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Capillary method for measuring near-infrared spectra of microlitre volume liquids

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Abstract: The present study theoretically explored the feasibility of the capillary method for measuring near-infrared (NIR) spectra of liquid or solution samples with microlitre volume, which was proposed in our previous studies. Lambert-Beer absorbance rule was applied to establish a model for the integral absorbance of capillary, which was then implemented in numerical analyses of the effects of capillary on various spectral features and dynamic range of absorption measurement. The theoretical speculations indicated that the capillary method might be used in NIR spectroscopy, which was further supported by the empirical data collected from our experiments by comparison between capillary NIR spectra of several organic solvents and cuvette cell NIR spectra.

Key words: Near-infrared (NIR) spectroscopy, Capillary, Cuvette cell, Numerical analysis
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INTRODUCTION

The bands of near-infrared (NIR) spectra have low absorptivities, which allow in situ analysis of chemical and biological materials with no or little sample preparation. Therefore, as one of the most important nondestructive analytical techniques, NIR spectroscopy is being extensively applied in the fields of agriculture, industry, biotechnology and medicine (Siesler *et al*., 2002; Scarff *et al*., 2006; Ward *et al.*, 2006). Recently, with instrumental developments such as Fourier-transform (FT) spectrometers, diode arrays and light-fiber probes (Siesler *et al*., 2002) and data manipulation progress such as chemometrics (Ozaki *et al*., 2001; Yuan *et al.*, 2003; Wu and Chen, 2004; van der Greef and Smilde, 2005) and 2D correlation spectroscopy (Noda, 2000; Dou *et al*., 2004), NIR spectroscopy has become more useful and versatile.

In spite of the wide recognition of the importance of NIR spectroscopy in the analytical fields, spectral measurement techniques for small amounts of liquid or solution samples have not been well developed. Nevertheless, the requirements for such techniques are especially high in some research fields. For example, in the clinical and medical studies, it is always difficult to obtain sufficient bio-fluids such as cerebrospinal fluid, interstitial fluid, aqueous humor, saliva, and serous fluid to analyze.

In our previous studies (Murayama *et al.*, 2003a; 2003b), a capillary tube method for NIR spectroscopy was proposed in the analysis of solutions with microliter volume in which the spectra of liquid samples in capillary were compared with those in cuvette cell. It was recognized that this technique might expand the potential of NIR spectroscopy into new fields such as laboratory medicine, but due to limitation in time and energy, few discussions have been carried out in this respect.

The present study attempts to fill the gap by exploring the feasibility of capillary method for meas-

uring NIR spectra of liquid or solution samples at microlitre volume from a theoretical perspective. Taking the capillary geometry into consideration, the absorbance model of liquid samples in capillary has been established by using Lambert-Beer absorbance rule. On the basis of this model, numerical calculations were carried out for straight line, absorption peaks of singlet, doublet and shoulder, and experimental spectrum, respectively. Additionally, the effect of capillary cell on the dynamic range of absorption measurement was evaluated by comparison with that of cuvette cell. The numerical results and empirical data obtained from comparison of spectra of several organic solvents used in capillary method and cuvette cell method proved that capillary method can be directly applied in NIR spectroscopy.

THEORETICAL ANALYSIS

The absorbance model of capillary

Figs.1a and 1b show the cross sections of optics for measurement of NIR spectroscopy using cuvette cell and capillary, respectively. We can see clearly from Fig.1 that there is obvious difference between the two in that the optical path length of cuvette cell is uniform, while that of capillary is variable as a function of distance from optical axis. Let us suppose that origin is the center of capillary, and x-axis is perpendicular to light beam and passing the origin. Then optical path length of capillary d_c is given by

$$d_c = \sqrt{R^2 - 4x^2} \quad (x \le R/2), \tag{1}$$

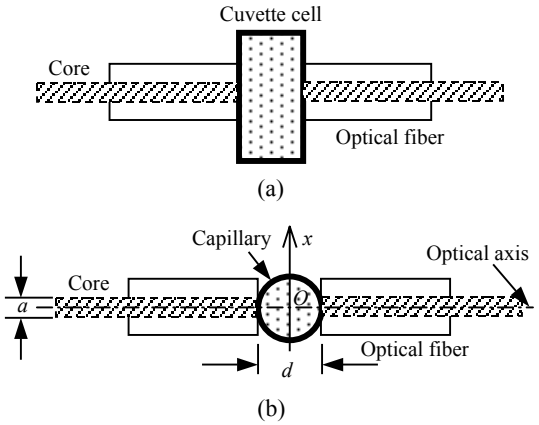

Fig.1 Cross-sections of optics for (a) cuvette cell and (b) capillary

where R is inner diameter of capillary.

