Dissolved oxygen and pH detection system based on the fluorescence characteristics of coumarin-modified CdSe quantum dots

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Abstract. In the field of biomedicine, the pH and dissolved oxygen (DO) content of the cell metabolism fluid can reflect the health of the cell and provide an auxiliary reference for the early detection of cancer. Based on the preliminary research results of the same group, a dual-parameter synchronous detection system for cell metabolic fluid was established. The system is based on the fluorescence characteristics of 7-Amino-4-methylcoumarin-modified CdSe quantum dots. Both of pH and DO are solved by the voltage signal of the photodetection module. Firstly, the characteristics of 7-Amino-4-methylcoumarin-modified CdSe quantum dots were introduced. Then the dual-parameter detection method was briefly proposed. Next, the hardware components and software functions of the system were introduced. Finally, the detection algorithm was proposed and the solution model was built according to the calibration experiment. The verification experiment showed that the sensitivity for detecting pH was 0.05 and the accuracy was 0.07. The sensitivity for detecting DO was 0.04 mg/L, and the accuracy was 0.07 mg/L. As the prototype machine, the system can basically realize the dual-parameter detection of the cell metabolism liquid with high accuracy, fast speed and small loss of the original liquid.

1. Introduction

Cancer is one of the most serious diseases threatening human health. “Cancer statistics, 2018” predicted that the number of new cancer cases in the United States would be 173,350 and the number of new cancer deaths would be 609,640 in 2018 [1]. Early screening for cancer is one way to reduce cancer mortality. The abnormal metabolic process of cancerous cells leads to different parameters of the cell metabolism fluid than normal cells. The most representative parameters are pH and DO. The pH of most cancer cell metabolites is 6.0-7.0, which is lower than that of normal cells by 7.3-7.4 [2]. The DO of abnormal cell metabolizing fluid is lower than that of normal cell [3]. Therefore, the pH and DO of the cell metabolism fluid can reflect the health of cells and provide a reliable basis for early diagnosis of cancer [4].

At present, the detection methods of the pH of the cell metabolism liquid mainly include MRI method, microelectrode method, fiber colour modulation method and fluorescence spectroscopy. The MRI method has low measurement accuracy and a large volume of equipment, which is not suitable for general laboratory. The microelectrode method has many sources of error, high measurement cost and poor reliability. The colour modulation of optical fiber has many research results. Tou et al. realized the...
optical fiber pH sensor by means of interferometry [5]. Fan S F used the dual LED for reference and produced a commercial fiber pH sensor with high measurement accuracy, but the production cost is high [6]. Fluorescence spectroscopy method is widely used, but the commonly used organic dyes have poor optical stability, wide and overlapping emission spectrum, so the signal is difficult to obtain [7].

The methods of measuring DO mainly include iodometry, electrochemical probes and optical sensors. The iodometry method requires much liquid to be tested, and the operation is very complicated. The electrochemical probe requires the test solution to ensure a certain flow rate. The optical DO sensor is widely used, but they must be stored in a humid environment when not working, which leads to some trouble in using it. The DO measurement chip proposed by Shao et al is effective for DO measurement in solution [8], but it is difficult to use for a small amount of solution. Boonya et al. developed a method for measuring DO under extremely acidic conditions [9], but not advisable to cell living environments.

However, simultaneous detection of DO and pH has not been reported. A method for detecting DO and pH is proposed in this paper, which is based on the fluorescence characteristics of 7-Amino-4-methylcoumarin-modified CdSe quantum dots. The fluorescence signal of two wavelengths can be obtained by excitation of one wavelength, and these can be calculated to obtain the pH and DO. Based on this, two photomultiplier tubes are used to construct a dual-parameter detection system.

