

Real-time in-situ simultaneous monitoring of dissolved oxygen and materials movements at vicinities of an aquatic plant by fluorescence quenching/deflection with an improved calculation method

DOI: 10.25177/JPS.2.2.2

Research

Author: Xing-Zheng Wu

December 2017

Received Date: 14th Nov 2017Accepted Date: 05th Dec 2017Published Date: 15th Dec 2017

Copy rights: © This is an Open access article distributed under the terms of Creative Commons Attribution 4.0 International License.

Luowei Huang^{1,2}, Xing-Zheng Wu,^{1†}

1. Department of Life, Environment and Materials Science, Fukuoka Institute of Technology, 3-30-1 Wajirohigashi, Higashi, Fukuoka 811-0295, Japan

2. School of Chemical Engineering, Nanjing University of Science and Technology, No.200, Xiaolingwei Road, Nanjing 210094, China

CORRESPONDENCE AUTHOR

Xing-Zheng Wu

Email: wu@fit.ac.jp

CONFLICTS OF INTEREST

There are no conflicts of interest for any of the authors.

ABSTRACT

Recently we developed a novel optical detection system that allowed real-time *in-situ* simultaneous monitoring of dissolved oxygen and materials movements at a vicinity of micrometers from an aquatic plant by combining deflection of a probe beam and fluorescence quenching. In the detection system, dissolved oxygen (DO)-quenched fluorescence was monitored together with beam deflection signals at the vicinity of the plant, then DO concentration was calculated from the fluorescence intensity. Here, three calculation methods for obtaining DO concentrations from the fluorescence intensity were examined. Results showed that exponential equations method, where fluorescent quenching coefficient and measured fluorescence intensity either with or without DO were approximated with exponential equations of temperature, gave the closest results to the measured ones by a DO sensor. The method was further applied to monitoring of oxygen and materials movement across the aquatic plants surface.

Keyword: fluorescence quenching, deflection, dissolved oxygen, aquatic plant, *in situ* monitoring

INTRODUCTION

Research on plants is becoming more and more important due to not only by population growth but also environmental problems such as the greenhouse effect. Most studies on plants are carried out by analyzing components involved in the plants activities with various analytical methods. For example, concentration changes of CO₂ and O₂ due to plants activities have been determined by Infrared spectroscopy¹⁾ and fiber optic micro-optrode²⁾, respectively. However, only average concentration changes over a defined sampling interval and within a sample-cell or sample-vessel that hold the plants have been obtained. The resulting average concentration changes reflect spatial- and temporal-average changes caused by the whole plants. It is difficult to distinguish the concentration changes caused from various organs such as leaves from stems. However, activities of the different or-

gans in a plant are usually different.³⁾

Recently, we have developed a novel optical detection method which allows real-time in-situ simultaneous monitoring of the dissolved oxygen and material movements at a vicinity of micrometers from the aquatic plant surface.⁴⁾ In the method, a blue semiconductor diode-laser was used as the light source of both the probe beam and excitation light for fluorescence of Ru-complex (Tris (2, 2'-bipyridyl) ruthenium (II) chloride). The dissolved oxygen (DO) quenched fluorescence and material movement-induced deflection signals at a vicinity of a plant *Egeria densa* Planch were monitored simultaneously and compared at different distances from the aquatic plant surface. The results show that the optical detection system can monitor DO and the material movements at the vicinity of the aquatic plants not only much more sensitively, but also much closer to real time than those analytical methods that monitor concentration changes in the bulk solution.⁴⁾

In the method⁴⁾, DO concentration was calculated from the DO-quenched fluorescence according to the Stern-Volmer equation:

$$\frac{F_0}{F} = 1 + K_{SV}C_{DO} \quad (1)$$

Where C_{DO} is the DO concentration, F_0 and F are measured fluorescence intensities of the Ru-complex solution without and with DO, and K_{SV} is the quenching coefficient, respectively.

Before measuring F at a vicinity of the plant, K_{SV} has to be known. Because temperature T affects F_0 , F , and C_{DO} , K_{SV} is depend on T . The following algorithm for obtaining $K_{SV}(T)$ was used.⁴⁾ Firstly, $C_{DO}(T)$ and $F(T)$ in a 10^{-6} M Ru-complex solution without the aquatic plant were monitored for 2 hours. The values of $C_{DO}(T)$ and $F(T)$ were approximated as a linear and quadric equations of T , respectively. Secondly, $F_0(T)$ was monitored with an anaerobic 10^{-6} M Ru-complex solution for 2 hours, and the measured $F_0(T)$ was also expressed by a quadric equation of T . Thirdly, with above $F_0(T)$, $F(T)$ and $C_{DO}(T)$, $K_{SV}(T)$ was calculated by

$$K_{SV}(T) = \frac{\left(\frac{F_0(T)}{F(T)} - 1\right)}{C_{DO}(T)} \quad (2)$$

The calculated values of $K_{SV}(T)$ was approximated as a quadric equation too.

Then, F and T at the vicinity of the aquatic plant were monitored, and C_{DO} at the vicinity were calculated by Eq. (1) from the monitored $F(T)$ and calculated $F_0(T)$, $K_{SV}(T)$. The previous experiments were carried out over a temperature range of 25~27°C. However, further experimental results showed that the calculation of C_{DO} had relatively larger experimental error, especially when temperature was exceeded 25 ~ 27 °C in summer season. Therefore, the calculation method has to be improved. Here, three different calculation methods for C_{DO} were tried and compared. Furthermore, the detection system with the improved calculation method is applied to monitor changes of DO and materials movement-induced deflection signal at vicinities of aquatic plant *Cabomba*.

Basic considerations of the novel calculation methods

Cubic equation (CE) method

As stated above, quadric equations were used to approximate $F_0(T)$, $F(T)$ and $K_{SV}(T)$ in previous work. Mathematically, when using polynomial equations to fit experimental data, the higher the polynomial degree is, the more the accuracy can be obtained. Therefore, cubic equations were tried to approximate the experimental relationship of F , F_0 and K_{SV} to T .

Exponential equation (EE) method

It was reported⁵⁻⁶⁾ that $\ln(K_{SV})$ was linear to $1/T$. The measured relationships of F_0 and F to T were found closer to exponential equations than polynomial ones. Hence exponential equations were used to fit the measured $F_0(T)$, $F(T)$, and the calculated values of $K_{SV}(T)$.

Three dimensional diagram (3DD) method

In phase fluorometric technique⁷⁻⁸⁾, fluorescence lifetime τ or phase shift of fluorescence was a function of C_{DO} . A three dimensional diagram (3DD) of phase shift, C_{DO} and temperature T was proposed to obtain C_{DO} from measured phase shift and temperature.⁹⁾ Here, a 3DD of F_0/F , C_{DO} , and T was tried to obtain C_{DO} . Moreover, the 3DD was tried to be expressed by a binary quadratic equation.

Experimental

Experimental setup was similar to the previous one⁴. As shown in Fig. 1, a semiconductor laser (wavelength 405nm) was used as the source of both the excitation light and the probe beam. The laser light was reflected by a dichroic mirror and focused at a vicinity of a short piece of aquatic plant *Cabomba* (about 3.0 cm long) in a culture dish with 20 ml of 10^{-6} M Ru-complex solution. A short piece of slide glass was placed on the aquatic plants to prevent any possible movement or motion. The culture dish was placed on a holder mounted on an X-Y-Z micro-stage. The fluorescence of the Ru-complex passed through the dichroic-mirror was detected by a PMT. Simultaneously, deflection signal of laser beam was detected by a deflection detector. A commercial DO/Temperature sensor (pyro science GmbH Hubertusstr. 35 D-52064 Aachen) was placed in the culture dish.

As the experimental procedures, C_{DO} , F , and T were firstly monitored for 2 hour with a 10^{-6} M Ru-complex solution without the plant. Secondly, F_0 and T were monitored for 2 hours with anaerobic 10^{-6} M Ru-complex solution without the plant. Thirdly, F and T at vicinities of the aquatic plants in the 10^{-6} M Ru-complex solution were monitored for 2 hours in a dark room. In all experiments, DO concentration at 1cm away from the plants was monitored by the commercial DO sensor too.

