Rapid Detection of Sodium Hexametaphosphate by Visualization of Ratiometric Fluorescent Probes Based on Carbon Dots and Copper Nanoclusters

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Abstract: A method for ratiometric fluorescence detection of sodium hexametaphosphate based on carbon dots and copper nanoclusters was established. In this probe, aggregation-induced emission (AIEE) between Al3+ and CuNCs resulted in fluorescence enhancement accompanied by carbon dot fluorescence burst. When sodium hexametaphosphate was added, Al3+ was coordinated with sodium hexametaphosphate to release Al3+ from the surface of Cu nanoclusters to achieve Cu nanocluster fluorescence burst. The different response of the dual emission to sodium hexametaphosphate leads to a change in fluorescence color, which makes macroscopic visualization easy. With a smartphone, the fluorescence color change can be observed more clearly than with a single fluorescent nanoprobe, and the response is linearly increasing, so the nanoprobe can be applied for rapid measurements. In addition, the dual-emission nanoprobe was successfully used for beverage analysis. Accurate recoveries ranged from 97.5% to 102.5% with a relative standard deviation of less than 3.1%. Thus, this dual-emission detection system integrated with a smartphone holds promise as a new portable method for on-site measurement of sodium hexametaphosphate.

Keywords: Sodium hexametaphosphate; Copper nanoclusters; Carbon point; Ratiometric fluorescence; Aggregation induced luminescence; Smartphone app.

1. Introduction

Sodium hexametaphosphate (SHMP) is a traditional and commonly used food-grade quality improver. It is often used in the food industry to improve the water-holding and caking properties of meat products, to prevent fat oxidation, to improve the juice yield and viscosity of fruit beverages, and to inhibit the decomposition of vitamin C; it is also used to stabilize the natural color and color in other foods [1,2]. However, food-grade sodium hexametaphosphate often contains traces of heavy metals (arsenic, lead, etc.) and a certain amount of fluoride, and its excessive addition not only destroys the nutritional elements in food, but also poses a serious health risk [3,4]. Therefore, it is important to establish a simple and convenient method to detect the content of sodium hexametaphosphate in food additives. Currently, the methods for the determination of sodium hexametaphosphate include ion chromatography [5], atomic absorption photometry, gravimetric method and spectrophotometric method. Most of the traditional detection methods require not only expensive equipment, tedious pretreatment and skilled operators, but also cumbersome and time-consuming determination steps, which limit their application areas. Fluorescence analysis for trace analytes had been proven to be a more powerful optical technique. (reference?)

Compared with traditional methods, fluorescence detection assays have various advantages, such as operative simplicity, high detection sensitivity, good selectivity for the target, fast response time, and wide range of application [6]. Carbon dots have excellent optical properties, size tunability, high quantum yield, high photostability, high absorption coefficient, resistance to photobleaching and good water solubility [7,8]. Copper nanoclusters have large Stoke shift and very unique optical properties such as tunable size, very high quantum yield, photostability properties and absorption coefficient [9,10]. Carbon dots and copper nanoclusters have emerged as highly promising inorganic nano-materials in the field of food analysis. In recent years, there’s a variety of studies shed lights on the effectiveness of using carbon dots and copper nanoclusters as novel fluorescent probes for the analytical detection of various sensing materials. However, the complex matrix of the sample itself and the presence of substances with very similar luminescence response properties to the target to be measured led to a reduction in the fluorescence specific selectivity of carbon dots and copper nanoclusters. To address this critical issue, ratiometric fluorescent probes were chosen. The ratio probe is based on the intensity ratio of two fluorescence wavelengths as the output signal, and has a self-calibration function that can eliminate the effect of non-specific background and further improve the sensitivity, selectivity and accuracy of the detection [11,12]. Commonly used sensing strategies for proportional detection are generally based on fluorescence burst or enhancement, such as the internal filtration effect, fluorescence resonance energy transfer, and aggregation-induced emission effect.

In this study, we prepared blue-light carbon dots with red-light copper nanoclusters to construct nanoprobe...
emission peaks, allowing them to be captured by the naked eye with a significant change in fluorescence color. When Al\textsuperscript{3+} is present in the system, Al\textsuperscript{3+} can bind to the carboxyl group in the copper nanoclusters. The fluorescence intensity of the copper nanoclusters was enhanced at 610 nm, while the fluorescence of the carbon dots was burst at 444 nm. The affinity of sodium hexametaphosphate for Al\textsuperscript{3+} was higher than that of the copper nanoclusters. It caused the fluorescence burst of copper nanoclusters. Therefore, the ratio of fluorescence intensity of copper nanoclusters to carbon dots (F610/F444) could be used for the sensitive and selective detection of sodium hexametaphosphate. In particular, this ratio nanoprobe can be successfully applied to the immediate detection of sodium hexametaphosphate using smartphones. The smartphone-assisted visual results had greater accuracy on capturing small changes in RGB values than the naked eye, and the results showed high selectivity, sensitivity, and interference resistance performance.

2. Experimental Part

2.1. Apparatus and reagents

Sodium hexametaphosphate citrate, urea, glutathione, copper chloride, aluminum chloride, sodium hydroxide, and 2-morpholine ethanesulfonic acid were provided by Aladdin Reagent (Shanghai) Co. The fluorescence spectrophotometer was a Lumina FL-4500 model from Thermo, USA.

2.2. Experimental methods

2.2.1. Synthesis of carbon dots

Carbon dots were prepared with reference to the literature [13]. 0.21 g (1 mmol) citric acid and 0.18 g (3 mmol) urea were dissolved in 5 ml of water and stirred to form a clear solution. The solution was then transferred to a 20 ml Teflon-lined stainless steel autoclave. The sealed autoclave was heated to 160 °C in an electric oven and held for an additional 4 hours. Ethanol was added to the solution to collect the final product and centrifuged at 5000 rpm for 5 minutes. The solid could be easily redispersed into water.

2.2.2. Preparation of Al\textsuperscript{3+}-CuNCs

Copper nanoclusters were prepared according to the referenced literature [14]. Firstly, a solution of glutathione (50 mg/mL) was prepared with a volume of 5 mL, and a solution of CuSO\textsubscript{4} (10 mmol/L) was prepared with a volume of 5 mL. The glutathione solution was slowly added dropwise to the CuSO\textsubscript{4} solution, resulting in the formation of flocculent precipitate. Then, a solution of NaOH (1 mol/L) was added dropwise while continuously stirring until the solution became transparent and light yellow. The temperature of a thermostatic water bath was set to 37 ℃, and the mixture was placed in the water bath and shaken for 1 hour. Afterward, the mixture was removed and kept in a freezer for later use.

To the CuNCs solution (100 μL), 100 μL of Al\textsuperscript{3+} solution with a concentration of 20 μM and 2-mercaptoethanesulfonic acid buffer solution (pH=6) were added for mixing. The fluorescence intensity was measured using a fluorescence spectrophotometer in the presence of different concentrations of Al\textsuperscript{3+}. Excitation was performed at 320 nm, and the emission wavelength range recorded was 400-800 nm.

2.2.3. Preparation and Determination of Carbon Dots/Al\textsuperscript{3+}-CuNCs in Sodium Hexametaphosphate

The prepared carbon dots and Al\textsuperscript{3+}-CuNCs were diluted to concentrations of 50 μg/mL and 100 μg/mL, respectively. Then, the carbon dots and Al\textsuperscript{3+}-CuNCs were mixed in different volume ratios (Al\textsuperscript{3+}-CuNCs:carbon dots = 2:1, 1:1, 1:2, 1:4, 1:6) to optimize the detection conditions. Based on the fluorescence color and spectrum, the optimal mixing ratio was selected, and this ratio was used to construct the fluorescence probe for ratio determination.

A volume of 200 μL of the carbon dots and Al\textsuperscript{3+}-CuNCs solution with the optimal volume ratio was mixed with 200 μL of sodium hexametaphosphate solution at different concentrations and 600 μL of 2-methyl-2-thiopseudourea buffer (pH = 6). The mixture was then subjected to fluorescence intensity detection using a fluorescence spectrophotometer, with excitation at 320 nm and recording of emission wavelengths in the range of 400-800 nm. The solution was also photographed using a smartphone, and the colors in the photos were quantitatively analyzed by extracting the red (R), green (G), and blue (B) values, using a color recognition extractor in the WeChat mini-program.

2.2.4. Sample Processing

Three types of tea beverages containing sodium hexametaphosphate from the ingredient tables were taken, and the tea beverages were then filtered and diluted by 10 times, using a 0.22 μm membrane. 200 μL of the processed tea beverage were taken and added to 200 μL of carbon dots and Al\textsuperscript{3+}-CuNCs solution in the optimal volume ratio. The solutions were mixed with 600 μL of 2-aminoethyl pyridine ethanesulfonic acid buffer (pH=6), and placed in a fluorescence spectrophotometer to measure the fluorescence intensity. The emission wavelength ranged from 400 to 800 nm was recorded after exciting it at 320 nm.

3. Results and Discussion

3.1. Optical Properties of Carbon Dots and Al\textsuperscript{3+}-CuNCs

In this paper, the fluorescence properties of carbon dots/Al\textsuperscript{3+}-CuNCs for the determination of hexametaphosphate sodium (HMP) were investigated, as well as the fluorescence properties of carbon dots and copper nanoclusters (CuNCs), as shown in Figure 1. The emission peak of carbon dots was observed at 444 nm, exhibiting a high blue fluorescence under optimal excitation at 340 nm (Figure 1A). Al\textsuperscript{3+}-CuNCs emitted red fluorescence when excited at 280 nm, with an emission peak at 610 nm (Figure 1B). Compared to individual carbon dots, the ratio-type nanoprobe showed two emission peaks. It is worth noting that the characteristic fluorescence peaks of Al\textsuperscript{3+}-CuNCs and carbon dots could be triggered at different excitation wavelengths. Figure 1C illustrates the fluorescence emission peaks of carbon dots/Al\textsuperscript{3+}-CuNCs under various excitation wavelengths. Both carbon dots and Al\textsuperscript{3+}-CuNCs exhibited clear single emission peaks at the given excitation wavelength, while carbon dots/Al\textsuperscript{3+}-CuNCs displayed dual emission peaks when the excitation wavelength reached 320 nm. Therefore, 320 nm was selected as the excitation wavelength for fluorescence spectrometer analysis. The responses of the individual fluorescence system, ratio fluorescence system, and hexametaphosphate sodium were also investigated. It could be observed that the peak at 444 nm increased while the peak at 610 nm decreased upon the addition of hexametaphosphate sodium (Figure 1D). Therefore, this system could be used for the detection of hexametaphosphate sodium.
Figure 1. shows the fluorescence emission spectra of (A) carbon dots, (B) Al\(^{3+}\)-CuNCs, and (C) carbon dots/Al\(^{3+}\)-CuNCs at different excitation wavelengths. The insets display corresponding fluorescence photographs under UV light. (D) The fluorescence emission spectra of carbon dots, Al\(^{3+}\)-CuNCs, carbon dots/Al\(^{3+}\)-CuNCs, and carbon dots/Al\(^{3+}\)-CuNCs with SHMP.

### 3.2. