness values provided a sufficient path length for us to eliminate from the spectra the effect of the multiple reflections. Since windowing the data can introduce incorrect information at low frequencies, we do not use the spectra in 0 to 0.5 THz region for further analysis. The final weights of the tablets were also measured to determine the quantity of each amino acid that they contained. Wrapped samples for use in the nondestructive inspection of a packaged tablet were prepared by

Table 1 Concentration of amino acids and other ingredients in Supplements A and B

	Concentration, %	
	Supplement A	Supplement B
Amino acids		
L-Arg	17.3	7.5
L-Glu	18.0	7.5
L-Ile	12.3	10.0
L-Leu	15.3	15.0
L-Val	10.3	10.0
Other ingredients		
Maltitol	Unknown	Unknown
Citric acid	Unknown	Unknown
Sugars	Unknown	Unknown
Natural flavors	Unknown	Unknown
Vitamin C	0.63	1.25
Niacin	0.13	0.21
Pantothenic acid	0.08	0.06
Vitamin B6	0.01	0.02
Vitamin B2	0.01	0.02
Vitamin B1	0.01	0.01
Vitamin A	<0.01	<0.01
Vitamin D	<0.01	<0.01
Vitamin E	0.54	0.13

placing the above tablets of Supplements A and B between sheets of weighing paper (~30 μm thick).

To obtain the standard THz spectra, that is, the molar absorption coefficient in a certain THz region, standard tablets of the major constituents (L-arginine (L-Arg), L-glutamine (L-Gln), L-isoleucine (L-Ile), L-leucine (L-Leu), L-valine (L-Val), maltitol, and citric acid monohydrate) were prepared by mixing a certain amount of each chemical in powder form with high-density polyethylene (PE) powder (Aldrich).

Measurement

We measured the THz spectra by using a THz-TDS2004 (Aispec) combined with a near-infrared pulse laser to generate optical pulses with a duration time of 10 fs and a wavelength of 800 nm (Integral, FEMTOLASERS). The repetition frequency was 80 MHz. We used a 50-V biased low temperature-grown GaAs (LT-GaAs) photoconductive antenna for both the generation and detection of THz pulses. Other optical set-ups are described in detail elsewhere.[14] A sample tablet was attached at the center of the rear of the holder (ϕ7 mm) so as to allow the THz beam to pass through it. The data for the sample were collected by a detector. The reference data consisted of THz pulses that passed through the same aperture in the absence of a tablet. An accumulation number of 32 was used for both the sample and the reference to improve the *S*/*N*. The measurement time was within 5 min. The spectral resolution was 0.96 cm^{-1}. All of the measurements were acquired at room temperature under a pressure of 50 Pa to avoid water vapor absorption.

We measured the Raman spectra using an NIR Raman microprobe system (InVia Reflex, Ranishaw). The excitation light source was the 785-nm line of a laser-diode continuous-wave laser (Ranishaw). The approximately 30 mW NIR laser light was focused on the sample with an objective lens (×50, NA 0.55). The laser power at the sample was about 3 mW for all of the measurements. The laser was focused on top of a tablet of amino acid supplement or amino acid powder. The spot size diameter and the focal depth on the sample were about 1 μm and <10 μm, respectively. The scattered light was collected by using the 180° backscattering geometry, and the grating in the polychromator was 1200 line/mm. The exposure times for the Raman measurement were 10 to 30 s. The spectral resolution was <2 cm^{-1}. We performed all of the measurements at room temperature.

Method of quantitative analysis

Before obtaining the standard spectra of the major constituents (L-Arg, L-Gln, L-Ile, L-Leu, L-Val, maltitol, and citric acid monohydrate), we calculated the concentrations of the constituents in each standard tablet by using the following equations;

$$C = \frac{W \cdot w}{(w + w_{pe}) \cdot M \cdot V}, \quad (1)$$

$$V = \pi r^2 d, \quad (2)$$

where C, W, w, w_{pe}, M, d and r are the concentration, the weight of the tablet, the weight of the mixed amino acid powder, the weight of the mixed PE powder, the molecular weight of the amino acid, and the thickness and radius of the tablet, respectively.

