Fundamental study on compact absorptiometer for water quality testing

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Abstract
A compact absorptiometer for water quality testing that does not need a special technique to take measurements was studied where the absorbance of a sample solution could be stably measured without decreasing the sensitivity over time. Three types of devices were developed, namely, horizontal straight-line type, vertical straight-line type, and multiple reflection type, that consisted of a light emitting diode (LED), a photodiode (PD), and a glass capillary used as a sample cell. The results from our experiments conducted to detect the nitrite nitrogen in water, which is a factor in water pollution, were used to evaluate how these three devices perform. The shapes of the liquid surfaces at the end of the capillaries in the horizontal and vertical straight-line types, which had the LED, PD, and the glass capillary in line horizontally and vertically, respectively, significantly influenced the output voltage of the PD and this output significantly changed over time, so it was difficult to precisely measure the absorbance. The change in the shape of the liquid surface at the end of the capillary was particularly considerable in the horizontal straight-line type. On the other hand, in the multiple reflection type, which had reflective plates surrounding the glass capillary, the absorbance was measured quite stably because the light emitted by the LED did not transmit through the liquid surface at the end of the capillary and the output voltage of the PD was uninfluenced by the change in the shape of the liquid surface. Additionally, controlling the sensitivity of the absorbance was possible by adjusting the length of the reflective plates, i.e., adjusting the light path. The sample solution in this device did not come into direct contact with the reflective plate, so the device can suppress the decrease in the sensitivity caused by materials becoming directly attached to the reflection plate. The compact multiple reflection type device that was developed could be used in a manually operated portable absorptiometer and also in an automated water quality analysis system that incorporates mixing and flow devices.

Key words: Absorptiometer, Water quality testing, Reflection plate, Nitrite nitrogen, Optical detector

1. Introduction

The water industry, which includes systems such as water supply and sewerage systems, seawater desalination systems, and reclamation water systems, has been expanded with the increasing growth of the world’s population and progress of industrialization. As a result, water quality testing, such as the detection of heavy metals, organic compounds, and nitrate and nitrite nitrogen in water, has become increasingly more important.

In many water quality testing cases, such as in the detection of residual chlorine and nitrate and nitrite nitrogen in water, absorptiometry has been adopted in which some reagents are added to the measurement sample after pretreatment as necessary, and then a color reaction occurs and an optical detector is used to measure the absorbance of the sample. The measurement process, namely, pretreatment, mixing the reagents, color reaction and measurement of the absorbance, is usually manually conducted. Accordingly, the conventional water quality testing method requires a special laboratory and technique, and automation and acceleration of the measurements has been desired.

Flow systems for the water quality testing have been reported in several papers (Hirata et al., 2003, Steimle et al.,...
2002, Greenway et al., 1999, Moorcroft et al., 2001, Burakham et al., 2004) to solve these problems. In particular, micro-systems for chemical analysis, i.e., micro total analysis systems (μ-TAS), have attracted attention as a new approach (Manz, et al., 1990, 1994, Cefai, et al., 1994, Verpoorte et al., 1994). A microfluidic device used for conducting the water quality testing, which was fabricated by using micro electro mechanical systems (MEMS) technologies, was also reported (Miyake, et al., 1997). In this study, two 24 mm x 10 mm x 2 mm micro mixers and an absorptiometric flow cell were integrated into the one chip device, and it had reflection film coated onto the surface of the micro channel. The absorbance of the sample solution was continuously measured while the sample flew in the micro channel and the results showed good linearity between a concentration of phosphate solution and absorbance, and high sensitivity. However, certain materials in the sample may be attached to the reflection film over time, and thus the level of sensitivity in the device may decrease because the sample solution came into direct contact with the reflection film in the previous studies. Accordingly, a washing operation that involves flushing a wash solution into the device may be occasionally necessary.

The purpose of this study is to develop a compact absorptiometer for use in water quality testing that does not require a special technique for measuring the absorbance and that can stably measure it without decreasing the level of sensitivity due to residual material directly attached to the reflection film. The performance of the absorptiometer was evaluated from the results of detecting nitrite nitrogen in water.

2. Experiment

2.1 Configuration of compact absorptiometer

The basic concept of the absorptiometer is compact, inexpensive, and able to measure without needing special techniques. We accordingly developed an absorptiometer using a light emitting diode (LED) as a light source, a photodiode (PD) as a detector sensor, and a glass capillary as a sample cell, which were all inexpensive. Three kinds of configurations were investigated. The first one is a “horizontal straight-line type” in which a LED, a glass capillary, and a PD were set in line horizontally, as shown in Fig. 1(a). The second one is a “vertical straight-line type” in which a LED, a glass capillary, and a PD were set in line vertically, as shown in Fig. 1(b). The final one is a “multiple reflection type” in which a glass capillary is surrounded by reflective plates, and a LED and PD are arranged radially outside the reflective plates, as shown in Fig. 2. Table 1 shows the specifications of the LED and PD used in the experiments.

The straight-line type has a quite simple configuration. In this type, the lights emitted by the LED are incident on the one end of the glass capillary and arrive at the other end reflecting inside the capillary, and are detected by the PD. This type does not have a reflective plate, which would lead to a decrease in the sensitivity over time if some reagent comes into direct contact with them. Additionally, the manufacturing cost can be suppressed because the configuration is quite simple in the straight-line type.

When the refraction indexes of water, glass, and air are 1.33, 1.47, and 1.00, respectively, and the incidence angle to the center axis of the glass capillary is $\theta_1$, as shown in Figs. 1(a) and (b), the incident lights with less than 41.2º of incidence angle $\theta_1$ reflect totally on the interfacial surface between the glass capillary and the air outside the capillary. In addition, the lights arrive at the other end of the capillary reflecting on the interfacial surface and are detected by the PD.

In the multiple reflection type, the capillary is surrounded by reflective plates that form a pentagon, as shown in Figs. 2(a) and (b). The LED and PD were arranged radially outside the reflective plates. The lights emitted by the LED reflect on the inner surface of the reflective plates at various times. We designed it with the intention that the device would be able to measure the absorbance without any reagent coming into direct contact with the reflection plates, which would lead to a decrease in the sensitivity.

We mainly investigated the configuration of the optical device in this study, so it was necessary to manually fill the glass capillary with the sample solution. We used a glass capillary with a 0.8-mm inner diameter and filled it with the sample solution using a capillary action without needing a special flow system.
2.2 Preparation of sample solutions

We conducted experiments for detecting nitrite nitrogen in water, which is a factor in water pollution, in order to evaluate the performances of the devices. Various methods for detecting nitrite nitrogen have been reported from the past research (Moorcroft et al., 2001, Choi and Fung, 1980, Adornato et al, 2005). The naphthylethylenediamine photometric method (Japanese Industrial Standards, K0102, 43.1.1, 2013) was used to detect the nitrite nitrogen in this
study. Table 2 lists the chemicals used in the experiment. We supposed the measurements in the range from 0.025 to 1.000 mg/L of nitrite nitrogen concentration which is widely used in the water quality testing.

Table 2 shows the composition of prepared reagents. Reagent A consisted of 2.00 g of sulfanilamide and 10% hydrochloric acid and the total volume was 100 mL. Reagent B consisted of 0.100 g of N-1-naphthylethylenediamine and water and the total volume was 100 mL. Sample solutions from 0 to 0.700 mg/L of nitrite nitrogen concentration were prepared by diluting a 0.500-mol/L sodium nitrite solution into water. Then 1.00 mL of reagent A was added to 10.0 mL of the sample solution and the mixture was allowed to stand for five minutes while the nitrite ion and sulfanilamide reacted and diazotization occurred. Additionally, 1.00 mL of reagent B was added to the mixture and was allowed to stand for another 20 minutes while the diazo compound and N-1-naphthylethylenediamine reacted and a red colored azo compound was generated. The nitrite nitrogen concentration was determined by measuring the absorbance of the azo compound at 525 nm. Figure 3 shows a photograph of the prepared sample solutions.

