

Title	Distinction between some saccharides in scattered optical sum frequency intensity images
Author(s)	Mizutani, G.; Koyama, T.; Tomizawa, S.; Sano, H.
Citation	Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 62(4-5): 845-849
Issue Date	2005-12
Type	Journal Article
Text version	author
URL	http://hdl.handle.net/10119/4185
Rights	NOTICE: This is the author's version of a work accepted for publication by Elsevier. G. Mizutani, T. Koyama, S. Tomizawa and H. Sano, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 62(4-5), 2005,845-849, http://dx.doi.org/10.1016/j.saa.2005.03.014
Description	

Distinction between some saccharides in scattered optical sum frequency intensity images

G. Mizutani*, T. Koyama, S. Tomizawa, and H. Sano¹

*School of Materials Science, Japan Advanced Institute of Science and
Technology, 1-1 Asahidai, Tatsunokuchi, Ishikawa 923-1292, Japan*

(Received)

Abstract

Using an optical sum frequency (SF) microscope with visible and infrared light pulses for excitation, we have attempted to distinguish among four saccharide species. The saccharides we studied were D-glucose, amylopectin, β -cyclodextrin, and amylose. The wavelength of the infrared light was resonant to CH vibration. Amylose showed very weak sum frequency scattering for CH vibration among the four saccharides. As for the other three saccharides, we found a big difference in the sum frequency spectra of their CH stretching vibration near 2900cm^{-1} , when the incident visible and infrared light pulses were p- and s-polarized, respectively. Based on these facts, we have demonstrated a distinction between these three saccharides in the scattered SF intensity images.

Keywords: Optical sum frequency microscope, D-glucose, amylopectin, β -cyclodextrin, amylose

*Corresponding author: mizutani@jaist.ac.jp, tel: +81-761-51-1521, fax: +81-761-51-1149

¹E-mail address: h-sano@jaist.ac.jp

1. INTRODUCTION

Optical sum frequency (SF) spectroscopy has been used quite widely in recent years for studying molecular vibrations in various materials. The nonlinear susceptibility for the optical sum frequency generation (SFG) is proportional to the product of the infrared and Raman transition moments for the molecular vibration [1-3]. This fact, together with the effect of the non-resonant nonlinear process, complicates the spectral shapes of the SF intensity for some molecules [4].

In this paper we try to utilize the complexity of SF intensity spectra in order to distinguish among saccharides species in their scattered SF intensity images. There are a number of applications of microscopes using the other vibrational spectroscopies, i. e. Raman microscopes [5][6], and IR microscopes [5][6][7], and they show distinction between molecular species using the spectroscopic information. Thus it is desirable that such a trial should also be made with SF microscopes.

Several examples of using sum frequency microscopes for actual observation can be found [8][9][10][11][12][13]. Flörsheimer et al reported the first SF intensity image using a one-monolayer Langmuir-Blodgett film in the rear excitation geometry [8]. Schaller et al obtained images due to strain and impurities on a chemical vapor deposited (CVD) disk of ZnSe with near-field scanning optical microscope, varying the infrared (IR) wavelength from 3.1 to 4.4 μm [10][11]: they achieved a spatial resolution of 190 nm. Hoffmann et al discussed the optimization of the setup of a reflection-type sum-frequency microscope [12]. Kuhnke et al have demonstrated a distinction between different species of surface adsorbed thiolate in SF intensity images [13]. However, there has been very few examples of distinguishing between material species

using optical sum frequency microscopes except the last one.

In this study we have developed a microscope for observing scattered sum frequency light and have attempted to distinguish among four saccharides. The observation of saccharides is motivated by our previous finding that starch granules in water plants have large second-order nonlinear optical effect [14]. Also, the detection of saccharides in human body fluids is important for biomedical applications [15]. As will be shown in this paper, we took advantage of the difference in the complex spectral shapes of SF intensity among these saccharides.