The absorption spectra of a sample, $A(\nu)$, were calculated using

$$A(\nu) = -\ln\left|E_{sam}(\nu)/E_{ref}(\nu)\right|^2. \quad (3)$$

By Fourier transforming the time-domain signal, we obtained the amplitude spectra, $E_{sam}(\nu)$ and $E_{ref}(\nu)$, for a sample of thickness d placed in the beam and an empty reference, respectively. We removed the signal of multiple reflections prior to Fourier transforming. Since windowing the data can introduce incorrect information at low frequencies, we did not use the spectra in 0 to 0.5 THz region for further analysis. Our previous study[14] showed that the $A(\nu)$ resulting from the absorption of the sample is proportional to the concentration, and so $A(\nu)$ can be expressed by Beer's law,

$$A(\nu) = \varepsilon(\nu) C d, \quad (4)$$

where $\varepsilon(\nu)$ is the molar absorption coefficient. Thus, we calculated the $\varepsilon(\nu)$ values of the standard chemicals by averaging the $\varepsilon(\nu)$ curves for several different concentrations of standard tablets to obtain a standard spectrum. We used the standard spectra set ($\varepsilon_{L\text{-}Arg}(\nu)$, $\varepsilon_{L\text{-}Glu}(\nu)$, $\varepsilon_{L\text{-}Ile}(\nu)$, $\varepsilon_{L\text{-}Leu}(\nu)$, $\varepsilon_{L\text{-}Val}(\nu)$, $\varepsilon_{maltitol}(\nu)$, and $\varepsilon_{citric\ acid}(\nu)$) for further quantitative analysis.

We can calculate the spectrum ($S_{calc}(\nu)$) for any mixing ratio of L-Arg, L-Gln, L-Ile, L-Leu, L-Val, maltitol, and citric acid monohydrate by using the standard spectra set,

$$S_{calc}(\nu) = k_{L\text{-}Arg} \cdot \varepsilon_{L\text{-}Arg}(\nu) + k_{L\text{-}Glu} \cdot \varepsilon_{L\text{-}Glu}(\nu) + \cdots + k_{citric\ acid} \cdot \varepsilon_{citric\ acid}(\nu), \quad (5)$$

where $k_{L\text{-}Arg}$, $k_{L\text{-}Glu}$, $k_{L\text{-}Ile}$, $k_{L\text{-}Leu}$, $k_{L\text{-}Val}$, $k_{maltitol}$, and $k_{citric\ acid}$ are the coefficients for the constituents. To obtain the coefficients that correspond to the most likely ratio of the amino acids in Supplements A and B, we minimized the error between the calculated and experimental spectra.

Results and Discussion

Supplement A, unwrapped

The solid line in Fig. 1(a) shows the THz absorption spectrum

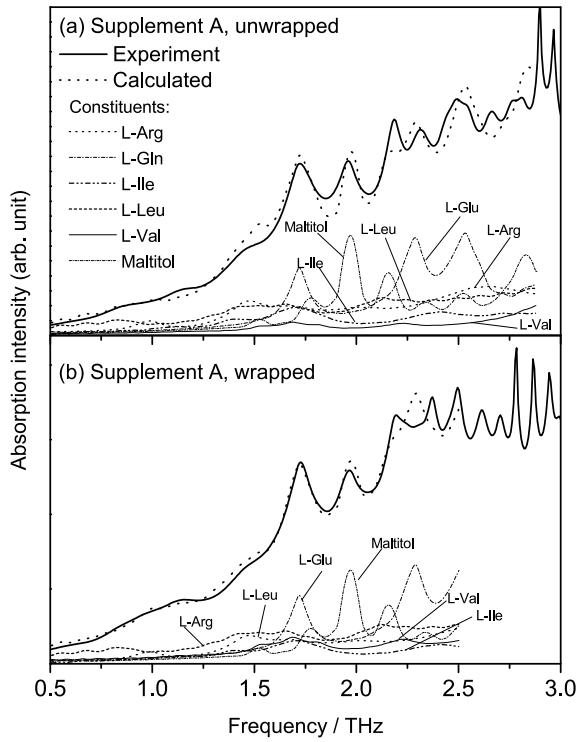

Fig. 1 THz spectra of unwrapped Supplement A tablet (a) and tablet wrapped in a weighting paper (b). Experimental (bold solid line) and calculated (bold dotted line) spectra are compared. Spectra of the constituents are overlaid to show their contributions to the calculated spectra.