Preparations of either air-saturated or anaerobic Ru-complex solutions were prepared as previously described.⁴

Results and discussion

CE method

Figure 2(a) showed the monitored C_{DO} in the Ru-complex solution without the aquatic plant by the commercial DO sensor at room temperature. The experiments were carried out three times in different days, and every time the monitoring was carried out for 2 hours. The room temperature changed in $19.7 \sim 22.0$ °C in winter season, much different from previous experiments in summer season. The result showed that the C_{DO} linearly changed with T . The experimental relationship between C_{DO} and T was expressed as follows:

$$C_{DO}(T) = -0.125T + 11.2 \quad (3)$$

Figures 2(b) and 2(c) showed the monitored F and F_0 for an air-saturated and anaerobic 10^{-6} M Ru (II) complex solution, respectively. The experiments were also carried out 3 times on different days. Both F and F_0 were found to decrease with temperature. Relationships of F and F_0 to T were approximately expressed by cubic equations (4) and (5), respectively.

$$F(T) = -0.00610T^3 + 0.3547T^2 - 6.81T + 45.7 \quad (4)$$

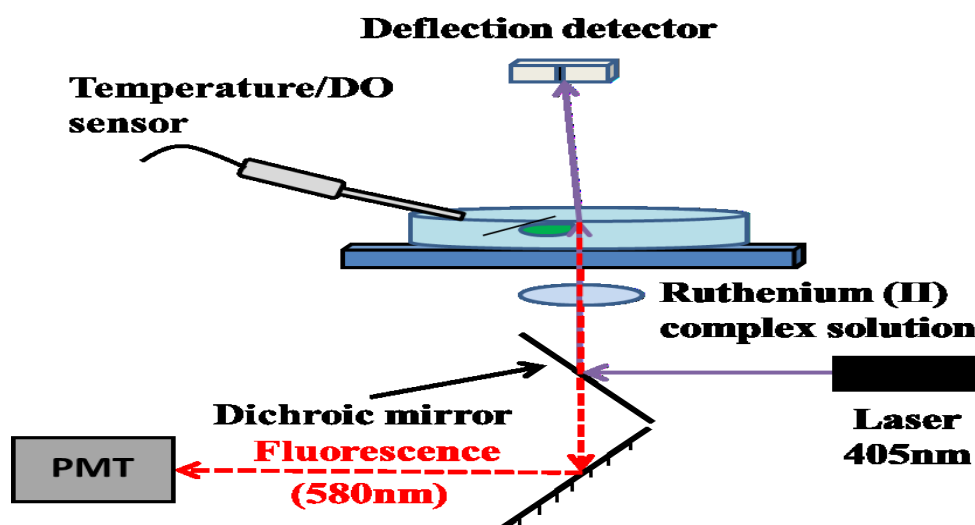
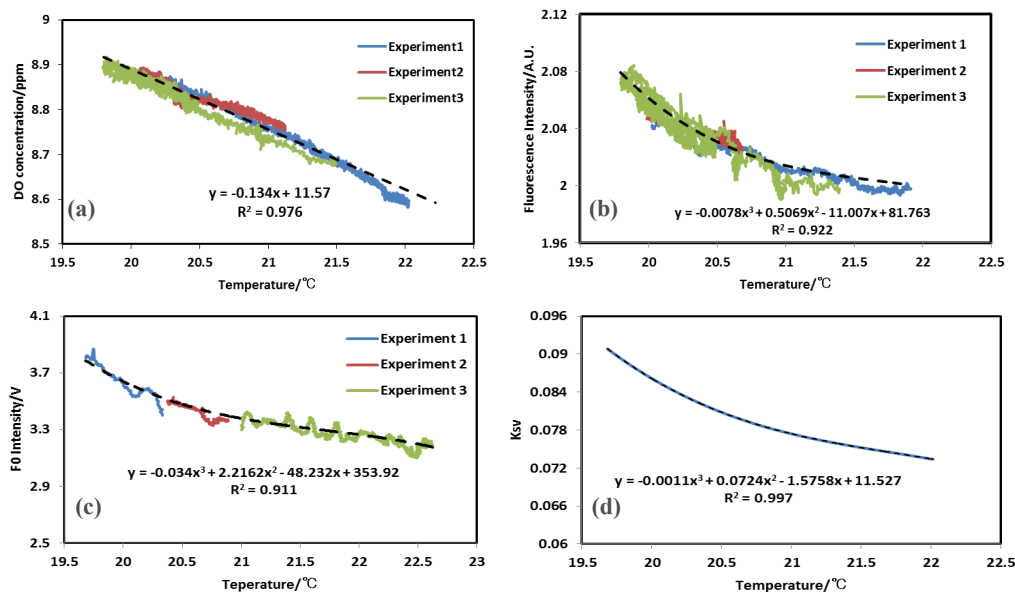


Fig. 1 Experimental setup of the optical system for deflection/fluorescence measurement.