2. EXPERIMENT

The experimental setups used for scattered sum frequency intensity spectra and sum frequency images are shown in Fig. 1. The visible light of wavelength 532nm is the doubled frequency output from a mode-locked cavity damped Nd:YAG laser (Continuum PY61) operating at 10 Hz and with the pulse width of 35 ps, and the wavelength tunable infrared light is an output from an optical parametric generator and amplifier system (Continuum Mirage 8000) driven by the fundamental and second harmonic output of the same Nd:YAG laser. The spectral bandwidth of the IR beam is 6cm^{-1} .

When measuring the scattered SF intensity spectra (Fig. 1(b)), the visible beam and the IR beam of frequencies from 2750cm^{-1} to 3030cm^{-1} were incident on the sample with incident angles of 45° and 50° , respectively. The pulse energies of the two beams were $10\mu\text{J}$ and $70\mu\text{J}$. The visible beam was either p- or s-polarized and the IR beam was always s-polarized. The scattered sum frequency light with no polarization

selection was passed through a double monochromator in a subtractive dispersion mode serving as a band-pass filter with energy window of 300 cm^{-1} , and was detected by a photomultiplier (Fig. 1(b)). The wavelength of the IR beam was scanned. The accumulation time at each IR frequency was 30 sec.

When recording the scattered SF intensity images, the samples were set in front of an objective lens of microscope optics as shown in Fig. 1(c). Visible light was focused on the sample with a lens of 450 mm focal length and the infrared light was focused with a gold concave mirror with the radius of curvature of 800 mm. A filter was inserted between the sample and the objective lens to block the Rayleigh scattering of the visible excitation light from the sample. The power of the visible and IR light pulses was $20 \mu\text{J}$ and $65 \mu\text{J}/\text{pulse}$, respectively. The visible beam was either p-polarized and the IR beam was s-polarized. The polarization of the scattered sum frequency light was not selected. The imaging optics was a commercial microscope (Olympus BX60) equipped with a band-pass filter with the center wavelength at 460nm and the band width of 10nm (Asahi MX460). The microscope was equipped with a charge-coupled device camera with a time-gated image intensifier (II-CCD). The size of the observed area was $200 \mu\text{m}\phi$ to $2 \text{ mm}\phi$ depending on the magnification of the objective lens from X50 down to X5. The size of each pixel of the CCD was $20 \mu\text{m} \times 20 \mu\text{m}$. With the objective lens of magnification X50, the pixel size on the CCD chip corresponds to an area of $0.67 \mu\text{m} \times 0.67 \mu\text{m}$ on the sample surface, and it is roughly equal to the diffraction limit of the generated SF light. The effective spatial resolution of the system was worse, because there is a correlation between the electric signals from adjacent CCD pixels. The spatial resolution of the system was confirmed to be better

than 2.2 μm . The accumulation time for one image was 3600 sec.

The samples were research grade powders of D(+)-glucose (Nacalai Tesque), amylopectin from maize (Fluke), amylose (type III) from potato (Sigma), and β -cyclodextrin (Sigma Aldrich). They were first dissolved into methanol and the resultant slurry was spread with the thickness of several hundred microns on Si(100) wafers and was dried. The excitation light beams were incident onto the wafers. The focal size of the visible light was approximately 5 mm. The focal size of the infrared light was difficult to measure, but it was larger than that of the visible light. The laser power was set well below the damage threshold of the samples.

3. RESULTS AND DISCUSSION

3.1 SF intensity spectra of saccharides

In Figs. 2 and 3 we show the scattered sum frequency intensity spectra of D-glucose, amylopectin, β -cyclodextrin, and amylose as a function of the IR frequencies for the polarization combinations of sum frequency, visible, and IR light (all, s, s) and (all, p,s), respectively. We find that amylose shows very weak SF intensity for both polarization combinations. For the (all, s, s) polarization combination the other three saccharides show peaks around 2900cm^{-1} with different background intensity (Fig. 2). D-glucose shows a peak at 2880 cm^{-1} with a shoulder at 2860 cm^{-1} , and a peak at 2940cm^{-1} , amylopectin shows peaks at 2890 cm^{-1} , and 2950cm^{-1} , and β -cyclodextrin shows a peak centered at 2910cm^{-1} with a slight structure at 2880cm^{-1} . The absolute SF intensity from amylopectin for both polarization combinations is much weaker than from D-glucose and β -cyclodextrin.