of Supplement A in the 0.5 to 3.0 THz region. Several characteristic peaks were clearly observed. The dotted line shows the calculated spectrum obtained by adding the standard spectra, which were scaled in proportion to the calculated concentrations of five amino acids and maltitol. The spectra of the five amino acid constituents and maltitol are overlaid to show their contributions to the calculated spectra. The experimental spectrum was well reproduced by the calculated spectrum. Figure 3(a) and Table 2 summarize the concentrations of the five amino acids shown in the nutrition fact sheet and calculated results for Supplement A. The recovery ratios (calculated concentration over concentration given in nutrition fact sheet) revealed that the estimation errors, defined by ((recovery ratio) – 1)× 100, were within ±12% for the three constituents (L-Arg, L-Gln, and L-Ile). For L-Leu and L-Val, the estimation errors were 59 and 29%, respectively. The order of the concentration was correctly predicted, expect as regards L-Leu. Thus, despite employing a rough analysis that did not take account of the contribution of minor components, such as vitamins, we successfully analyzed the actual supplement by using THz-TDS.

Supplement B, unwrapped

The solid line in Fig. 2(a) shows the THz absorption spectrum of Supplement B in the 0.5 to 3.0 THz region. Several characteristic peaks were clearly observed, and the spectral shape was different from that of Supplement A. The dotted line shows the calculated spectrum obtained by adding the standard spectra, which were scaled in proportion to the calculated concentration of five amino acids, maltitol, and citric acid. The order written in the nutrition fact sheet for Supplement B

Table 2 Calculated concentration and recovery ratio of amino acid in Supplements A and B

	Concentration, %				
	Nutrition sheet	Calculated			
		Unwrapped	Recovery ratio	Wrapped	Recovery ratio
Supplement A					
L-Arg	17.3	15.3	0.88	16.7	0.97
L-Glu	18.0	19.6	1.09	17.8	0.99
L-Ile	12.3	12.2	0.99	16.4	1.33
L-Leu	15.3	24.4	1.59	7.7	0.50
L-Val	10.3	13.3	1.29	1.7	0.17
Supplement B					
L-Arg	7.5	19.5	2.60	19.9	2.65
L-Glu	7.5	10.3	1.37	10.5	1.40
L-Ile	10.0	9.0	0.90	12.8	1.28
L-Leu	15.0	6.6	0.44	38.4	2.56
L-Val	10.0	10.3	1.03	11.7	1.17

