Photochemical Reactions between Methylene Blue and Tri-, Di- and Monomethylamine. I

By Hiroshi OBATA and Masao KOIZUMI

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Introduction

Imamura and Koizumi[1], one of the present authors, have found that eosine is photobleached in the alcoholic solution in vacuo but not in the aqueous solution, while in the aerobic condition it bleaches in the latter case but not in the former case. The bleaching of eosine in these cases is irreversible and the reaction as a whole is quite complicated. Now in contrast to eosine, it has long been known that methylene blue in the evacuated alcoholic solution is photobleached and the reaction in this case is the formation of a leuco dye, which returns to the original dye when the solution is exposed to the atmospheric air. The aqueous solution of methylene blue is of course stable against illumination in vacuo, but a notable fact has been found by Weil[2] that the addition of some suitable oxidizable compounds causes the photobleaching to take place in the evacuated aqueous solution. The aim of his work, however, was not to study the bleaching reaction but to study the photosensitized oxidation of various organic substances from the biochemical standpoint using methylene blue as a sensitizer. He therefore measured the rate of oxygen absorption when the aqueous solution of various organic substances in the presence of oxygen and methylene blue[2,3]. In the case of trimethylamine, he particularly analyzed the reaction product and identified it to be trimethylamine oxide. Hence he considered that the overall reaction in this case is

$$\mathrm{MB} + \mathrm{TA} + \frac{1}{2}\mathrm{O_2} \xrightarrow{h\nu} \mathrm{MB} + \mathrm{TAO},$$

where TA is trimethylamine.

The above mentioned experiments of Weil seem to have been done merely for a supplementary purpose, and no scheme for the reaction in vacuo was given. There seems to be no doubt that the reaction in vacuo was given. These seems to be no doubt that the reaction consists of the formation of leuco dye and TAO (trimethylamine oxide), since the coloration is regained when the solution is exposed to air. But if so, it is very natural to suppose that the hydrogen donor in this case is water itself and not trimethylamine, since it is very difficult to detach the hydrogen atom from the latter compound. Thus the bleaching reaction in this case would be interpreted as a result of the photochemical fission of water molecule. An interesting analogue to the first process of photosynthesis by chloroplast as postulated by van Niel[4] was known.

The object of the present papers is to establish the above scheme definitely and to make clear the mechanism of the reaction. It is to be added here, that a certain attempt has been made[5] recently to explain the photobleaching of methylene blue in the deoxygenated solution by dint of ethylenediamine tetraacetic acid, on the postulate that the primary process is the photosensitized decomposition of water, but no conclusive evidence has yet been reported.

Experimental Method

Materials.—*Methylene Blue*.—Grübler's reagent was used without further purification.

Trimethylamine[6].—Trimethylamine hydrochloride was prepared by the reaction of paraformaldehyde with ammonium chloride at about 160°C. From the reaction mixture, a free base was evolved by the neutralization with NaOH and was liquified by a condenser, using dry ice as a cooling reagent.

Dimethylamine[7].—Dimethylamine hydrochloride was prepared by the reaction of ammonium chloride with formaldehyde. The product was reocystallized five times from absolute alcohol, m.p. 165°C. The Free base was prepared from its hydrochloride. The neutralization was done by the same method as for trimethylamine.

Monomethylamine[8].—Monomethylamine hydro-

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[1] M. Imamura and M. Koizumi, This Bulletin, **28**, 117 (1955).

[2] L. Weil, *Science* **107**, 426 (1948) ; L. Weil and J. Maher, *Arch. Biochem.*, **29**, 241 (1950).

[3] For example. L. Weil, W. G. Gordon and A. R. Buchert, *Arch. Biochem.*, **33**, 90 (1951).

[4] Van Niel, *Advances in Enzymology*, **1**, 263 (1941).

[5] J. R. Merkel and W. J. Nickerson, *Biochim. et Biophys. Acta*, **14**, 303 (1954).

[6] "Organic. Syntheses" Collect. vol. **1**, p. 517, 514.

[7] "Beilstein Handbuch" Erst. Ergänz. III-IV, p. 320.

[8] Organic Syntheses" Collect. vol. **1**, p. 340.

chloride was prepared from ammonium chloride and formaldehyde. The product was recrystallized from absolute alcohol washed with cold chloroform and once more recrystallized from butanol, m.p. 224°C. The free base was prepared from its hydrochloride in the same way as for trimethylamine.