Fig. 2 Relationships of C_{DO} (a), F (b), F_0 (c), and calculated K_{SV} (d) to T , respectively. The data were obtained from 10^{-6} M Ru-complex solution without the aquatic plant.



$$F_0(T) = -0.0340T^3 + 2.22T^2 - 48.2T + 354 \quad (5)$$

Then, $K_{SV}(T)$ was calculated by Eq. (2) with above $F_0(T)$, $F(T)$ and $C_{DO}(T)$ at 19.7~22.0°C. Figure 2(d) showed results of the calculated $K_{SV}(T)$. The values of $K_{SV}(T)$ also decreased with temperature. The calculated $K_{SV}(T)$ was fitted with a cubic equation as follows:

$$K_{SV}(T) = -0.000900T^3 + 0.0668T^2 - 1.56T + 12.1 \quad (6)$$

With above $K_{SV}(T)$ and $F_0(T)$, C_{DO} at vicinity of a plants or another Ru-complex solution could be calculated by Stern-Volmer equation from monitored fluorescence intensity F .

EE method

Figures 3(a) and 3(b) showed the exponential fitting of $F(T)$ and $F_0(T)$, respectively. The exponential fitting equations were as follows:

$$F(T) = 1.99 + 6.23 \times 10^{-12} \times e^{\frac{463}{T}} \quad (7)$$

$$F_0(T) = 3.10 + 2.21 \times 10^{-7} \times e^{\frac{297}{T}} \quad (8)$$

Then, values of $K_{SV}(T)$ were calculated from above $F_0(T)$, $F(T)$ and C_{DO} by Eq. (2) and the results were shown in Fig. 3(C). The calculated

$K_{SV}(T)$ was also fitted with an exponential equation as follows:

$$K_{SV}(T) = 0.0586 + 7.45 \times 10^{-7} \times e^{\frac{214}{T}} \quad (9)$$

With above $K_{SV}(T)$ and $F_0(T)$, C_{DO} at vicinity of a plant or another Ru-complex solution also could be calculated by Stern-Volmer equation from monitored fluorescence intensity F .

3DD method

Figure 4 showed the measured 3DD of F_0/F , C_{DO} and T from the 10^{-6} M Ru-complex solution without the plant. Furthermore, a binary quadratic equation was used to fit the 3DD in Fig. 4. A MATLAB program for the fitting calculations gave the following binary quadratic equation:

$$\frac{F_0}{F} = f(C_{DO}, T) = 0.857 \times C_{DO}^2 + 0.0555 \times T^2 + 0.277 \times C_{DO} \times T - 20.8 \times C_{DO} - 4.83 \times T + 144 \quad (10)$$

Then, C_{DO} at vicinity of the plant can be either found from the 3DD or calculated from the Eq. (10) for a measured F_0/F and T .

Fig. 3 Exponential fitting results of $F(T)$ (a) and $F_0(T)$ (b), and the calculated $K_{SV}(T)$ (c).

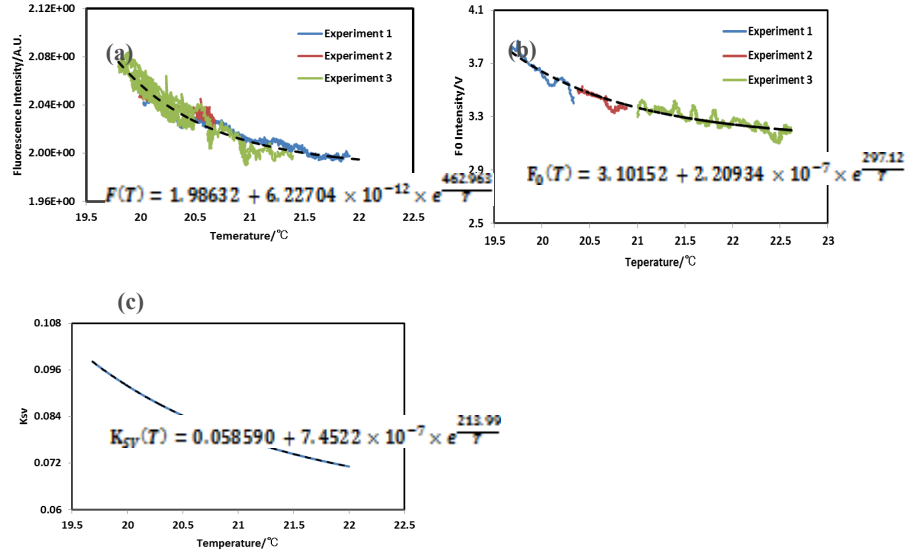


Fig. 4 Three dimensional diagram of the F_0/F , C_{DO} and T .