2. Principle of Dual-parameter Detection
The coumarin-modified CdSe quantum dots have a large Stokes shift, a wide excitation wavelength range and a narrow emission wavelength range. The fluorescence intensity and stability are better than ordinary fluorescent dyes, and they have good biocompatibility. A method based on this for detecting pH and DO is proposed, and the principle is shown as figure 1. CdSe quantum dots containing ZnS nucleocapsid were modified by 7-Amino-4-methylcoumarin. Under the ultraviolet light with the wavelength of 365 nm, CdSe quantum dots will produce fluorescence with the wave peak of 625 nm, and 7-Amino-4-methylcoumarin will produce fluorescence with the wave peak of 485 nm. The fluorescence of CdSe quantum dots is sensitive to pH but not sensitive to DO. The fluorescence will be quenched to different degrees due to pH. The fluorescence of 7-Amino-4-methylcoumarin is sensitive to DO and pH. The pH and DO of the solution can be solved by measuring the fluorescence intensity of the 7-Amino-4-methylcoumarin-modified CdSe quantum dots at these two wavelengths.

![Figure 1. Principle of dual-parameter detection.](image)

3. Dual-parameter Detection System
Figure 2 is a block diagram of the dual-parameter detection system. The system frame is made of black plexiglass and evenly painted with black matt lacquer. The light source is selected from CUN6GF1A UVLED353 violet lamp bead of Seoul SVC. The two photomultiplier tube modules are selected from the same batch of Hamamatsu H10493-013 photomultiplier tube module. The cuvette is 400 μL slit cuvette, and the data acquisition card is MP411L Module. The self-made constant voltage power supply supplies power to the light source and two photomultiplier modules. The light source uses 3.1 V, the two photomultiplier modules use ±15 V for power and 0.9 V for control voltage. The exit tube and the incident tube are in contrast to the cuvette, preventing the beam from diffusing into other areas and minimizing the effects of other light that may be present in the environment. A 365 nm filter is mounted at the rear end of the exit barrel. The rear ends of the two incident cylinders are symmetrically mounted
with a 625 nm filter and a 485 nm filter respectively. Two photomultiplier tubes are symmetrically mounted at the rear of the two entrance tubes and connected to the data acquisition module. The data acquisition module is connected to the PC. The data is transmitted to the host computer and processed.

Figure 3 is a physical diagram of the detection system.

![Figure 3](image3.png)

**Figure 3.** Image of two-parameter measuring system.

The software of dual-parameter detection system displays the two voltage values collected by the data acquisition module in real time, and separately filters and processes the two voltage signals for subsequent analysis. Figure 4 shows the interface of the host computer software.

The sensitive substance used in the dual-parameter detection system is the self-made 7-Amino-4-methylcoumarin-modified CdSe quantum dots, as shown in figure 5.

![Figure 4](image4.png)

**Figure 4.** PC interface of two-parameter measuring system.

![Figure 5](image5.png)

**Figure 5.** 7-Amino-4-methylcoumarin-modified CdSe quantum dots.

When the system works, a stable power supply will supply power to the light source and the two photomultiplier modules. The light source emits the light with a wavelength of about 365 nm, which passes through the 365 nm filter and is irradiated onto the cuvette side of the detection chamber through the exit tube. The solution in the cuvette will fluoresce when excited. Fluorescence is detected by two identical photomultiplier modules after passing through symmetric filters on both sides of the detection chamber. When the working condition is stable, the voltage signal output by the photomultiplier module is proportional to the collected light intensity, so the pH and DO of the solution can be calculated synchronously by the voltage signals of the two photomultiplier modules. The calculation process is: firstly, the pH value of the solution is calculated from the voltage corresponding to the wavelength of 625 nm, then the DO value of the solution is calculated according to the pH result and the voltage corresponding to 485 nm.
4. Calibration and Verification

In the dual-parameter detection system, the photomultiplier module is used to detect the fluorescence intensity. It is necessary to establish a detection model by using the output voltage of the two photomultiplier modules and the two parameters of the solution. The fluorescence intensity of 625 nm is mainly related to the pH of the solution. It is necessary to establish a detection model of the pH and the output voltage corresponding to the 625 nm, which is called Model 1. The fluorescence intensity of 485 nm is mainly related to the pH and DO of the solution. Under the condition of knowing the pH, it is necessary to establish a detection model of pH, DO and the output voltage corresponding to the 485nm, which is called Model 2. By substituting Model 1 into Model 2, the final model for dual-parameter simultaneous detection can be obtained.