It is convenient to compare the effects to absorbance between cuvette cell method and capillary method by supposing that the optical path length of cuvette cell d is equal to the capillary radius R. Furthermore, let us assume that the light beam is strictly parallel and that the cell is uniformly illuminated. According to Lambert-Beer absorbance rule, absorbance obtained by use of cuvette cell can be simply written as

$$A=\log(I_0/I)=\alpha d, \tag{2}$$

where α is extinction coefficient, I_0 is optical intensity of incident beam and I is that of transmission beam.

If the homogeneously illuminated capillary region is limited at $-a/2 \le x < a/2$, by use of Lamber-Beer absorbance rule and Eq.(2), integral mean absorbance of capillary A_c can be calculated to be

$$A_c = -\log \frac{\int_{-a/2}^{a/2} 10^{-\frac{A}{d}\sqrt{d^2-4x^2}}\,\mathrm{d}x}{a}. \tag{3}$$

It can be inferred directly from the above equation that effective absorbance measured using capillary does not satisfy the Lambert-Beer absorbance rule. However, we cannot obtain more information on the effect of capillary to absorption since Eq.(3) is non-integrable. Therefore, numerical analysis must be carried out to further investigate the capillary method.

Capillary effect on spectral features

The principle of numerical analysis is that the original spectrum obtained using cuvette cell is given first and spectrum measured by use of capillary is calculated point by point according to the capillary absorbance model described above. Taking into account the sizes of optical fiber and capillary in our previous studies, the ratio of a/d=3/5 was used. Numerical calculations were performed using software Matlab 7.0.

Although the flat feature in the spectroscopy contributes little to spectral analysis, to carry out numerical calculation for a straight line is beneficial for gaining understanding on the capillary effect. Fig.2 shows the capillary behavior to a linear spectrum, from which we observed that the calculated spectrum is slightly bent, which indicates that the

effect of the capillary on the spectrum is nonlinear. It seems that it is inappropriate to apply the capillary method in spectroscopic techniques, as will be shown in the following section.

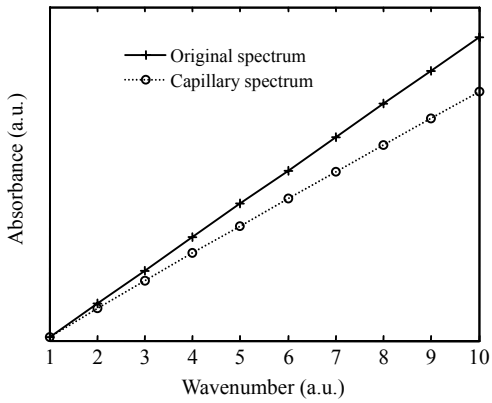

Fig.2 Numerical result for a straight line

To investigate the feasibility of capillary method in NIR spectroscopy, it is necessary to analyze the capillary effects on the absorption peaks, which are the most important features in spectral analysis. Since the absorption peaks of singlet, doublet and shoulder are very common spectral features, numerical calculations were carried out for them and the results are shown in Fig.3. It is observed that calculated capillary spectra can well preserve the shapes of absorption peaks of original spectra except for a little decreasing in the absorption intensity. To further study the capillary effects on the positions of absorption peaks, we calculated the corresponding secondary derivatives of the spectra in Fig.3, which are shown in Fig.4. It can be derived from Fig.4 that capillary method does not cause the shifts of absorption peaks. Therefore, we can conclude that introduction of capillary in the measurement does not distort or obscure the absorption peaks.

To further investigate the capillary effect on arbitrary spectral shapes, the NIR spectrum of methanol was measured using cuvette cell as original spectrum in the numerical analysis and the corresponding capillary spectrum was calculated as shown in Fig.5. We observed that the calculated spectral features are almost identical with original spectral features, which indicates that capillary method does not affect the spectral analysis.