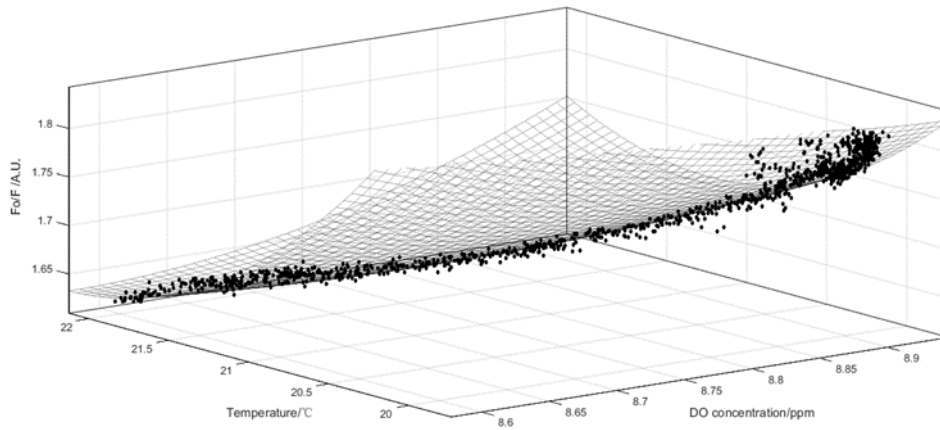
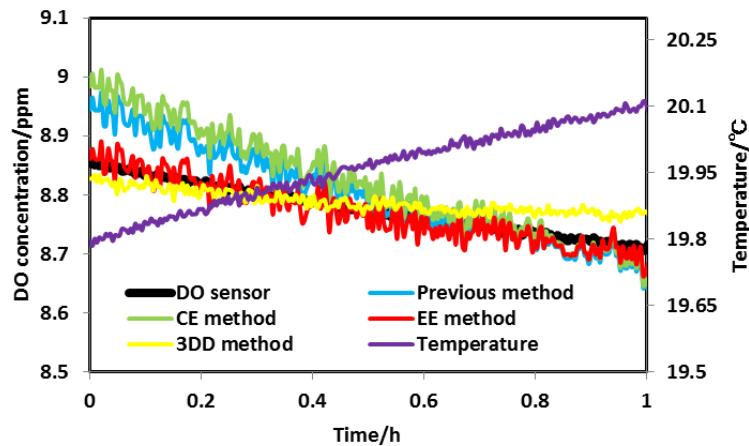


Fig. 5 Comparisons of the three calculation methods and the previous method with the DO-sensor measured results.



Comparison of the three methods with the previous one.

A new prepared 10^{-6} M Ru-complex solution without the aquatic plant was used for comparison of the three calculation methods. Fluorescence intensity F was monitored, and meanwhile temperature T and DO concentration were also monitored by the commercial DO sensor. Then, C_{DO} was calculated by the three methods, and the results were shown in Fig. 5. Figure 5 also shows calculation results of C_{DO} by the previous method and the measured C_{DO} by the DO sensor.

Figure 5 showed that the *EE method* gave the closest DO concentration to the measured ones by the DO sensor in the three calculation methods. In Fig 5, results of the FE method were also closer to measured ones by the DO sensor than the previous method. This meant that the *EE method* was the most accurate to obtain the DO concentration from the fluorescence quenching. Therefore, the *EE method* was used to determine the DO concentration at vicinity of the aquatic plants.

Monitoring of DO and deflection signals at the vicinity of the aquatic plant

Figure 6 showed a typical example of monitoring results of DO concentration and deflection signals at vicinities of different distance away from the aquatic plant surface in a dark room. As shown in Fig. 6(a), the DO concentration decreased with time at $0 \mu\text{m}$. After 2 hours, the DO concentration at $0 \mu\text{m}$ decreased to about 8.1 ppm from 9.3 ppm. Because respiration processes in the dark room consumed oxygen, DO at the plant surface was consumed firstly, then DO in solution diffused to the plant surface to supply the oxygen consumption.⁴⁾ On the other hand, DO changed little at $100 \mu\text{m}$ away from the plant. The DO concentration at this distance was nearly the same as that at 1 cm away from the plant surface monitored by the DO sensor. Figure 6(b) showed that the deflection signals also changed greatly at $0 \mu\text{m}$, while on the similar level as background solution at $100 \mu\text{m}$. These results indicated that the monitoring of both the DO and materials movements-induced deflection were with the most highest sensitivity at the plant surface.