4.1. pH Detection Calibration

The experiment was carried out by selecting eight groups of PBS buffers having pH of 6.1, 6.3, 6.52, 6.72, 7.07, 7.33, 7.63, 7.87. The pH was measured by a pH meter as standard values. After extensive experiments, it was found that the best results were obtained by mixing 297 μL of PBS buffer with 3 μL of 7-Amino-4-methylcoumarin-modified CdSe quantum dots. 297 μL of the buffer was mixed with 3 μL of these quantum dots and placed in a cuvette of the detection system. The output voltage corresponding to the wavelength of 625 nm was recorded, and the relationship between the voltage and the pH at this wavelength was investigated. Figure 6 shows the test results for each set of experiments. The average of each set was taken as a stable output voltage, and the relationship with the pH of each group was established, as shown in figure 7.

![Figure 6. Experimental results of eight groups.](image)

![Figure 7. Relationship between pH and voltage.](image)

The results of figure 7 can be fitted to obtain the linear relationship between the fluorescence intensity at 625 nm and the output voltage of the photomultiplier modules, which is Model 1 as follows.

\[ U_{625} = 214.432x - 532.746 \]  

(1)

Where x is pH, \( U_{625} \) is the voltage of photomultiplier module corresponding to 625nm. \( R^2 \) is 0.9922. The standard deviations of the eight experimental data were 8.463 mV, 7.763 mV, 10.740 mV, 5.097 mV, 6.447 mV, 8.815 mV, 6.476 mV, 7.164 mV. The maximum standard deviation was taken to calculate and the sensitivity limit for detecting pH was 0.05.

4.2. DO Detection Calibration

19 sets of PBS buffers of different pH were selected, and each set of buffers was taken out in four equal portions for deoxygenation to obtain different DO content. The deoxygenation operation is to introduce nitrogen into the solution for different time. 76 groups of liquid to be tested were obtained with different pH and DO. The DO content of each group of solutions was measured using a Hash Polymetron 9582.
Dissolved Oxygen Analyzer and used as a standard value. For each set of experiments, 297 μL of buffer was mixed with 3 μL of such quantum dots and placed in the cuvette of the detection system. The relationship between the output voltage corresponding to the wavelength of 485 nm and two parameters of the solution was recorded. The output voltage of each set of experiments was averaged and shown in figure 8. It can be seen from the data that when the pH is constant, the output voltage has a good linear relationship with DO, but the slope and intercept of the linear relationship vary with pH. The data at each pH was linearly fitted and expanded. The measured DO maximum of 9.25 mg/L and the measured minimum of 8.18 mg/L were used as the endpoints, as shown in figure 9.

Taking the pH and the voltage as input and DO as output, a mathematical model was established, and the establishment process is as shown in figure 10. The detection model of the voltage, pH and DO was finally obtained as follows, which is Model 2.

