Communications to the Editor

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LASER PHOTOACOUSTIC MICROSCOPY FOR THE DETERMINATION OF DYE ON A SOLID BIOPOLYMER

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Laser (He-Ne, 25 mW) photoacoustic microscopy was applied to the determination of alcian blue 8GS on the mucin layer. Determination range in 40 μm diameter area was 40-1200 pg. In ca. 2 mm diameter area it was 80-1500 ng by the integration of signals with an error less than 15% irrespective of uniformity of the dye distribution. This method was applied to the analysis of alcian blue 8GS on a stained rat rectum specimen of 3 μm thickness, and its photoacoustic microscopic image showed a good agreement with the histochemical pattern.

KEYWORDS——photoacoustic microscopy; solid surface analysis ; alcian blue; stained rat rectum

The photoacoustic method[1] detects acoustic (thermal) waves generated by intermittent light absorption and thermal relaxation. With a sharply focused light beam for irradiation and X-Y scanning of the sample stage a photoacoustic microscopic (PAM) image is obtained. This method is especially suitable for the analysis of highly light-scattering solid samples, such as ceramics,[2,3] metals[4,5] and others.[6,7] Our purpose is to apply this method to the analysis of solid biological components, and in the present paper the applicability is studied by using a model sample of stained mucin layer.

Alcian blue 8GS which was known to stain mucus in the histochemistry was used. The dye solution (0.167-1.0 mg/ml, 0.5-1.5 μl) was spotted on the mucin layer[8] and dried. The photoacoustic apparatus was similar to our previous report[9] with three additional modifications: 1) Irradiation light beam (He-Ne laser, 25 mW, NEC, Tokyo, Japan) was focused in a spot with a diameter (ø) of 40 μm by a lens and by a pinhole. 2) A photoacoustic cell with a detector microphone was set on the X-Y stage which was driven by a pulse motor. 3) Photoacoustic signal, obtained at a modulating frequency 43 Hz and at a phase

102° which gave the maximum signal intensity, was analyzed by a data processor, Chromatopac C-R3A (Shimadzu, Kyoto, Japan).

Typical PAM images of the alcian blue 8GS spot (ca. 2 mmø) on the mucin layer are shown in Fig. 1, in which (a) and (b) represent uniform and non-uniform distribution of dye, respectively.

The averaged individual PAM signal intensity of the uniform dye is shown as the signal intensity / 40 μmø area in Fig. 2. The data showed non-linear dependence on the dye quantity as shown in Fig. 2(a). However, the least-squares relation between logarithmic value of signal intensity and that of the dye quantity gave the following equation:

$$\log[\text{signal intensity}] = 5.29 + 0.816 \times \log[\text{dye quantity}] \quad (1)$$

This equation turns out as follows:

$$[\text{dye quantity}] = 3.30 \times 10^{-7} \times [\text{signal intensity}]^{1.23} \quad (2)$$

and gave the calibration curve shown in Fig. 2(b) and was used for the integration of PAM signal by the data processor.

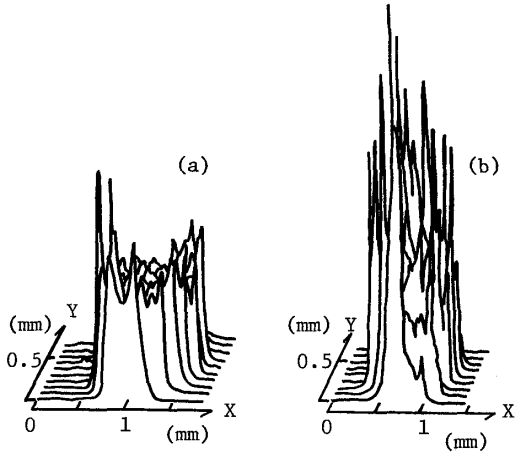

Fig. 1. PAM Images of Spotted Alcian Blue 8GS on the Mucin Layer (a) uniform distribution with 500 ng dye, (b) non-uniform distribution with same 500 ng dye. In the spot of (a) averaged quantity of dye in the area of 40 μmø was ca. 360 pg.

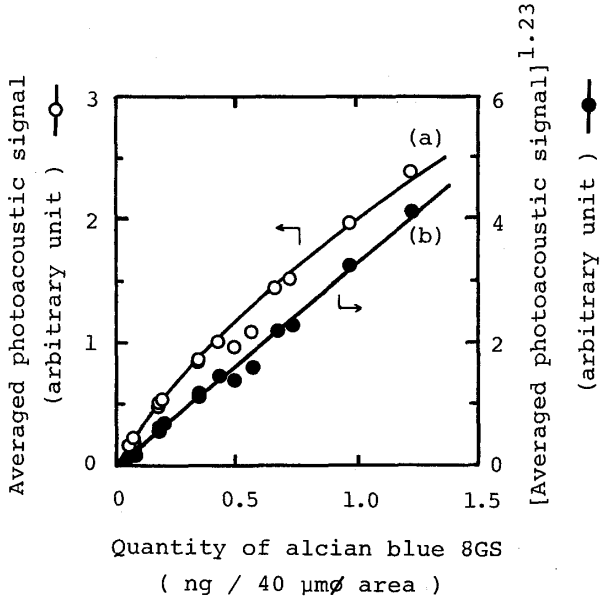

Fig. 2. Calibration Curve for Alcian Blue 8GS on the Mucin Layer
(a) averaged photoacoustic signal versus dye quantity, (b) [averaged photoacoustic signal]$^{1.23}$ versus dye quantity.