In summary, the above theoretical analyses showed that capillary method is absolutely reliable for spectral analysis, which will be evidenced by the experimental results in the following section.

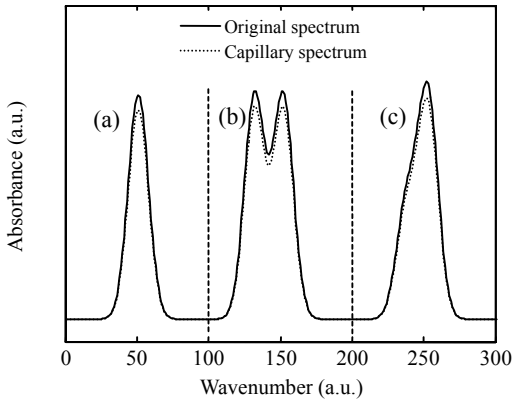

Fig.3 Numerical results for absorption peaks of (a) singlet, (b) doublet and (c) shoulder

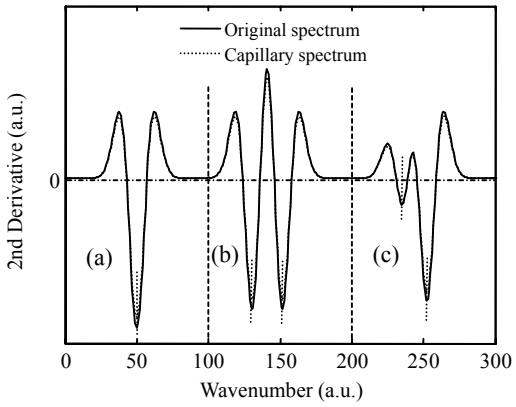

Fig.4 Corresponding secondary derivatives of the spectra of (a) singlet, (b) doublet and (c) shoulder in Fig.3

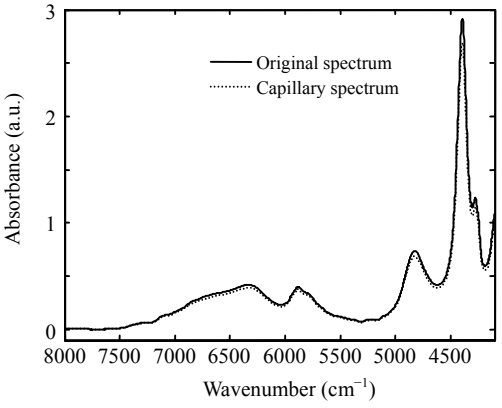

Fig.5 Numerical result of NIR spectrum of methanol in the spectral region of 8000~4100 cm^{-1}

Dynamic range improvement of absorption measurement

The dynamic range of absorption measurement is limited by the increase in relative absorbance error at extremely high or low transmittance. Since the linear absorption spectrum was obtained by converting transmittance in the instrument's computer, the absorption accuracy can be evaluated by defining an accuracy ratio for absorption spectroscopy as described by Hirschfeld (1985):

$$M=\frac{SNR_{\mathrm{A}}}{SNR_{\mathrm{T}}}=A\frac{\Delta T}{\Delta A}, \tag{4}$$

where ΔA is absorbance's uncertainty, ΔT is that of the photometry, SNR_{A} and SNR_{T} are the signal-to-noise ratios of the absorbance and transmittance, respectively.

For the conventional cuvette cell, we can directly calculate

$$M=10^{-A}A\ln 10, \tag{5}$$

while for the combined effect of the capillary cell and its mathematical rectification we have

$$M_{\mathrm{c}}=A\frac{\partial A_{\mathrm{c}}/\partial A}{\partial A_{\mathrm{c}}/\partial T_{\mathrm{c}}}, \tag{6}$$

giving

$$M_{\mathrm{c}}=\frac{\ln 10}{a}\int_{-a/2}^{a/2}10^{-A\sqrt{1-4x^2/d^2}}\sqrt{1-4x^2/d^2}\,\mathrm{d}x. \tag{7}$$

Given a minimum accuracy ratio, the dynamic ranges of absorption measurement for cuvette cell and capillary cell are compared in Fig.6. Clearly, introduction of capillary in the measurement substantially improves the dynamic range for absorption measurement, which indicates that a higher signal-to-noise ratio of spectrum will be obtained by using capillary cell instead of cuvette cell.