Table 2  Reagents used in experiments

| Chemicals                          | Manufacturer                                      | Product number |
|------------------------------------|---------------------------------------------------|----------------|
| Sulfanilamide                      | Wako Pure Chemical Industries, Ltd.               | 191–04502      |
| 10% hydrochloric acid              | Wako Pure Chemical Industries, Ltd.               | 085–07535      |
| N-1-naphthylethylenediamine       | Wako Pure Chemical Industries, Ltd.               | 147–00763      |
| 0.500 mol/L sodium nitrite solution| Wako Pure Chemical Industries, Ltd.               | 192–12565      |

Table 3  Composition of prepared reagents

| Reagent A          | Solute: 2.00 g of sulfanilamide | Solvent: 10% hydrochloric acid | Total volume: 100 mL |
|--------------------|---------------------------------|--------------------------------|----------------------|
| Reagent B          | Solute: 0.100 g of N-1-naphthylethylenediamine | Solvent: Water | Total volume: 100 mL |

![Fig. 3 Prepared sample solutions completing color reaction](image)

2.3 Experimental method

The end of the glass capillary with a 0.8-mm inner diameter, a 1.3-mm outer diameter, and 20.0 mm in length was dipped in the prepared sample solution and the glass capillary was filled with the sample solution through capillary action. Then, the glass capillary was set in the compact absorptiometer and the absorbance was then measured. The absorbance was determined by using Eq. (1) where \( A \) is the absorbance, \( V_1 \) is the amplified output voltage of the PD when the water that filled in the glass capillary was measured, \( V_2 \) is the amplified output voltage of the PD when the sample solution that filled in the glass capillary was measured. The absorbance is generally expressed by Eq. (2) which is called the Beer–Lambert law, where \( \varepsilon \) is molar extinction coefficient, \( c \) is concentration, and \( l \) is light path. The absorbance is proportional to concentration of sample and light path.

\[
A = -\log \frac{V_2}{V_1}
\] (1)
A = αcl  \quad (2)

3. Results and discussion

3.1 Horizontal straight-line type

Figure 4 shows a photograph and a cross-sectional view of the fabricated horizontal straight-line type device. The device was fabricated using black acrylonitrile-butadiene-styrene resin (ABS resin) so that the light exiting from the capillary does not radially return inside the capillary. Figure 4(b) shows the cross-sectional view of the device. The cover is not shown in Fig. 4(a) to show the internal structure, but the actual measurements were conducted with the cover in fact attached as shown in Fig. 4(b). The device had a light shield plate at the end of the capillary because we found that the influence of light through only the glass without transmitting the sample solution was significant and the output voltage of the PD was not different for the sample solution concentration when there was no light shield plate.

![Photograph of straight-line type device](image1)

(a) Photograph of straight-line type device  
(b) Cross-sectional view

Fig. 4  Photograph and cross-sectional view of straight-line type device

Figure 5 plots the results of the relation between the output voltage of the PD and the measurement time. The results indicate that the output voltage of the PD significantly decreased over time when water and the low concentration sample solutions were measured, whereas the output voltage of the PD remained almost unchanged when the high concentration sample solutions were measured.

Figure 6(a) plots the absorbance calculated from Eq. (1) using the output voltage of the PD at 7 min (420 s) in measurement time when the voltage of the PD was approximately constant. The black circles indicate the average absorbance, the triangles indicate the measurement values, and the error bars indicate the standard deviation (S.D.). Linearity between the concentration and the absorbance average was obtained within the concentration range from 0 to 0.250 mg/L, but was broken above a concentration of 0.250 mg/L. It was believed that the linearity was broken in the range above a concentration of 0.250 mg/L because the proportion of the noise which means the light transmitting through the glass was significant in the range. The absorbance should be proportional to the concentration from Eq. (2), so it was impossible to determine the concentration from the absorbance in the range above 0.250 mg/L. Figure 6(b) plots the absorbance within the concentration range from 0 to 0.250 mg/L. The contribution rate $R^2$ was 0.882 and the reproducibility was not very good in this range.
The instability in the output voltage of the PD over time was due to the change in the liquid surface shape at the end of the glass capillary. For example, when the glass capillary was filled with water, the shape of the liquid surface at the end of the capillary was convex, as shown in Fig. 7(a), but the shape of the liquid surface changed with vaporization, and finally became concave, as shown in Fig. 7(b). Since the shape of liquid surface at the end of the capillary was convex shortly after the low concentration sample solution filled in the capillary, the liquid surface acted as a convex lens and the lights were focused on the PD due to the convex liquid surface. However, the shape of the liquid surface gradually changed to concave, and the lights gradually diffused and the amount of the light reaching the PD decreased over time, and the output voltage of the PD gradually decreased over time. Finally, it was believed that the vaporization stopped when the pressure in the space between the capillary and the PD reached the saturated vapor pressure, and the output voltage of the PD became stable.