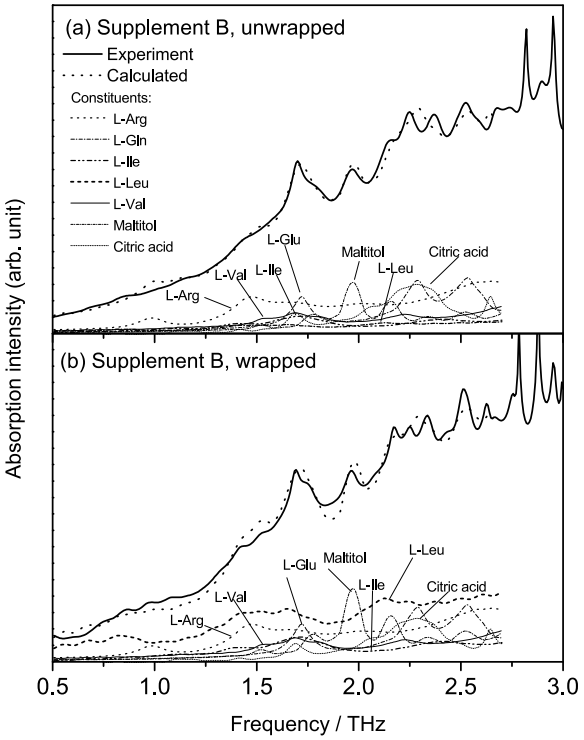

Fig. 2 THz spectra of unwrapped Supplement B tablet (a) and tablet wrapped in a weighting paper (b). Experimental (bold solid line) and calculated (bold dotted line) spectra are compared. Spectra of the constituents are overlaid to show their contributions to the calculated spectra.

indicates that the percentage of citric acid is between those of L-Leu and L-Val, although the concentration was not stated. We took citric acid into consideration in the analysis. The spectra of the five amino acid constituents, maltitol, and citric acid are overlaid to show their contributions to the calculated spectra. The experimental spectrum was well reproduced by the calculated spectrum. Figure 3(b) and Table 2 summarize the concentrations of the five amino acids shown in the nutrition fact sheet and the calculated results for Supplement B. The

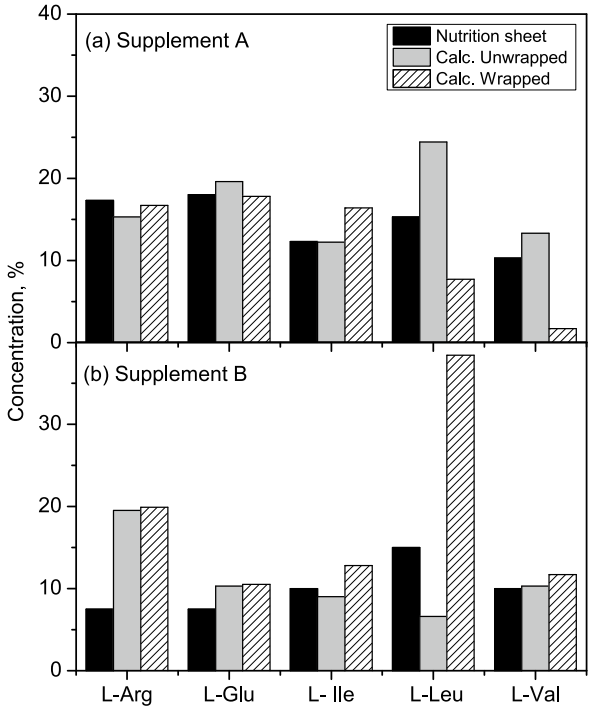

Fig. 3 Comparison of concentrations of five amino acids provided by a nutrition fact sheet (black), calculated results of unwrapped (gray) and wrapped (hatch) samples of Supplements A and B.

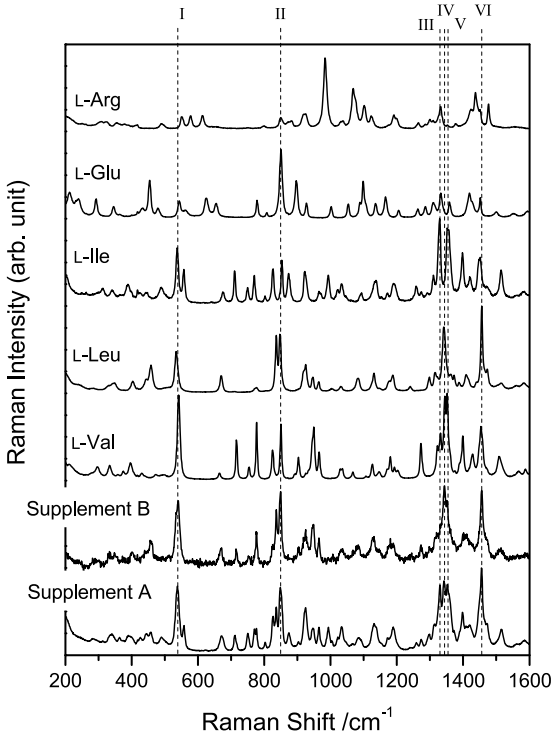

Fig. 4 Raman spectra of five amino acids, and unwrapped Supplements A and B.