EXPERIMENTAL DETAILS

In order to verify the above conclusions from theoretical analysis and illustrate advantages of capillary method, NIR spectra of liquid or solution samples were measured and analyzed. Experimental procedures and spectral manipulations are the same as those described in our previous study (Murayama *et al.*, 2003a; 2003b). Here, we carefully compared the NIR spectra of organic solvents in the capillary and cuvette cell.

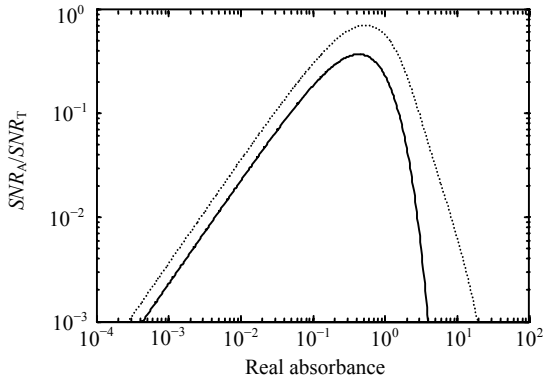

Fig.6 Dynamic ranges of absorption measurement for cuvette cell (solid line) and capillary (dotted line)

Comparison of NIR spectra measured using capillary and cuvette cell

Fig.7 shows FT-NIR spectra in the 7500~4000 cm^{-1} region of several organic solvents measured by using capillary and cuvette cell, respectively. It is clearly observed that spectral profiles of organic solvents obtained by capillary method are quite similar to those obtained by cuvette cell method. The results demonstrate the potential of capillary NIR spectroscopy in the spectral analysis of liquid samples.

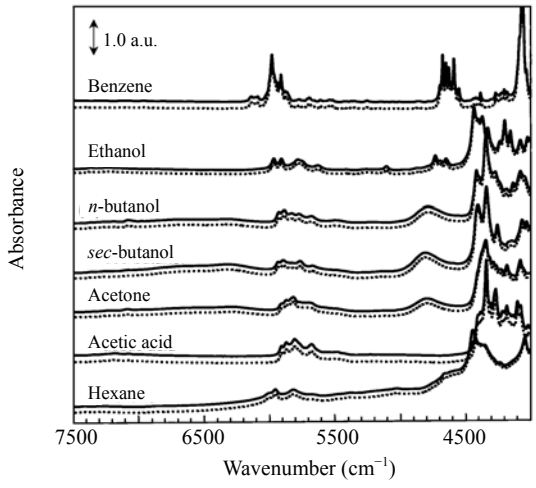

Fig.7 FT-NIR spectra in the 7500~4000 cm^{-1} region of benzene, ethanol, *n*-butanol, *sec*-butanol, acetone, acetic acid and hexane in cuvette cell (solid lines) and capillary (dotted lines), respectively

NIR bands in Fig.7 can be assigned on the basis of NIR studies of various materials (Maeda *et al*., 1999; Siesler *et al.*, 2002; Katsumoto *et al.*, 2002; Yuan and Dou, 2004). The bands in the 4600~4000 cm^{-1} region and the bands in the 6200~5550 cm^{-1} region are both due to CH_n vibrations. The former arises from combination modes, while the latter results from the first overtones. The bands in the 5200~4800 cm^{-1} region are assigned to combination modes of OH groups, and those between 7300 and 6200 cm^{-1} are due to the first overtones of the OH stretching mode.

Note that amount of sample consumed in the measurement by capillary method is only 2.5 μl, while that for cuvette cell is 0.4 ml. Therefore, once one cannot obtain a considerable amount of solutions, the capillary method can make it possible to perform NIR spectroscopic measurement, which is the key point of capillary method in NIR spectroscopy. Furthermore, capillary method has advantage over cuvette cell method in that the cuvette cell must be washed before it is used in the experiment, while it is possible to discard the capillary tube because of its low cost.

CONCLUSION

In this paper the capillary method in NIR spectroscopy for small amounts of liquid or solution samples, which was proposed in our previous studies, has been proven to be useful in theory on the basis of the established absorbance model and numerical analyses, and by the experimental data also. Although the effect of capillary on the spectrum is nonlinear, capillary method does not distort or obscure the spectral features but improve the dynamic range of absorption measurement compared with cuvette cell method. Moreover, capillary NIR spectra were well measured for several organic solvents and used in spectral analysis, which further demonstrates the potential of capillary method in the application of NIR spectroscopy for liquids with microliter volume.