The shoulder at 2860cm^{-1} and peaks at 2880cm^{-1} and 2940cm^{-1} for D-glucose in Fig. 2 could be assigned either to the vibrations of the CH and CH_2 groups, but the details of the assignment is a future problem. The spectra from amylopectin and β -cyclodextrin are different from that of D-glucose, and the origin of this difference is not clear, either.

The spectra from the three saccharides for the polarization combination (all, p, s) are different from those for (all, s, s), as shown in Fig. 3. Specifically, D-glucose shows a dip at the IR frequency of 2880cm^{-1} . The energy separation of the two peaks for amylopectin is larger than that in Fig. 2. The spectra for β -cyclodextrin are similar in the two polarization combinations. Here, we notice that the SF intensity at 2960cm^{-1} is larger than, similar to, and smaller than those at 2910cm^{-1} for D-glucose, amylopectin, and β -cyclodextrin, respectively. These interesting intensity relations is used later in the analysis of the SF intensity images of these saccharides.

Mizutani et al suggested that the intense second-order nonlinear optical response from starch is due to the periodic and well-oriented arrangement of D-glucopyranose in the starch granule [14]. Thus the difference in the SF spectra of the four saccharides in Figs. 2 and 3 are suggested to result from the higher order structure of the D-glucopyranose units in the saccharides' microcrystals. The reason for the weak SF intensity from amylopectin and amylose may be that the arrangement of CH bonds in their crystal structures could have total or partial inversion symmetry or disorder.

3.2 Distinction between saccharide species in scattered SF intensity images

In Fig. 4 (c) and (d) we show scattered SF intensity images of the CH vibration of three saccharides, D-glucose, β -cyclodextrin, and amylopectin for the polarization combinations (all, p, s) with the frequency of the IR light at 2910cm^{-1} and 2960cm^{-1} , together with a normal microscopic image of the saccharides illuminated by white light (Fig. 4 (a)) and one illuminated only by the visible exciting light at 532nm (Fig. 4(b)) with the experimental setup in Fig. 1(a) and (c). Fig. 4(b) indicates that the Rayleigh scattering of the visible excitation beam reaching the detector is negligible. The Rayleigh scattering of the IR beam is not the origin of the image in Fig. 4(c) to (d), either, since our detector does not have a sensitivity to infrared light.

Fig. 4(c) and (d) show that the β -cyclodextrin shows much stronger SF intensity at 2910cm^{-1} than at 2960cm^{-1} . α -D-glucose and amylopectin show a little stronger and weaker SF intensity at 2960cm^{-1} than at 2910cm^{-1} , respectively, as can be found from the intensity integrated over each saccharide sample. The ratios of the integrated SF intensity at 2960cm^{-1} to those at 2910cm^{-1} are 1.08, 0.30 and 0.92 for α -D-glucose, β -cyclodextrin and amylopectin, respectively. The corresponding values read in the spectra in Fig. 3 are 1.64, 0.28 and 1.26. Normalizing these values to those of amylopectin, considering the dependence of the sensitivity of the microscope on the wavelength, we have ratios of 1.17, 0.33 and 1.0 from Fig. 4 and 1.26, 0.22 and 1.0 from Fig. 3. These results are roughly consistent with each other.

Using the above results, the four saccharides can be distinguished in their SF intensity images by comparing the intensities at 2910cm^{-1} and 2960cm^{-1} in the (all, p, s) polarization combination. Namely, the sample emitting negligible SF light is amylose, the one emitting SF light of similar intensity at 2910cm^{-1} and 2960cm^{-1} is amylopectin, the one emitting a little stronger SF light at 2960cm^{-1} than at 2910cm^{-1} is D-glucose,

and the one emitting much weaker SF light at 2960cm^{-1} than at 2910cm^{-1} is β -cyclodextrin. This distinction method is valid only for this combination of saccharides, but it is suggestive of its capability of distinguishing between other combination of molecules.