Table I. Experimental Error by Changing Scanning Mode (%)

Quantity of alcian blue 8GS (ng)	Error by scanning interval 80	160	320(μm)	Deviation between two scanning axes (changed by 90°)
125	1.5	5.4	8.0	2.1
250	*	2.9	5.6	4.9
500	1.0	4.8	5.7	2.4
750	*	3.1	6.6	4.1

* : not studied.

The quantity shown in brackets represents the value in the area of 40 μmø. The factors in the equations are variable due to the difference in optical and thermal characteristics of samples. The determination range of dye in the area of 40 μmø was 40-1200 pg. The upper limit was 1200 pg due to the difficulty of making a more concentrated uniform dye spot. In the spot of ca. 2 mmø area, the total quantity of dye could be determined in the range of 80-1500 ng by the integration of PAM signal. The determination by this method was irrespective of the distribution of dye; for example, spots in Figs. 1(a) and (b) could be determined with almost the same results by the integration of individual PAM signals / 40 μmø area using equation (2).

On the PAM measurement of these spotted dyes (ca. 2 mmø), as shown in Table I, a scanning interval less than 80 μm was necessary in order to determine the dye quantity with less than 2% error. A change of scanning axis had little effect on the error. For example, the deviation between two scanning axises on the same non-uniform spot was less than 5%. The precision for the determination of the spots with various concentration of dye was less than 15%. The difference in the local thickness of the dye spot might be responsible for the error.

This method was applied to the alcian blue 8GS in the stained rat rectum specimen of 3 μm thickness.[10] Since the phase lag of the specimen was almost the same as that of the mucin layer, optical and thermal characteristics of the specimen seemed to be similar to those of the mucin layer, and the calibration curves in Fig. 2 could be used for this case. A PAM image of a typical sample is shown in Fig. 3. The image represented the distribution of dye mostly in the mucosal layer, which was in good agreement with the histochemical pattern. The individual dye quantity in the area of 40 μmø was in the determination range. Alcian blue 8GS in the rat rectum (oval shape of 2.8 x 3.5 mm with 3 μm thickness) shown in Fig. 3 was determined to be totally 1.8 μg with the micro-distribution of ca. 40-600 pg of alcian blue 8GS / 40 μmø area. Since the binding ratio of mucin / alcian blue 8GS by weight was ca. 40,[11] the dye quantity of 40-600 pg corresponds to 1.6-24 ng of mucin / 40 μmø area.

In the present work we suggest that the PAM has the ability to determine the biological polymer in the stained sample with its micro-distribution. For the quantitative analysis of biological tissues, a PAM method with higher resolution and higher sensitivity is being developed in our laboratory.

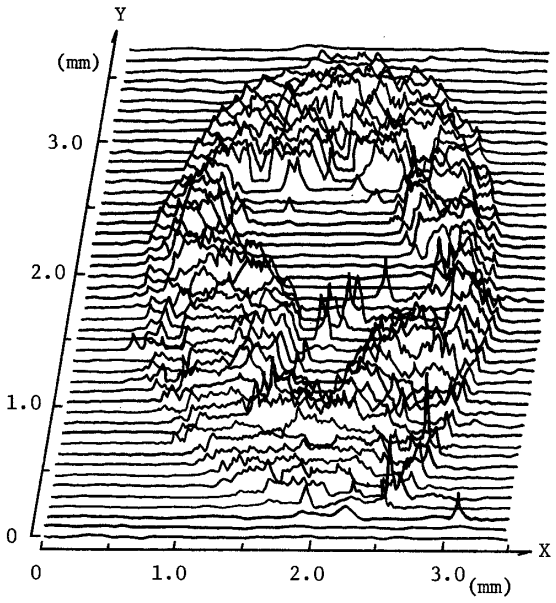

Fig. 3. PAM Image of Stained Rat Rectum Specimen (Scan Interval = 80 μm)

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REFERENCES AND NOTES

1) A. Rosencwaig, "Photoacoustics and Photoacoustic Spectroscopy," John Wiley and Sons Inc., New York, 1980.
2) Y. H. Wong, R. L. Thomas and G. F. Hawkins, Appl. Phys. Lett., 32, 538 (1978).
3) Y. H. Wong, R. L. Thomas and J. J. Pouch, Appl. Phys. Lett., 35, 368 (1979).
4) G. Busse and A. Rosencwaig, Appl. Phys. Lett., 36, 815 (1980).
5) T. Sawada, H. Shimizu and S. Oda, Jpn. J. Appl. Phys., 20, L25 (1981).
6) A. Rosencwaig and G. Busse, Appl. Phys. Lett., 36, 725 (1980).
7) G. Veith, Appl. Phys. Lett., 41, 1045 (1982).
8) The mucin layer on the thin glass plate was made by spotting mucin (Sigma Chemical Co., USA) solution (10 mg/ml, 60 μl) followed by immediate drying.
9) T. Masujima, K. Wada, H. Yoshida and H. Imai, J. Photoacoustics, 1, 347 (1982-1983).
10) A rat rectum was fixed with 10% formaldehyde, embedded with paraffin, sectioned 3 μm thick by a microtome and stained with alcian blue 8GS.
11) The binding ratio in the solution was estimated by filtrating the incubated solution of mucin with dye and measuring the absorption of the filtrate containing only free dye.

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