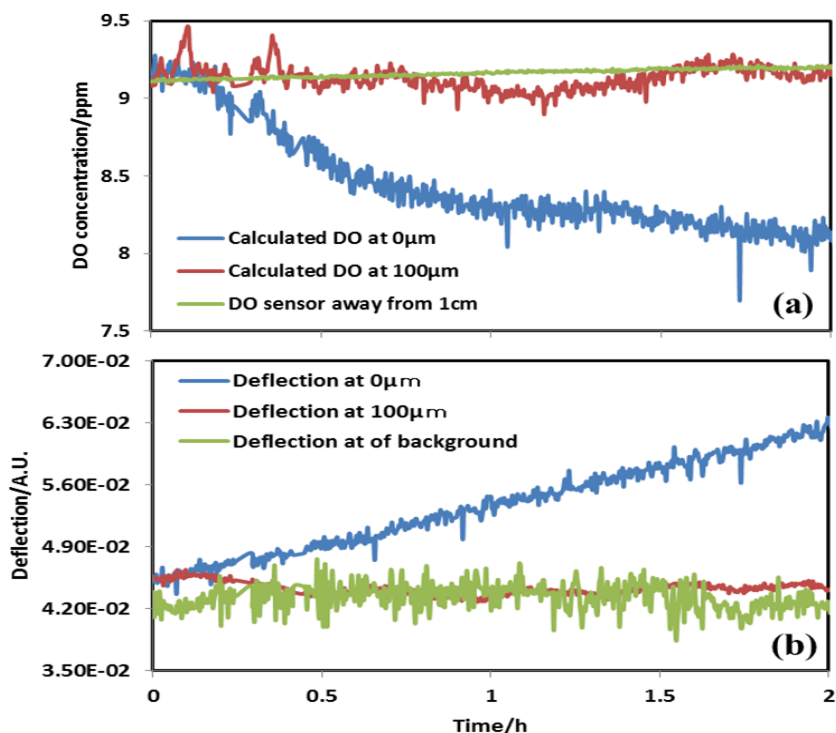


Fig. 6 Real time in-situ monitoring of DO concentration (a) and deflection signals (b) at the vicinity of different distance away from the leaf surface of the aquatic plant in a dark room.

As a conclusion, three calculation methods for obtaining DO concentration at vicinity of a plant from the oxygen-quenched fluorescence were examined. The results show that the *EE method* gave the best results. The optical detection system with the improved calculation method is more accurate in monitoring of DO at vicinity of the aquatic plants. This method is expected to be powerful in monitoring aquatic plants activities because of its higher sensitivity and more real-time than those conventional analytical methods by measuring the spatial- and temporal-average concentration changes.

REFERENCES

1. Schumacher T E, Smucker A J M, *Plant Physiology.*, 1983, 72(1), 212-214.
2. McCulloch S, Uttamchandani D, *Measurement and Technology.*, 1999, 146(3), 123-127.
3. Wu, X. Z., Nakaoka, T., Inoue, T., & Inoue, H. *Sensors and Actuators A: Physical.*, 2009, 155(2), 241-245.
4. Wu, Xing-Zheng, Xiaoyan Wu, and Tomomi Inoue. *Anal. Sci.*, 2017, 33(3), 351-357.
5. Wiczak W M, Latowski T, *Zeitschrift für Naturforschung A.*, 1992, 47(3), 533-535.
6. Patil, N., Melavanki, R., Patil, H., Nagaraja, D., & Sanningannavar R, F. *Life.*, 2013, 50, 64.
7. McDonagh, C., Kolle, C., McEvoy, A. K., Dowling, D. L., Cafolla, A. A., Cullen, S. J., & Mac-Craith, B. D. *Sensors and Actuators B: Chemical.*, 2001, 74(1), 124-130.
8. Lippitsch M E, Draxler S. *Sensors and Actuators B: Chemical.*, 1993, 11(1-3), 97-101.
9. Holst, G. A., Köster, T., Voges, E., & Lübbers, D. W. *Sensors and Actuators B: Chemical.*, 1995, 29 (1-3), 231-239.

Contact Us: SIFT DESK

Deerpark Dr, #75, Fullerton, CA, 92831, United States.

E-mail: helpdesk@siftdesk.org

Visit us on the web at: www.siftdesk.org