recovery ratios showed that the estimation errors were within ±10% for L-Ile and L-Val. For L-Glu and L-Leu, the estimation errors were +37 and –56 %, respectively. The estimation error for L-Arg was as large as +160%. Since the amino acid concentration in Supplement B is smaller than that in Supplement A, and the concentrations of the other ingredients in Supplement B were mostly larger than that in Supplement A, the errors were larger in the quantitative analysis of the amino acids in Supplement B. The results indicate that the detection limit of THz spectroscopy is currently around a few percent, that is, about 0.01 to 0.1 μM/mm^2 for the molecules whose molecular size is about 10^2, and the accuracy of the quantitative analysis, is also several percent. This means that, unfortunately, THz spectroscopy is incapable of trace analysis and has no advantage in terms of the detectable range over other spectroscopic methods. However, advances in optical design, brighter light sources and more sensitive detectors may improve the detection limit, and thus make THz spectroscopy a more powerful analytical tool.

Supplement A, wrapped

We also measured wrapped samples of Supplements A and B by placing a tablet between sheets of weighing paper (~30 μm thick) as an example of the nondestructive inspection of a packaged tablet. The solid line in Fig. 1(b) shows the THz spectra of wrapped tablets of Supplement A in the 0.5 to 3.0 THz region. We found that wrapping in paper had very little effect on the spectral shape because the differential spectra calculated by subtracting the absorption intensity of wrapped sample from that of unwrapped sample was satisfactory flat. The peak positions and spectral shapes did not change, although the absorbance increased slightly in the overall frequency (the ratio of the absorption intensity of the difference spectra to that of unwrapped sample was 0.11 at ~1.7 THz), and thus the noise was larger at frequencies above ~2.5 THz. Thanks to the ability of THz waves to pass through paper, we can perform a quantitative analysis by using the same standard spectra as those used for the unwrapped sample. The dotted line shows the calculated spectrum obtained by adding the standard spectra, which were scaled in proportion to the calculated concentrations of five amino acids and maltitol. We did not use the noisy region above ~2.5 THz for the calculation. The experimental spectrum was well reproduced by the calculated spectrum. The recovery ratios in Table 2 show that the estimation errors were within ±33% for the three constituents (L-Arg, L-Gln, and L-Ile). For L-Leu and L-Val, the underestimation errors were 50 and 83%, respectively. Since L-Ile and L-Val both have peaks at around 1.7 THz, the estimation of L-Ile and the underestimation of L-Val can occur simultaneously by confusing the two components, which have similar THz absorption profiles. Using a wider spectral region for an unwrapped sample or changing the measurement temperature to see the difference in the spectral shapes will help to avoid confusion and allow us to improve the accuracy.

Supplement B, wrapped

The solid line in Fig. 2(b) shows the THz spectra of wrapped tablets of Supplement B in the 0.5 to 3.0 THz region. Again, we found that the effect of wrapping in paper on the spectral shape was very small, and the peak positions and the spectral shapes did not change. Due to the small overall frequency absorption, the noise was sufficiently small to allow us to observe a similar region to that for the unwrapped sample, we used the region up to 2.7 THz for the calculation for both the wrapped and unwrapped samples. The dotted line shows the calculated spectrum obtained by adding the standard spectra, which were scaled in proportion to the calculated concentrations of the five amino acids, maltitol, and citric acid. The experimental

Table 3 Comparison of the Raman peak positions of Supplements A and B with those of amino acids