Within the extent of this study it is not possible to judge whether sum frequency microscopy is a superior tool to other vibrational microscopies or not. However, in some cases such as the study of surface adsorbed molecules or the application to pump and probe experiments with ultrafast time resolution [16], sum frequency microscopy can be of powerful use. The information offered in this work will provide support in such cases.

4. CONCLUSION

We have obtained scattered optical sum frequency intensity spectra of CH vibration from four saccharides' powder. As far as the vibrational modes near 2900cm^{-1} are concerned, the SF spectra were found to be sensitive to the difference in saccharide species. Using the dependence of the scattered SF spectra of CH peaks on the saccharide species for the (all, p, s) polarization combinations, we have distinguished between the three saccharides in imagery from an optical sum frequency microscope.

REFERENCES

- [1] J. Miragliotta, R. S. Polizotti, P. Rabinowitz, S. D. Cameron, and R. B. Hall, *Appl. Phys. A* **51** (1990) 221.
- [2] A. Morita and J. T. Hynes, *Chem. Phys.* **258** (2000) 371.
- [3] J. Y. Huang and Y. R. Shen, *Phys. Rev.* **A49** (1994) 3973.
- [4] C. Humbert, L. Dreesen, A. A. Mani, Y. Caudano, J. -J. Lemaire, P. A. Thiry, and A. Peremans, *Surf. Sci.* **502-503** (2002) 203.
- [5] P. J. Treado and M. D. Morris, *Appl. Spectrosc. Rev.* **29** (1994) 1.
- [6] V. F. Kalasinsky, *Appl. Spectrosc. Rev.* **31** (1996) 193.
- [7] H. H. Rotermund, *phys. stat. sol. A* **188** (2001) 1537.
- [8] M. Flörsheimer, C. Brillert, and H. Fuchs, *Mater. Sci. and Eng.* **C8-9** (1999) 335.
- [9] Y. Shen, J. Swiatkiewicz, J. Winiarz, P. Markowicz, and P. N. Prasad, *Appl. Phys. Lett.* **77** (2000) 2946.
- [10] R. D. Schaller and R. J. Saykally, *Langmuir* **17** (2001) 2055.
- [11] R. D. Schaller, J. C. Johnson, K. R. Wilson, L. F. Lee, L. H. Haber, and R. J. Saykally, *J. Phys. Chem.* **B106** (2002) 5143.
- [12] D. M. P. Hoffmann, K. Kuhnke, and K. Kern, *Rev. Sci. Instrum.* **73** (2002) 3221.
- [13] K. Kuhnke, D. M. P. Hoffmann, X. C. Wu, A. M. Bittner, and K. Kern, *Appl. Phys. Lett.* **83** (2003) 3830.
- [14] G. Mizutani, Y. Sonoda, H. Sano, M. Sakamoto, T. Takahashi, and S. Ushioda, *J. Lumin.* **87-89** (2000) 824.
- [15] J. L. Lambert, J. M. Morookian, S. J. Sirk, and M. S. Borchert, *J. Raman Spectrosc.* **33** (2002) 524.

[16] J. Kubota, A. Wada, S. S. Kano, and K. Domen, *Chem. Phys. Lett.* 377 (2003) 217.

FIGURE CAPTIONS

Figure 1 (a) Experimental setup for the optical sum frequency spectroscopy and microscopy. The detection apparatus in the dashed square is either (b) a monochromator and photomultiplier (PMT) for SF spectroscopy, or (c) a commercial microscope equipped with optical filters and gated image-intensified CCD camera for SF microscopy. OPG/OPA represents the optical parametric generator and amplifier. B. P. F. represents the band pass filter.

Figure 2 Scattered sum frequency intensity spectra from D-glucose, amylopectin, β -cyclodextrin, and amylose as a function of the frequency of the infrared light for the polarization combinations of SFG, visible, and infrared (all, s, s). The thick curves are guide to the eye.