\[
y = 9.25 - \frac{1.07}{f_1(x) - f_2(x)} \left( 3.95319U_{485} - 2349.47055 - f_2(x) \right)
\]

\[
f_1(x) = \begin{cases} 
-49668.50354x^4 + 1.26801 \times 10^6x^3 - 1.21316 \times 10^7x^2 \\ + 5.15547 \times 10^7x - 8.21073 \times 10^7(6.01 < x \leq 6.78) \\
-32719.13809x^5 + 1.21945 \times 10^8x^4 - 1.81686 \times 10^9x^3 + 1.35268 \times 10^8x^2 \\
-5.03245 \times 10^9x + 7.48472 \times 10^9(6.78 < x < 7.97)
\end{cases}
\]

\[
f_2(x) = \begin{cases} 
-4.60869 \times 10^5x^6 + 1.77387 \times 10^7x^5 - 2.84367 \times 10^8x^4 + 2.4303 \times 10^9x^3 \\
-1.16785 \times 10^{10}x^2 + 2.9918 \times 10^{10}x - 3.19219 \times 10^{10}(6.01 < x \leq 6.78) \\
-120.43972x^3 + 27538.43289x^2 - 210258.72245x + 538467.76128 \\
(6.78 < x < 7.97)
\end{cases}
\]

Where x is pH, y is DO (mg/L), and $U_{485}$ is the voltage corresponding to 485 nm (mV). In each set of data, the maximum voltage standard deviation is 12.823 mV, and the detection sensitivity limit is 0.04 mg/L. The pH x in this model was replaced by Model 1, and the whole model was obtained, whose input are $U_{625}$ and $U_{485}$, and the output are pH and DO. Process is shown in figure 11.

4.3. Dual-parameter Detection Verification

Eight groups of PBS buffers were selected for the verification. The pH values of the eight groups of solutions were respectively measured by a hand-held pH meter as standard values, and the DO values of the eight groups of solutions were respectively measured by a Hash Polymetron 9582 dissolved oxygen analyzer as a standard value. For each set of solutions, 297 μL of PBS buffer was mixed with 3 μL of 7-Amino-4-methylcoumarin-modified CdSe quantum dots and detected using the dual-
parameter detection system. The output voltages were recorded and calculated according to the above model and the pH and DO measured by the system were obtained. Table 1 shows the system verification results.

| Group | pH standard value | pH measured value | Error | DO standard value (mg/L) | DO measured value | Error (mg/L) |
|-------|-------------------|-------------------|-------|--------------------------|------------------|-------------|
| 1     | 6.10              | 6.079             | -0.021| 9.04                     | 9.017            | -0.023      |
| 2     | 6.30              | 6.251             | -0.049| 9.12                     | 9.103            | -0.017      |
| 3     | 6.52              | 6.589             | 0.069 | 8.97                     | 9.004            | 0.034       |
| 4     | 6.72              | 6.686             | -0.034| 9.03                     | 9.002            | -0.028      |
| 5     | 7.07              | 7.135             | 0.065 | 8.82                     | 8.755            | -0.065      |
| 6     | 7.33              | 7.375             | 0.045 | 9.03                     | 8.982            | -0.048      |
| 7     | 7.63              | 7.566             | -0.064| 8.94                     | 8.879            | -0.061      |
| 8     | 7.87              | 7.860             | -0.010| 9.05                     | 9.106            | 0.056       |

It can be seen from the above results that the dual-parameter detection system can realize the simultaneous measurement of pH and DO. The sensitivity of measuring pH satisfies 0.05, the accuracy satisfies 0.07, the sensitivity of detecting DO satisfies 0.04 mg/L, and the accuracy satisfies 0.07 mg/L.

5. Conclusion

In this paper, a dual-parameter detection system based on the analysis of fluorescence characteristics of 7-Amino-4-methylcoumarin-modified CdSe quantum dots was established. The hardware structure, software functions, and the work principle were introduced. Then the detection algorithms of pH and DO were proposed respectively, and the two parameters were decoupled. Finally, the mathematical calculation formula and detection sensitivity limit of dual-parameter detection were given. The experimental results showed that the sensitivity of the two-parameter detection system was 0.05, the accuracy was 0.07, the sensitivity of detecting DO was 0.04 mg/L, and the accuracy was 0.07 mg/L. As a prototype machine, the system basically realized the dual-parameter detection of liquid, with high accuracy, fast speed, small loss of the original liquid and high reference value.

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