On the other hand, the shape of the liquid surface at the end of the capillary became concave shortly after the capillary filled with the high concentration sample solution because the surface tension of this solution was low. Accordingly, the change in the liquid surface at the end of the capillary was only slight, and the output voltage of the PD was stable when the high concentration sample solution was measured.
3.2 Vertical straight-line type

The glass capillary was set vertically and the other experimental conditions were the same as those in the horizontal straight-line type experiments. Figure 8 plots the results between the amplified output voltage of the PD and the measurement time. The time required for the value to become constant was shorter than that in the horizontal straight-line type.

Figure 9(a) plots the absorbance calculated from Eq. (1) using the voltage of the PD at 7 min (420 s) in measurement time when the voltage of the PD was approximately constant. The black circles indicate the average absorbance, the triangles indicate the measurement values, and the error bars indicate the standard deviation (S.D.). Linearity between the concentration and the absorbance average was obtained within the concentration range from 0 to 0.250 mg/L, but was broken above a concentration of 0.250 mg/L. These results indicate the same tendency to those obtained in the horizontal straight-line type experiments. Figure 9(b) plots the absorbance in the concentration range from 0 to 0.250 mg/L. The contribution rate \( R^2 \) was 0.945 within this range, and this indicates that the correlation between the concentration and the absorbance in the vertical straight-line type was improved more than that obtained in the horizontal straight-line type experiments.

In the vertical straight-line type, the shape of the liquid surface at the end of the capillary stabilized more promptly than that in the horizontal straight-line type due to gravity. It was believed that the shape of the liquid surface at the bottom of the capillary was convex and the shape of the liquid surface at the top of the capillary was concave due to gravity shortly after the capillary was set vertically, as shown in Fig. 10, and gravity suppressed the change in the shape of the liquid surface.
Fig. 9  Relation between concentration and absorbance calculated from data 420 s after measurement

(a) Absorbance between 0 to 0.700 mg/L  
(b) Absorbance between 0 to 0.250 mg/L

3.3 Multiple reflection type

Figure 11(a) shows a schematic overview of the fabricated multiple reflection type device and (b) shows a schematic of the top view of the bottom fixture jig. The length of the reflective plate between the entrance and exit slits, which was defined as \( L \), was 3.0 or 7.0 mm. The fixture jig was fabricated using black ABS resin, and the reflective plane was designed to be a pentagon, where the reflective plates with the deposited layer of silver were attached, as shown in Fig. 11(c). Figure 12 shows photographs of the actual fabricated device.
Figure 13 plots the results between the amplified output voltage of the PD and the measurement time when $L$ is 3.0 mm. The output voltage of the PD was significantly constant and stable, whereas in the horizontal or vertical straight-line type experiments, the output voltage of the PD significantly decreased over time when the low concentration sample solutions were measured.

In the straight-line type experiments, approximately 7 min (420 s) was needed for the voltage of the PD to become constant, while only several seconds was needed for the voltage to become constant in the multiple reflection type experiments. Figure 14(a) plots the absorbance when Eq. (1) was used for the calculation using the voltage of the PD at 30 s in measurement time. The black circles indicate the average absorbance, the triangles indicate the measurement values, and the error bars indicate the standard deviation (S.D.). Linearity between the concentration and the absorbance average was obtained within the concentration range from 0 to 1.000 mg/L, but was broken above a concentration of 1.000 mg/L.