Figure 3 Scattered sum frequency intensity spectra from D-glucose, amylopectin, β -cyclodextrin, and amylose as a function of the frequency of the infrared light for the polarization combinations of SFG, visible, and infrared (all, p, s). The IR frequencies indicated by the vertical dashed lines are adopted for the microscopic observation in Fig. 4. The thick curves are guide to the eye.

Figure 4 (a) Normal image of powdery amylopectin, β -cyclodextrin, and α -D-glucose distributed on a Si substrate under illumination by white light. The thickness of the powder layer was several hundreds of microns. (b) An

image of the same sample illuminated by the exciting light beam at 532nm only. (c), (d) The SF intensity image of the sample with the polarization combination of (all, p, s). The polarization of the sum frequency output was not selected. The frequencies of the infrared beam are indicated in the figures.

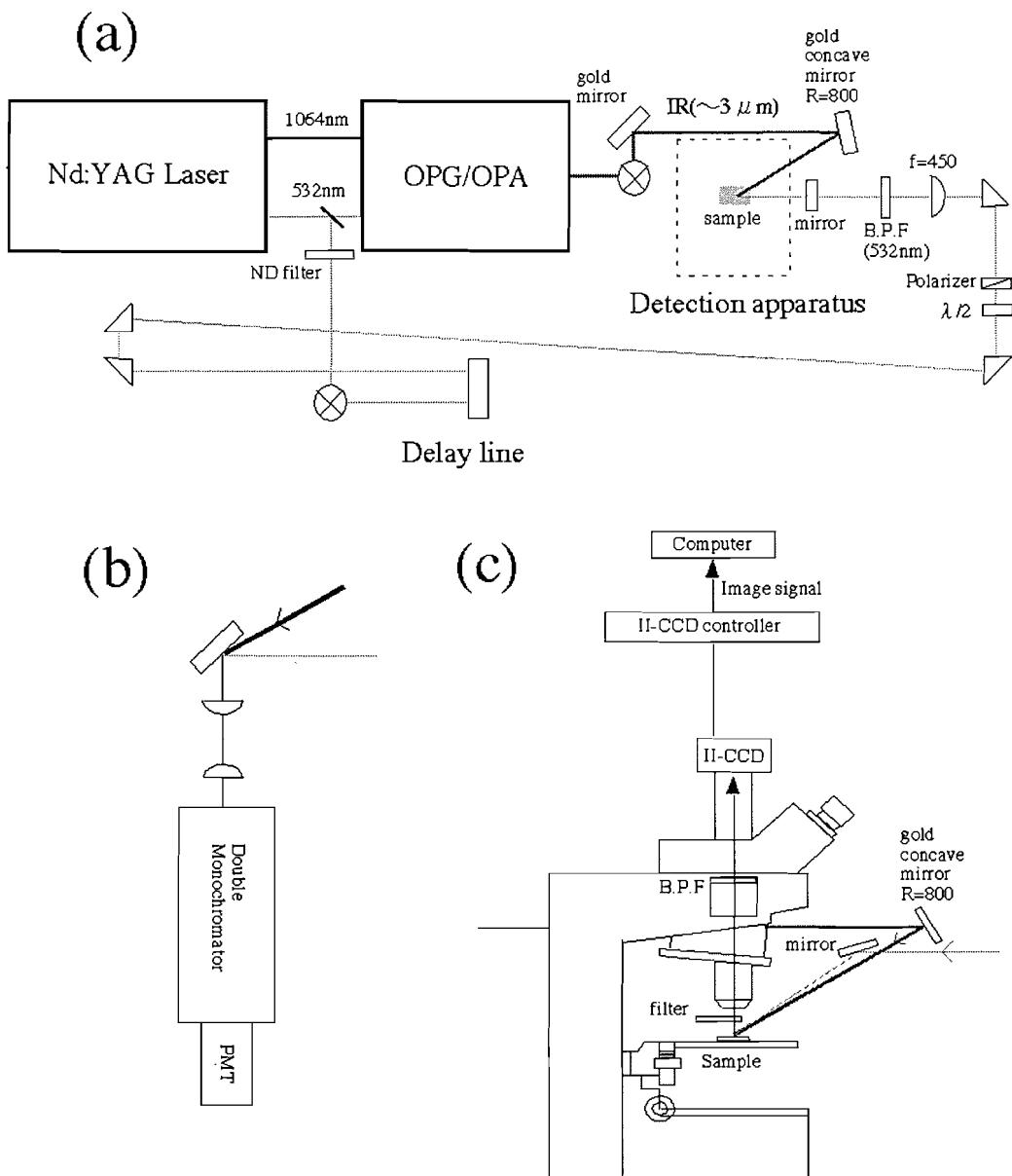


Figure 1 Mizutani et al

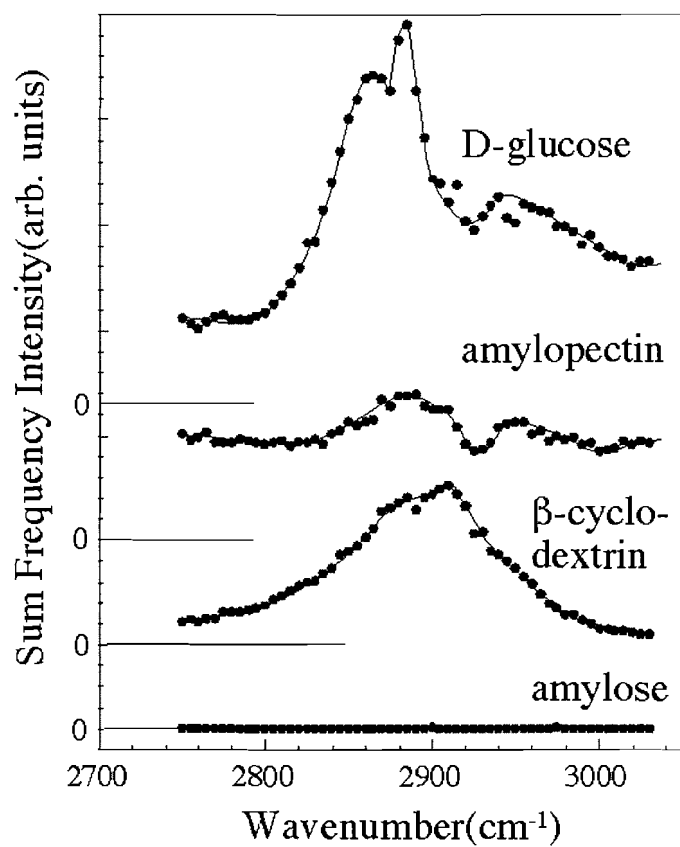


Figure 2 Mizutani et al

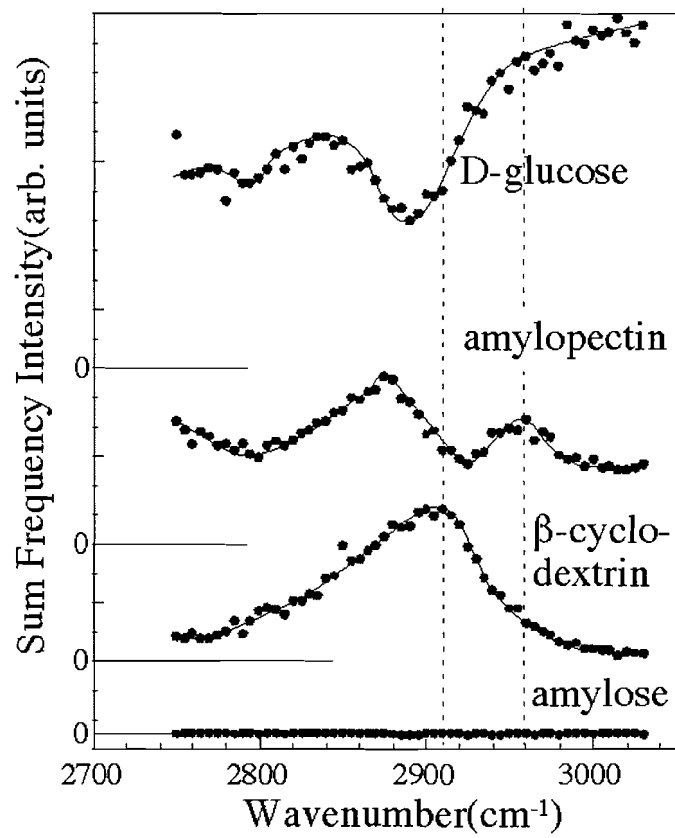


Figure 3 Mizutani et al

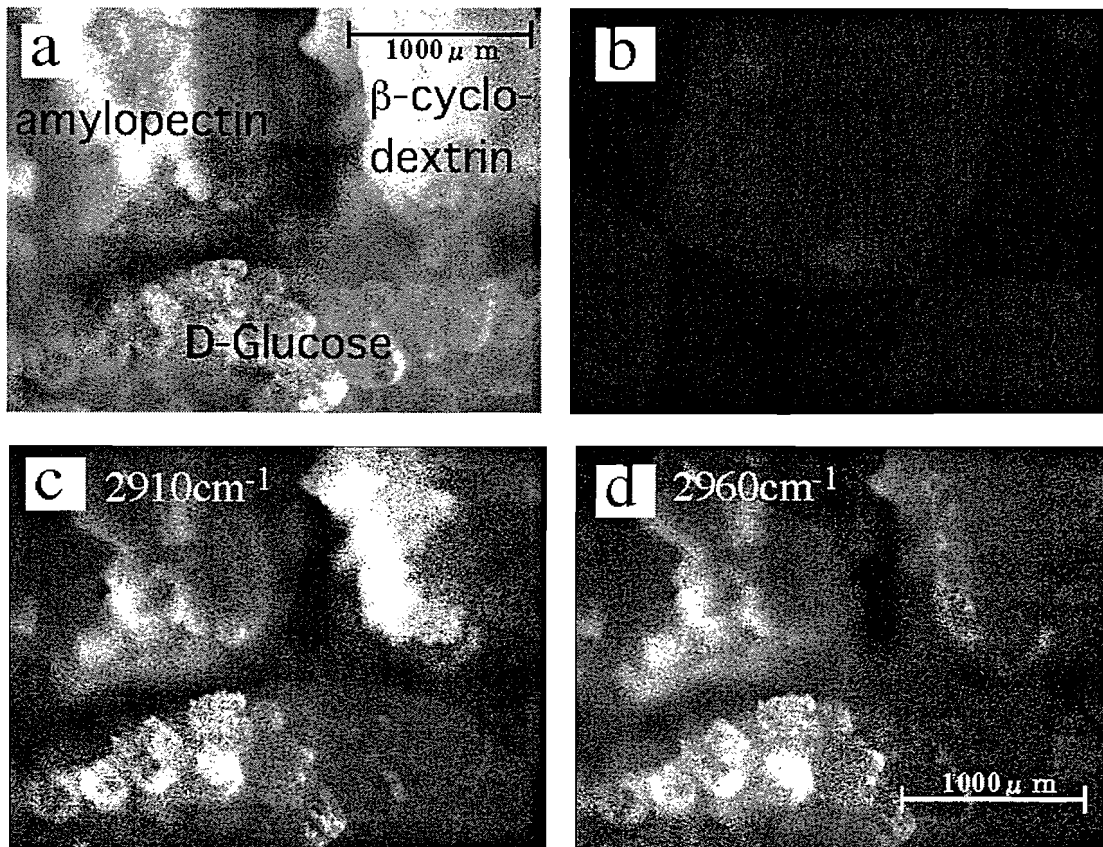


Figure 4 Mizutani et al.