Trimethylamine Oxide[9].—It was prepared from trimethylamine and hydrogen peroxide. The product was crystallized from alcohol-ether and then recrystallized twice from methanol. $(CH_3)_3NO2H_2O$, m.p. 96°C.

Since the solutions of tri-, di-, and monomethylamine are all autoöxidized in the air, it is necessary to degass the solutions and maintain them in the evacuated ampoules. Caution had to be taken to employ the fresh solution in each experiment.

Apparatus and Procedures for Measuring the Rate of Photobleaching.—Fig. 1 shows the main part of the apparatus. As a light source, a tungsten lamp for automobile (6 V, 35 W./35 W.) was employed and the range of wave lengths was adequately chosen by means of a filter for photography, the transmission of which is shown in Fig. 2. A drum-shaped reaction cell had an inner diameter of 27 mm. and a thickness of 10 mm. The light intensity was measured by Se-photocell.

The aqueous solutions of methylene blue and trimethylamine which were separately degassed in a vacuum system were poured into a reaction cell. The procedure for evacuation is quite analogous to that employed by Imamura[1]. The bleaching can be pursued continuously by measuring the intensity of a purallel beam which has passed through the reaction cell. A calibration curve between methylene blue concentration and photocurrent is shown in Fig. 3. The number of photons entering the reaction cell was usually ca. 10^{16} when the metallic nets as shields were not employed.

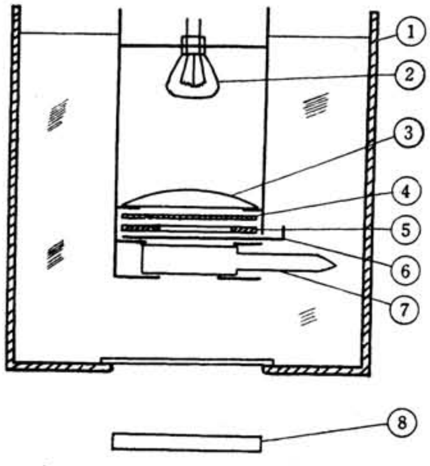

Fig. 1. The apparatus for measuring the photobleaching.

① Thermostat
② Light source (6 V, 35 W./35 W.)
③ Condensor lens
④ Metallic net to adjust the light intensity
⑤ Filter
⑥ Shutter
⑦ Reaction cell
⑧ Se-photocell

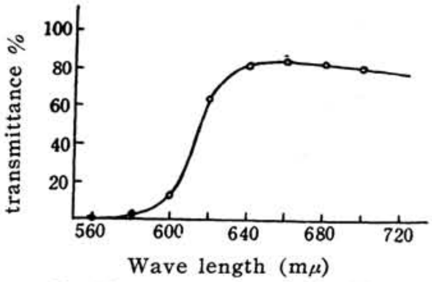

Fig. 2. The transmittance of a filter used.

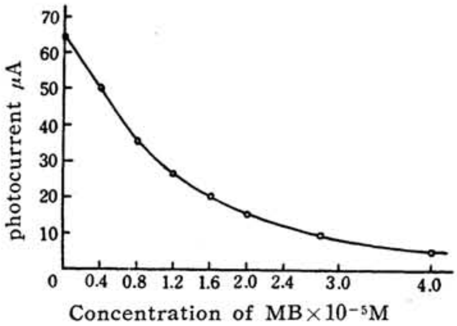

Fig. 3. Calibration curve for photocurrent.

Experimental Results

(A) Preliminary Observations.—**(a) The Influences of Solvents.**—Photobleaching of methylene blue with trimethylamine was examined in various solvents: namely water, ethanol, isopropanol, butanol, acetone, methyl-ethyl ketone, acetic acid, and acetic anhydride. The bleaching was observed in all solvents except acetic acid and acetic anhydride, but its rate depended very much upon the kind of solvent. For example, the bleaching proceeded remarkably faster in butanol than in water and ethanol, and in acetone and methyl ethyl ketone a considerable dark reaction took place. After the photobleaching was complete, the solution was kept in dark for a long time, but the coloration of the solution was not observed in all cases. But when the solution was exposed to air, the solution regained its original color sooner or later. Similar experiments were carried out with monomethylamine and dimethylamine, and the results were the same at least qualitatively. In the aqueous solution the rate of photobleaching was the lowest in the case of monomethylamine and somewhat slower in

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[9] Meisenheimer, *Ann.* **397**, 286 (1913) ; Dunstan, *J. Chem. Soc.*, **75**, 1004 (1899).

dimethylamine. From the above results, it is clear that the rate depends very much on solvents, and this is natural since the solvent molecule is considered to participate in the reaction.