Figure 14(b) plots the absorbance within the concentration range from 0 to 1.000 mg/L when the $L$ is 3.0 and 7.0 mm. Within this range, the contribution rate $R^2$ was 0.992 and 0.993 when the $L$ was 3.0 mm and 7.0 mm, respectively, and the correlation between the concentration and the absorbance in the multiple reflection type was significantly improved compared to those obtained in the horizontal and vertical straight-line type experiments.

In the multiple reflection type, the light does not transmit through the liquid surface at the end of the capillary, so
the light is unaffected by the change in the shape of the liquid surface, and it was possible to stably measure the absorbance. Additionally, it was found that the sensitivity, which means the absorbance to the concentration, can be controlled by adjusting the length of \( L \), which corresponds to a change in the light path.

![Figure 13](image)

**Figure 13** Relation between output voltage of PD and time in multiple reflection type when \( L \) is 3.0 mm

![Figure 14](image)

**Fig. 14** Relation between concentration and absorbance calculated from data 30 s after measurement

Table 4 summarizes the light path in each type. It indicates the theoretical light path of the light emitted from the center of the LED. The theoretical light path in horizontal and vertical straight-line type was 20.0 mm and that in the multiple reflection type was approximately 4.5 mm when the \( L \) was 3.0 mm whereas that in the normal type where the light transmits across the capillary radially was 0.8 mm. Accordingly, we can obtain longer light path in the devices proposed in this study than that in the normal type. The absorbance to the concentration in straight-line type was theoretically approximately 4.4 times higher than that in multiple reflection type, and it corresponded to the results of the experiments. In this study, the light path of the multiple reflection type was shorter than that of straight-line type, but it can be improved by optimizing mounting angle of the LED and PD and adjusting the length of \( L \), and the size of the capillary.

In the batch method like the devices studied in this paper where the sample solution was kept in a capillary and did not flow, the straight-line type could not measure the absorbance precisely because the change in the shape of the liquid
surface considerably influenced the measurement accuracy. However, it has a potential to be useful in a flow system where the capillary and flow tube are connected and the sample solution flows continuously and there is not the interface between air and sample solution.

### Table 4  Theoretical light path in each type

| Measuring method | Straight-line type | Multiple reflection type (when $L$ is 3.0 mm) | Normal type |
|------------------|-------------------|---------------------------------------------|-------------|
| Schematic        | ![Schematic](image) | ![Schematic](image)                        | ![Schematic](image) |
| Theoretical light path [mm] | 20.0 | 4.5 | 0.8 |

#### 4. Conclusion

We have developed a compact absorptiometer for water quality testing that uses a micro glass capillary. We actually developed three types of devices, namely, horizontal straight-line type, vertical straight-line type, and multiple reflection type, and evaluated them by conducting experiments to detect the nitrite nitrogen in water, which is a factor in water pollution. The devices consisted of a LED, a PD, and a glass capillary. In the horizontal and vertical straight-line type, the shape of the liquid surface at the end of the capillary significantly influenced the output voltage of the PD and it was difficult to precisely measure the absorbance, especially in the horizontal straight-line type, because the change in the shape of the liquid surface was considerable. On the other hand, in the multiple reflection type, we could quite stably measure the absorbance because the light emitted by the LED did not transmit through the liquid surface at the end of the capillary and the output voltage of the PD was uninfluenced by the change in the shape of the liquid surface. Additionally, controlling the sensitivity of the absorbance was possible by adjusting the length of the reflective plates, i.e., adjusting the light path. This device has a potential to make the absorptiometer smaller by optimizing mounting angle of the LED and PD and the reflective plates because we can obtain long light path in small region by reflecting light at various times in the device.

This compact absorptiometer needs only 10 μL of sample solution for measuring the absorbance. In addition, the sample solution in this device did not come into direct contact with the reflective plate, so it is believed that the device can suppress the decrease in the sensitivity caused by materials becoming directly attached to the reflection plate. Additionally, the device can be easily cleaned if perchance materials become attached to the reflection plate because it has an open structure and exchanging the glass capillary for a new one is also quite easy.

The absorptiometer was mainly studied for this paper, so the process for filling the capillary with the sample solutions was a manual operation. An automated water quality testing system could be created if a preprocessing device or system, e.g., a micro mixer and a flow control unit, is attached to the compact absorptiometer.

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