Supplement A	Supplement B	L-Arg	L-Gln	L-Ile	L-Leu	L-Val
					404	
				418		
458	456		455		458	
			480			
		489		490		
539	541		544	538	534	542
		551				
558				557		
		577				
		614				
			626			
			655			
						665
671	671			675	670	
711	715			711		716
750				749		754
777	776		779		778	776
827				827		826
837	837				837	
848	849	850	850	853	847	850
875				874		
		882				
			897			
	905					904
925	925	923	928	922	925	
947	949				947	950
965	965			965	965	966
		983				
994				993		
			1003		1004	
				1023		
1033	1034	1037		1035	1032	1035
			1054			
		1068				1068
1086	1084				1083	
				1093		
		1101	1098			
		1124				
1131	1129		1136	1137	1131	1126
						1147
			1166			
				1172		
	1181					1181
1189	1190	1191		1191	1187	1193
			1205			1202
					1240	
				1258		
		1265	1263			
	1274					1273
			1286			
1299		1298			1298	
			1310	1310	1316	
	1319					1322
1330	1331	1333	1333	1329		1332
1342	1344				1341	1345
1352	1351			1355		1352
			1359			
1398	1401			1398		1399
					1409	
1419		1424	1419	1420		
						1429
		1437				
1455	1456		1452	1451	1456	1454
		1477				
						1508
1515	1514			1514	1514	

spectrum was well reproduced by the calculated spectrum. The recovery ratios revealed that the estimation errors were +40, +28, and +17 for L-Glu, L-Ile, and L-Val, respectively. The estimation errors for L-Arg and L-Leu were as large as +165 and +156%. Despite the significant change in the result for L-Leu, the degree of the estimation error was little changed by the wrapping. It is noteworthy that THz-TDS can realize almost the same level of accuracy as quantitative analysis for both packaged and unwrapped samples.

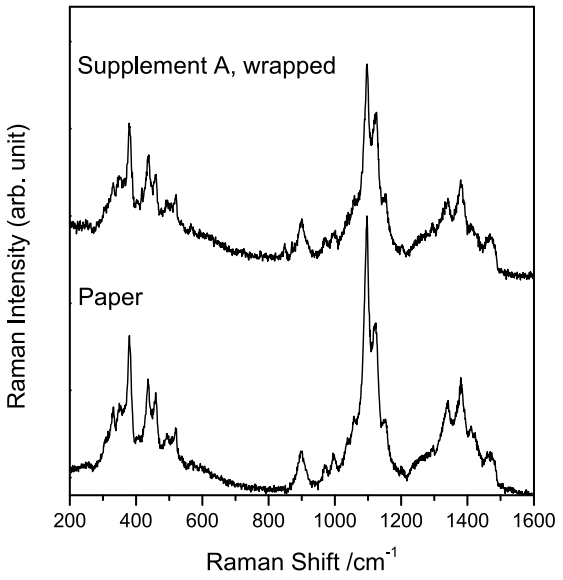

Fig. 5 Raman spectra of wrapped Supplement A and a weighting paper.

Lastly, we measured the Raman spectra of Supplements A and B and the five constituent amino acids (Fig. 4). Several sharp peaks were observed. The six large peaks are indicated with dashed lines and were observed in the spectra of both Supplements A and B. Table 3 compares the Raman peak positions. Considering the bandwidth, the peaks observed in the ±5 cm^{-1} region were regarded as being the same vibrational mode, and so are placed on the same rows in the table. The six marked peaks in Fig. 4 are shown as gray rows. We found that the contributor to the peaks cannot be identified as a single amino acid component, but is assigned as the sum of the peaks of two or more amino acids. Although a detailed analysis can reveal the degree to which each amino acid contributes to a certain peak, quantitative analysis based on a scattering method, such as Raman spectroscopy, is complicated because calibrations are often non-linear due to the variation in the intrinsic scattering efficiency with the concentration.[18] We also measured samples of Supplement A after placing the tablet between sheets of weighing paper. Figure 5 compares the Raman spectra of the wrapped Supplement A (top) and the weighing paper (bottom). The results indicate that the Raman spectra cannot detect any signals from the chemicals in Supplement A through a paper whose thickness (~30 μm) exceeds the focal depth (<10 μm). Therefore, we confirmed that compared with Raman spectroscopy THz-TDS is a powerful tool for the direct quantitative analysis of packaged samples when the concentration range of the target chemicals exceeds a few percent, that is, about 0.01 to 0.1 μM/mm^2 for the molecules whose molecular size is about 10^2.

Conclusions

We successfully analyzed five amino acid concentrations in actual dietary amino acid supplements with an error of ±12%

for the best-reproduced components. The detection limit with THz spectroscopy was around a few percent, and the accuracy of the quantitative analysis was also several percent. In addition, we succeeded to analyze tablets of commercially available dietary amino acid supplements wrapped in paper. The wrapping had a negligible effect on the spectral shape, and the peak positions and spectral shapes remained unchanged. The ability of THz waves to pass through the paper made it possible to perform a quantitative analysis using the same standard spectra as those used for the unwrapped sample. It is noteworthy that the accuracy of a direct quantitative analysis for a packaged sample was almost the same as that for an unwrapped sample. We confirmed that THz-TDS is a more powerful tool for the direct quantitative analysis of packaged samples than Raman spectroscopy, when the concentration range of the target chemicals exceeds a few percent, that is, about 0.01 to 0.1 μM/mm^2 for the molecules whose molecular size is about 10^2.

References

1. Y. Ueno and K. Ajito, *Anal. Sci*., **2008**, *24*, 185.
2. D. M. Mittleman, M. Gupta, R. Neelamani, R. G. Baraniuk, J. V. Rudd, and M. Koch, *Appl. Phys. B*, **1999**, *68*, 1085.
3. K. Kawase, Y. Ogawa, and Y. Watanabe, *Opt. Express*, **2003**, *11*, 2549.
4. E. Pickwell and V. P. Wallace, *J. Phys. D: Appl. Phys*., **2006**, *39*, R301.
5. B. M. Fischer, M. Walther, and P. U. Jepsen, *Phys. Med. Biol*., **2002**, *47*, 3807.
6. P. F. Taday, I. V. Bradley, and D. D. Arnone, *J. Bio. Phys*., **2003**, *29*, 109.
7. K. Yamamoto, K. Tominaga, H. Sasakawa, A. Tamura, H. Murakami, H. Ohtake, and N. Sarukura, *Biophys. J*., **2005**, *89*, L22.
8. B. M. Fischer, M. Hoffmann, H. Helm, R. Wilk, F. Rutz, T. Kleine-Ostmann, M. Koch, and P. U. Jepsen, *Opt. Express*, **2005**, *13*, 5205.
9. K. Yamamoto, M. H. Kabir, and K. Tominaga, *J. Opt. Soc. Am. B*, **2005**, *22*, 2417.
10. P. F. Taday, *Phil. Trans. R. Soc. Lond. A*, **2004**, *362*, 351.
11. K. L. Nguyen, T. Friščić, G. M. Day, L. F. Gladden, and W. Jones, *Nat. Mater*., **2007**, *6*, 206.
12. H. Wu, E. J. Heilweil, A. S. Hussain, and M. A. Khan, *Int. J. Pharm*., **2007**, *343*, 148.
13. J. Aaltonen, K. C. Gordon, C. J. Strachan, and T. Rades, *Int. J. Pharm*., **2008**, *364*, 159.
14. Y. Ueno, R. Rungsawang, I. Tomita, and K. Ajito, *Anal. Chem*., **2006**, *78*, 5424.
15. Z. Zhang, X. Yu, H. Zhao, T. Xiao, Z. Xi, and H. Xu, *Opt. Commun*., **2007**, *277*, 273.
16. R. Nishikiori, M. Yamaguchi, K. Takano, T. Enatsu, M. Tani, U. Chandimal de Silva, N. Kawashita, T. Takagi, S. Morimoto, M. Hangyo, and M. Kawase, *Chem. Pharm. Bull*., **2008**, *56*, 305.
17. A. R. Taulbee, J. A. Heuser, W. U. Spendel, and G. E. Pacey, *Anal. Chem*., **2009**, *81*, 2664.
18. N. Everall, "*Analytical Applications of Raman Spectroscopy*", ed. M. J. Pelletier, **1999**, Chap. 4, Blackwell Science, Oxford, 127.