(b) The Reaction Products between Methylene Blue and Trimethylamine.—The absorption spectrum of photobleached aqueous solution was examined by Beckman spectrophotometer and found to be the same as that of the aqueous solution of leuco dye which was produced by the action of sodium hydrosulfite upon the original dye (Fig. 4). Hence there is no doubt that one product of the bleaching reaction is leuco methylene blue. Another product is most certainly supposed to be trimethylamine oxide. To confirm it definitely, one cannot rely upon a spectroscopic method, since this compound has no absorption through visible to near ultraviolet region. Hence, a chemical method was employed which is based on the fact that TAO effectively separates iodine from potassium iodide in the acidic medium; a particular experiment was done for such an analytical purpose. A large quantity of the evacuated aqueous solution of methylene blue and trimethylamine was illuminated by the sun light and after the decoloration occured almost completely, the solution was treated with Japanese acid clay in situ to remove the bleached and unbleached dye. The colorless filtered solution was concentrated to some extent under reduced pressure, and then some quantity of dilute acidic solution of KI and a drop of 1% starch solution were added. An intense blue coloration could be observed at once. The above experiment, however, had to be further checked by a blank using the aqueous solution of trimethylamine without the addition of dye, since there was some doubt that unreacted trimethylamine would be oxidized by atmospheric oxygen during the process of concentrating the solution. The blank experiment also showed an iodine-starch reaction but its extent was much smaller.

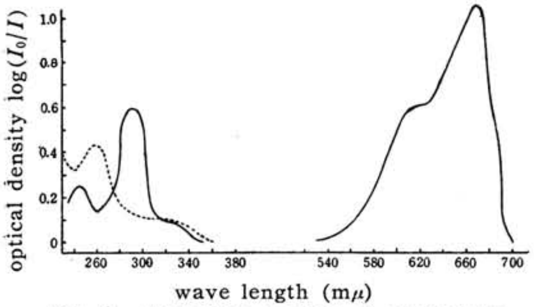

Fig. 4. Absoption spectra of methylene blue and leuco methylene blue.
full line: methylene blue
broken line: leuco methylene blue

Hence one can safely conclude the formation of trimethylamine oxide. Thus the overall reaction in the aqueous solution has been determined as follows:

$$\mathrm{MB} + \mathrm{H_2O} + \mathrm{TA} \longrightarrow \mathrm{MBH_2} + \mathrm{TAO}$$

The formation of leuco dye was also established in the case of ethanol and butanol solution, but no further analysis was undertaken in these cases.

(B) Kinetic Investigations.—Since the overall reaction in the aqueous solution has now become clear, the authors investigated next some general features of the reaction from kinetic viewpoints. The concentration of dye cannot be changed so much because the change in light intensity is sensitive to that of dye concentration only in a small range of the latter, as judged from the calibration curve in Fig. 3. Almost all the experiments were carried out in the concentration of 2.0×10^{-5} M of methylene blue since this concentration is suitable for the intensity measurement and moreover it is expected that the mechanism will be simpler in such a low concentration. Below will be summarized the chief results obtained by the various experiments kinetically in nature.

(1) General Features of the Bleaching Curves.—Fixing the pH of solution at 9.2 by using M/35 borate buffer, the effect of the trimethylamine concentration was investigated at 30°C. The results can be summarized as follows.

(a) At high concentration of trimethylamine (about 100 times as that of methylene blue), the bleaching curve is quite simple as shown in Fig. 5, and the bleaching is practically complete within about fifty minutes. The reaction can be expressed satisfactorily as a simple first order one in dye concentration.