References

Dou, X.M., Yuan, B., Zhao, H.Y., Yin, G.Z., Ozaki, Y., 2004. Generalized two-dimensional correlation spectroscopy: theory and applications in analytical field. *Science in China Ser. B*, **47**(3):257-266. [doi:10.1360/03yb0085]

Hirschfeld, T., 1985. Lens and wedge absorption cells for FT-IR spectroscopy. *Applied Spectroscopy*, **39**(3):426-430. [doi:10.1366/0003702854248494]

Katsumoto, Y., Adachi, D., Sato, H., Ozaki, Y., 2002. Usefulness of a curve fitting method in the analysis of overlapping overtones and combinations of CH stretching modes. *Journal of Near-Infrared Spectroscopy*, **10**:85-91.

Maeda, H., Wang, Y., Ozaki, Y., Suzuki, M., Czarnecki, M.A., Iwahashi, M., 1999. A near-infrared study of hydrogen bonds in alcohols—comparison of chemometrics and spectral analysis. *Chemometrics and Intelligent Laboratory Systems*, **45**(1-2):121-130. [doi:10.1016/S0169-7439(98)00096-3]

Murayama, K., Yuan, B., Tomida, M., Ozaki, Y., Era, S., 2003a. Capillary Near-infrared Spectroscopy for Microliter Solutions: Basic and Bio-medical Application. 11th International Conference on Near Infrared Spectroscopy, Cordoba, Spain.

Murayama, K., Yuan, B., Ozaki, Y., Tomida, M., Era, S., 2003b. Near-infrared spectroscopy for liquids of microliter volume using capillaries with wall transmission. *Analyst*, **128**(2):957-959. [doi:10.1039/b301142a]

Noda, I., 2000. Determination of two-dimensional correlation spectra using the Hilbert transform. *Applied Spectroscopy*, **54**(7):994-999. [doi:10.1366/0003702001950472]

Ozaki, Y., Šasic, S., Jiang, J.H., 2001. How can we unravel complicated near infrared spectra?—Recent progress in spectral analysis methods for resolution enhancement and band assignments in the near infrared region. *Journal of Near-Infrared Spectroscopy*, **9**:63-95.

Scarff, M., Arnold, S.A., Harvey, L.M., McNeil, B., 2006. Near-infrared spectroscopy for bioprocess monitoring and control: current status and future trends. *Critical Reviews in Biotechnology*, **26**(1):17-39. [doi:10.1080/07388550500513677]

Siesler, H.W., Ozaki, Y., Kawata, S., Heise, H.W., 2002. Near-infrared Spectroscopy: Principles, Instruments, Applications. Wiley-VCH, Weinheim, p.75-84, 179-269.

van der Greef, J., Smilde, A.K., 2005. Symbiosis of chemometrics and metabolomics: past, present, and future. *Journal of Chemometrics*, **19**(5-7):376-386. [doi:10.1002/cem.941]

Ward, K.R., Ivatury, R.R., Barbee, R.W., Terner, J., Pittman, R., Torres, I.P., Spiess, B., 2006. Near-infrared spectroscopy for evaluation of the trauma patient: A technology review. *Resuscitation*, **68**(1):27-44. [doi:10.1016/j.resuscitation.2005.06.022]

Wu, X.H., Chen, D.Z., 2004. Recent development of non-linear patrital least squares in chemometrics. *Chinese Journal of Analytical Chemistry*, **32**(4):534-540 (in Chinese).

Yuan, B., Dou, X.M., 2004. Near-infrared spectral studies of hydrogen-bond in water-methanol mixtures. *Spectroscopy and Spectral Analysis*, **24**(11):1319-1322 (in Chinese).

Yuan, B., Murayama, K., Wu, Y., Tsenkova, R., Dou, X.M., Era, S., Ozaki, Y., 2003. Temperature-dependent near-infrared spectra of bovine serum albumin in aqueous solution: spectral analysis by principal component analysis and evolving factor analysis. *Applied Spectroscopy*, **57**(10):1223-1229. [doi:10.1366/000370203769699072]