Capillary near-infrared spectroscopy for microliter solutions : basic and bio-medical application

K. Murayama,[a] B. Yuan,[b] M. Tomida,[c] Y. Ozaki[b] and S. Era[a]

[a]*Department of Biochemistry and Biophysics, Gifu University School of Medicine, 40, Tsukasa-machi, Gifu 500-8705 Japan.*

[b]*Department of Chemistry and Research Center for Near-Infrared Spectroscopy, School of Science and Technology, Kwansei Gakuin University, 2-1, Gakuen, Sanda, Hyogo 669-1337, Japan.*

[c]*Department of Physiology, School of Medicine, Tokyo Women's Medical University, 8-1, Kawatacho, Shinjuku-ku, Tokyo 162-8666, Japan.*

Introduction

Near-infrared (NIR) spectroscopy has become one of the most popular non-destructive analytical techniques and is being extensively used in the fields of agriculture, foods, pharmacy, polymers, textiles, biotechnology and medicine.[1–4] In spite of the importance of NIR spectroscopy as an analytical method, spectral measurement techniques for a small amount of liquid and solution samples have not been well developed. Requirements for such techniques are especially high in the clinical and medical fields where it is not easy to obtain sufficient amount of biofluids. In other spectroscopy such as Raman spectroscopy, ESR spectroscopy, and NMR spectroscopy, it is easy to measure high quality spectra with a high signal-to-noise ratio by use of a capillary tube. A few microlitre of sample is adequate for those spectral measurements by capillary sampling techniques.

In this article, we propose a capillary tube method for measuring NIR spectra of liquids and solutions of microlitre volume. We report the basic study of capillary NIR spectroscopy for several chemicals of microlitre volume, and present the bio-medical application of this technique. The method is very promising for applications of NIR spectroscopy to a variety of biomedical samples.

Material and method

Samples

All chemicals were purchased from Wako Pure Chemical Industry Co. (Osaka, Japan), and used without further purification. Human synovial fluids were obtained from subjects with and without teporomandibular joint disorder (TMD). The TMD samples were contained into a centrifuge tube, and it was centrifuged at 3,000 rpm for 15 min using a Himac CF 15R centrifugator (Hitachi, Japan). After centrifugation the supernatant was passed through a Millipore filter, and diluted by 50 times.

Near Infrared Spectroscopy: Proceedings of the 11th International Conference.

Instruments

FT-NIR spectra were measured with a Bruker Vector 22/N NIR spectrometer equipped with a Ge diode detector, quartz beam splitter and fibre-optic cables. The fused silica fibre used had a core diameter of 600 μm. Each spectrum was obtained by co-adding 256 scans at a spectral resolution of 4 cm^{-1} and was apodised with a Happ-Genzel function. For NIR measurement a capillary tube of silica glass with a 75 mm length and a 1 mm inner diameter (Drummond Scientific Co.) and quartz cuvette cell with a 1 mm optical pathlength (GL Science) were employed, respectively. Both ends of fibers were in contact with the surfaces of the capillary tube. Several chemicals and TMD samples were inserted in a capillary tube by using a Finnpipette Digital 20-200 μl (Finland). Both ends of the capillary tube were closed by piezo gas burner. Temperature of all the samples was kept at 25°C during the NIR measurements by means of circulating water.

Spectral analysis

Spectral analysis was performed by using Omnic software (ver. 4.0, Nicolet Instrument Co.) program. Minor spectral contributions in the NIR region from residual water vapour were eliminated by subtracting a set of water vapour spectrum. Next, the spectrum of each chemical was calculated by subtracting the spectrum of an empty capillary (cuvette) from the measured spectrum of each chemical in the capillary (cuvette). Then, all the spectra were subjected to Savitsky–Golay algorithm[5] of 15 points for smoothing and the calculation of 1st derivative, respectively.

PCA analysis by using The Unscrambler software (CAMO ASA., ver 6.01) was performed for the NIR spectra of TMD samples. For PCA calculations, 13 spectra from healthy persons and 44 spectra from patients with TMD were used.

Results and discussion

Basic

Figure 1 shows FT-NIR spectra in the 7500–4000 cm^{-1} region of several chemicals measured by using a quartz capillary tube (solid line) and a quartz cell (dotted line). Note that spectral feature of benzene in the 7500–4000 cm^{-1} region measured by using the capillary tube is almost identical with that by using the cuvette cell. This result demonstrates the potential of capillary NIR spectroscopy of liquid samples of microlitre volume. NIR bands observed in the spectra can be assigned on the basis of NIR studies of various materials[1–4,6–9] as follows; In the 4600–4000 cm^{-1} region; combination modes involving CH_n stretching vibrations, in the 5200–4800 cm^{-1} region; combination modes of OH groups, in the 6200–5550 cm^{-1} region; the first overtones of CH_n stretching vibrations, and in the 7300–6200 cm^{-1} region; the first overtones of the OH stretching modes.

To elucidate the minimum volume needed to measure a NIR spectrum of liquid, NIR spectra of benzene, changing the volume of a capillary tube from 50.0 μL to 2.5 μL, were shown in Figure 2. All the obtained spectra were essentially identical; all NIR bands of benzene are observed at the same positions and irrespective of the cells and sample volumes. In addition, there are two marked advantages of the capillary tube method over the cuvette cell method. (1) The amount of sample by capillary tube method needs much less than that by the quartz cell method. Even if one cannot obtain a considerable amount of solutions, the application of capillary tube can make it possible to perform NIR measurement. (2) Another advantage is easy to discard capillary tubes.

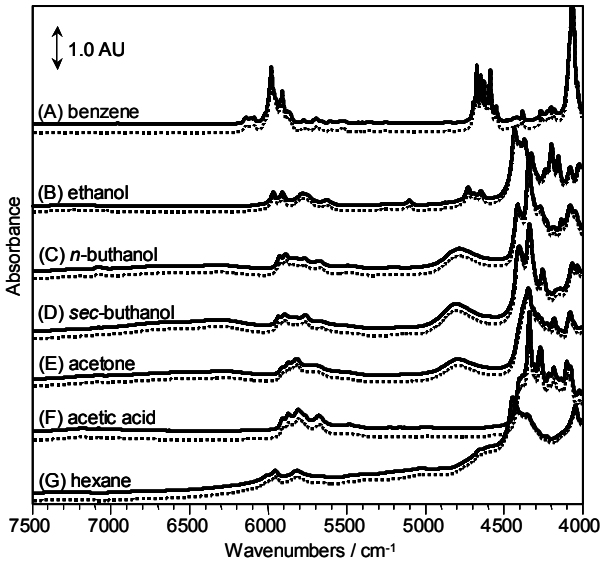

Figure 1. FT-NIR spectra in the 7500–4000 cm^{-1} region of (A) benzene, (B) ethanol, (C) *n*-butanol, (D) *sec*-butanol, (E) acetone, (F) acetic acid and (G) hexane. Solid lines; the spectra obtained using the capillary tube, dotted lines; the spectrum of benzene using the quartz cell. Spectra were moved up and down for clear representation.

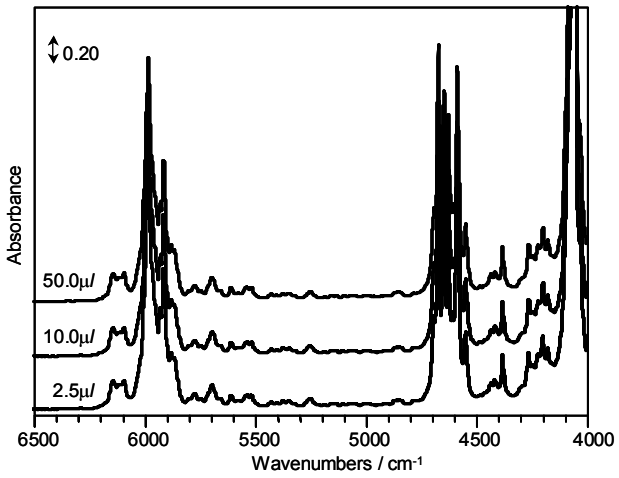

Figure 2. NIR spectra of benzene in the capillary tube in the 6500–4000 cm^{-1} region, which volumes were 50.0, 10.0 and 2.5 μL, respectively. Spectra were moved up and down for clear representation.

Biomedical application

Capillary NIR spectra of human synovial fluids are almost identical with a NIR spectrum of water.[10] For discrimination between healthy samples and those of TMD disease, PCA analysis was performed for the 1st derivatives of the capillary NIR spectra of human synovial fluids obtained from subjects with and without TMD. Figure 3 displays the 3D scatter plot of principle component of 2, 3 and 4 based on the 1st derivatives of capillary NIR spectra in the 8000–5500 cm^{-1} region. It can be seen from Figure 3 that each group of healthy and TMD is clearly discriminated in the 3D scatter plot. Hence, the result demonstrates that capillary NIR spectroscopy has great potential in bio-medical applications of very small amounts of bio-fluids.

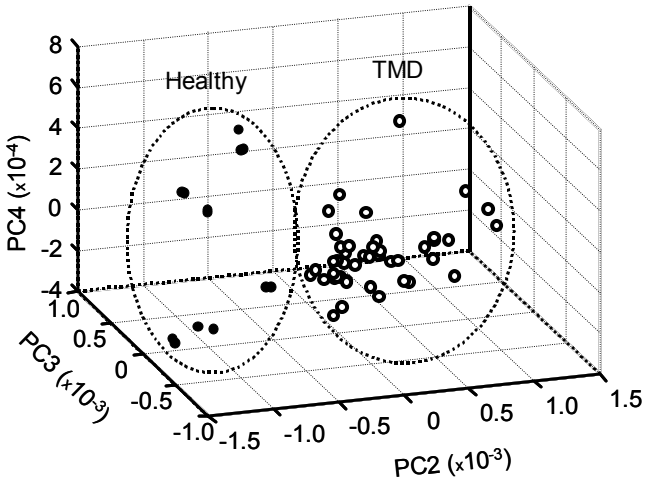

Figure 3. 3D scatter plot for principle components of 2, 3 and 4 calculated based on 1st derivatives of capillary NIR spectra in the 8000–5500 cm^{-1} region of human synovial fluid obtained from subjects with and without TMD. Closed and open circles indicate healthy and patiants, respectively.

Conclusion

We have proposed a capillary tube method for measuring NIR spectra of liquids and solutions of microlitre volume. This method enables us to measure NIR spectra of liquids of only 2.5 μL volume. In this paper, the usefulness of capillary NIR spectroscopy with chemometrics for bio-medical application has been demonstrated. PCA analysis based on capillary NIR spectra obtained from subjects with and without human synovial fluids has successfully discriminated between healthy samples and those of TMD disease. The present results suggest that capillary NIR spectroscopy will open to a new area of NIR spectroscopy for bio-medical applications.

References

1. P. Williams and K. Norris (Eds), *Near-Infrared Technology in the Agricultural and Food Industries,* 2nd Edn. American Association of Cereal Chemists, St Paul, MN, USA (1990).
2. D.A. Burns and E.W. Ciurczak (Eds), *Handbook of Near-Infrared Analysis*. Marcel Dekker, New York, USA (1992).

3. B.G. Osborne, T. Fearn and P.H. Hindle, *Practical Near Infrared Spectroscopy with Applications in Food and Beverage Analysis*. Longman Scientific & Technical, Essex, UK (1993).
4. H.W. Siesler, Y. Ozaki, S. Kawata and H.M. Heise (Eds), *Near Infrared Spectroscopy—Principles, Instrumentations, and Applications*. Wiley-VCH, Weinheim, Germany (2002).
5. A. Savitzky and M.J.E. Golay, *Anal. Chem*. **36,** 1627 (1964).
6. G. Socrates, *Infrared Characteristic Group Frequencies*. John Wiley & Sons, New York (1997).
7. H. Maeda, Y. Wang, Y. Ozaki, M. Suzuki, M.A. Czarnecki and M. Iwahashi, *Chemom. Intell. Lab. Syst*. **45,** 121 (1999).
8. Y. Katsumoto, D. Adachi, H. Sato and Y. Ozaki, *J. Near Infrared Spectrosc*. **10,** 85 (2002).
9. K. Murayama, K. Yamada, R. Tsenkova and Y. Ozaki, *Fresenius' J. Anal. Chem*. **362,** 155 (1998).
10. H. Maeda, Y. Ozaki, M. Tanaka, N. Hayashi and T. Kojima, *J. Near Infrared Spectrosc*. **3,** 191 (1995).