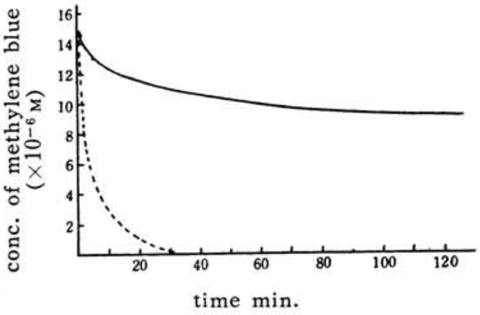

Fig. 5. The bleaching curve of methylene blue.
Methylene blue: 2.0×10^{-5} M.
Temp. 30 C, Buffer was not used.
Trimethylamine:
0.1×10^{-3} M (full line)
1.6×10^{-3} M (broken line)

(b) At low concentration of trimethylamine (about several to thirty times as much as that of methylene blue) the reaction practically came to an end at a certain stage of decoloration, and the rate cannot be expressed as a simple first order one in dye concentration. The kinetics of bleaching must be complicated since plots of $\log(-dc/dt)$ against $\log c$, c being the dye concentration, gives S-shaped curve. A typical example is shown in Fig. 5.

(2) The Existence of a Reverse Reaction.—The recovery of color was never observed under the above experimental condition when the photobleached solution was kept in the dark for a long time. Neither could the back reaction be perceived in the partially photobleached solution obtained under the experimental condition (b). Thus the dark back reaction does not exist at least in the above experimental conditions. To determine whether a reverse reaction occurs or not in other conditions, the following experiments were undertaken. By the vacuum distillation, water and unreacted trimethylamine were eliminated from the photobleached solution and then about the same quantity of pure water was added to the residue consisting of leuco methylene blue and trimethylamine oxide. All these procedures were of course done without any contact of air. When the obtained solution was kept in the dark for a few days, the blue color of methylene blue could clearly be observed. The following experiments demonstrate more definitely the existence of such a back reaction. Adding various quantities of trimethylamine oxide to the original solution, the quantity of photobleaching in vacuo was examined. The results are summarized as follows. From the bellow table it is clear that the addition of a large amount of TAO strongly suppresses the bleaching reaction, and the photobleaching takes place only partially even after a long illumination.

When the partially photobleached solution obtained in experiment (a) (4) in Table I was kept in the dark, the blue color became more dense within a few hours. Thus it is clear that when a large quantity of TAO exists, the dark back reaction can occur and that a certain photostationary state is realized.

(3) The Existence of an Intermediate Complex.—Judging from the type of overall reaction it seems natural to suppose that the reaction is successive in nature. Moreover, the results in (1) suggest the existence of a long lived intermediate. Hence such a possibility was examined spectroscopically. Immediately after the illumination of the aqueous solution (2.0×10^{-5} M of dye, 6.0×10^{-4} M of TA, pH 9.2) for about two minutes, the absorption spectra were carefully examined by Beckman spectrophotometer in the region from 750 to 1100 mμ, where methylene blue practically has no absorption. Repeated examinations showed that there really exist very small absorptions near 800 mμ, 850 mμ and 950 mμ as shown in Table II.

The above absorption was recognized even in the presence of oxygen. This will be discussed in the next paper.

When kept in the dark, the absorption in question slowly declines. Fig. 6 shows the optical density at 950 mμ as a function of time after two minutes illumination. That this intermediate slowly disappears in the dark was further confirmed by the recognition of a recovery in 665 mμ band of methylene blue slowly. The following data give the values of log (I_0/I) of the solution (methylene blue 2.0×10^{-5} M. trimethyl-

TABLE I

THE EFFECT OF THE ADDITION OF TAO

(a) Methylene Blue Conc. 2.0×10^{-5} M
M/35 Borate buffer (pH 9.2) was used.

	Conc. of TA, M	Conc. of TAO, M	Ratio of TAO/TA	Stationary conc. of MB, M	Note
(1)	6×10^{-4}	6×10^{-2}	100	0.65×10^{-5}	
(2)	6×10^{-4}	6×10^{-2}	100	0.80×10^{-5}	Intensity of light was 1/2.5.
(3)	4×10^{-4}	4×10^{-2}	100	0.6×10^{-5}	
(4)	4×10^{-4}	8×10^{-3}	20		The rate was much slower than that when no TAO was added.

(b)* Methylene Blue Conc. 2.0×10^{-5} M
Trimethylamine 1.7×10^{-3} M

	Conc. of TAO, M	Ratio of [TAO] to [TA]	Degree of bleaching
(1)	2.0×10^{-4}	0.1	Complete
(2)	2.0×10^{-3}	1	Almost complete
(3)	2.0×10^{-2}	10	Slight
(4)	2.0×10^{-1}	100	Not practically observed.

* These experiments were rather preliminary in nature and were done in quite different conditions from those for kinetic purposes.

TABLE II

ABSORPTION BAND OF AN INTERMEDIATE

Wave length (mμ)		750	800	850	900	950	1000	1100
log (I_0/I)	initial	0.020	0.018	0.017	0.017	0.017	0.017	0.019
	after 2 min. illumination	0.020	0.020	0.021	0.017	0.020	0.018	0.019

amine 1×10^{-3} M, pH 9.2) at 670 mμ, as a function of time after two minutes illumination.

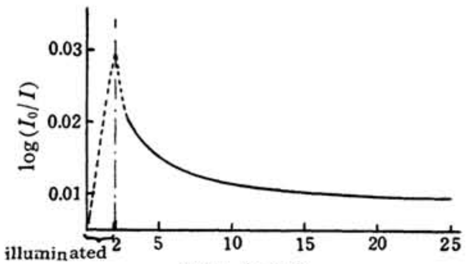

Fig. 6. Optical densities at 950 mμ after 2 min. illumination.
full line: The part experimentaly observed

TABLE III
THE RECOVERY OF 665 mμ BAND OF METHYLENE BLUE IN THE DARK AFTER 2 MIN. ILLUMINATION OF SOLUTION

	Optical Density
Initial	0.792
Immediately after 2 min. illumination	0.553
30 min. in the dark	0.599
60 min. 〃	0.610
120 min. 〃	0.628

The analogous phenomena were also observed in the cases of di- and monomethylamine.

Mechanism of the Reaction

From the above experiments, a probable scheme of the reaction would be

$$\mathrm{MB}^+ \xrightarrow{h\nu} \mathrm{MB}^{+*}$$
$$\mathrm{MB}^{+*} + \mathrm{H_2O} + \mathrm{TA} \underset{\mathrm{II}\ (k_2)}{\overset{\mathrm{I}\ (k_1)}{\rightleftharpoons}}$$
$$\text{Intermediate} \underset{\mathrm{IV}}{\overset{\mathrm{III}\ (k_3)}{\rightleftharpoons}} \mathrm{MBH} + \mathrm{TAO} \qquad (2)$$

and from the fact that the completely decolorized solution never regains its color in the dark, the process IV can be neglected practically for the photobleaching reaction. The order of the rate of the process IV can be estimated from the data given in the previous section (B) (2). From the data given in Table I (b) the apparent equilibrium constant of the photostationary state is estimated to be about 10, while from the data in Table I (a) it is to be of the order of 100. These constants, of course, depend upon the intensity of light and other experimental conditions, but the conditions in case (a) are quite similar to those employed for the bleaching experiment except the addition of TAO. Now the concentration of trimethylamine oxide in the experiments for Table I (a) is ca. 10^{-2} M while it is 2×10^{-5} M at most when trimethylamine oxide is not particularly added. The back reaction in the former case is perceived only after several hours, hence in the latter case it will be observed only after several thousand hours if one assumes that the rate is proportional to the concentration of TAO.

The genuine equilibrium constant $K=\dfrac{[\mathrm{MBH}]\,[\mathrm{TAO}]}{[\mathrm{MB}\ ]\,[\mathrm{TA}]}$ for the reactoin,

$$\mathrm{MB}^+ + \mathrm{H_2O} + \mathrm{TA} \rightleftharpoons \mathrm{MBH} + \mathrm{TAO}$$

has not yet been studied but it would be much less than one, since the thermal forward reaction is negligible in the present experimental condition. In fact, a preliminary experiment showed that the bleaching is observed even in the dark after about several hours if 10^5 times TA to MB is added. The magnitude of the rate seems to support the above estimation. Thus the bleaching reaction would be the one accompanied with the increase of free energy.

Next, a suitable rate formula will be derived. In most experiments the concentration of trimethylamine is much larger than that of the dye, hence it will be allowed to put it as a constant through the reaction. Then the concentration of dye is a sole quantity which must be expressed as a function of time.

If one assumes the scheme (2) and neglects the process IV, then the rate formula can easily be deduced as follows. Putting the concentration of dye and the intermediate as x and y respectively, the following differential equations can be written.

$$\begin{cases} -\mathrm{d}x/\mathrm{d}t = k_1x - k_2y \\ -\mathrm{d}y/\mathrm{d}t = (k_2+k_3)y - k_1x \end{cases}$$

The solution for x is

$$x = Ae^{-(k+s)t} + Be^{-(k-s)t}$$

where $k=\dfrac{1}{2}(k_1+k_2+k_3)$

$$s=\frac{1}{2}\sqrt{(k_1+k_2+k_3)^2-4k_1k_3}$$

and $A=\dfrac{c}{2}\left(1+\dfrac{k_1-k}{s}\right) \qquad B=\dfrac{c}{2}\left(1-\dfrac{k_1-k}{s}\right)$

(c is the initial concentration of dye.)

One can decide, by the method of trial and error, the values of A, B, k, and s from the bleaching curves experimentally obtained. In fact, all the data can be analyzed by the above method, though the

TABLE IV

pH 9.2 Temp. 30°C

	[MB]	[TA]	k_1	k_2	k_3	
Expt. A	2.0×10^{-5} M	6×10^{-4} M	0.157	0.0706	0.0604	$x=0.6e^{-0\cdot2t}+0.9e^{-0\cdot03t}$
Expt. B	2.0×10^{-5} M	1×10^{-4} M	0.0224	0.0321	0.0091	$x=0.5e^{-0\cdot2t}+1.0e^{-0\cdot0035t}$

accuracies for the four constants are not so good and some degree of arbitrariness cannot be avoided. A few examples are shown in Fig. 7 and Table IV.

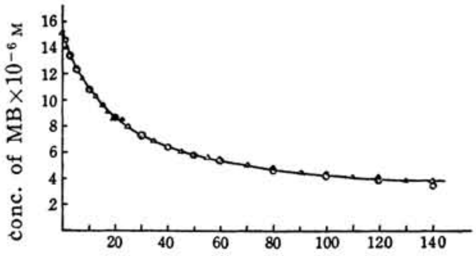

Fig. 7

MB 2.0×10^{-5} M TA 2.0×10^{-4} M
pH 9.2, Metallic net was not used,
Temp. 30°C
△: observed
○: calculated

When the concentration of TA is in large excess, the relation $(k+s)\gg(k-s)$ holds, and the first term becomes negligible after a short time, and almost the whole bleaching curve can be expressed only by the second term. This is the reason why the rate formula is a simple first order type when the concentration of TA is in large excess. In contrast to a very small rate for the disappearance of the intermediate in the dark, k_2 and k_3 are in fact not so small compared with k_1. This fact and the fact that the intermediate has very small absorption, suggest that the main process II and III are also photochemical.

In the next paper, systematic investigations under various experimental conditions will be reported and the above suggestion will be found to be true.

Summary

The photobleaching of the evacuated aqueous solution of methylene blue by dint of tri-, di- or monomethylamine was studied. The reaction products were identified to be leuco dye and amine oxide, the former by a spectroscopic method and the latter by a chemical one.

It was found that a long lived metastable intermediate is produced and this intermediate slowly returns to the original dye even in the dark. Furthermore it was found that this intermediate has its own absorption near infrared region. On the basis of the above observations, the following scheme was proposed for the reaction.

$$\mathrm{MB^+ + H_2O + TA} \underset{\mathrm{II}\,(k_2)}{\overset{\mathrm{I}\,(k_1)}{\rightleftharpoons}}$$

$$\text{Intermediate} \underset{\mathrm{IV}}{\overset{\mathrm{III}\,(k_3)}{\rightleftharpoons}} \mathrm{MBH + TAO}$$

in which the process IV can be neglected in the present experimental conditions.

From the above scheme, ths following rate formula was derived which can represent all the experimental results satisfactorily.

$$x=Ae^{-(k+s)t}+Be^{-(k-s)t}$$

where x is the concentration of methylene blue.

Institute of Polytechnics, Osaka City University, Osaka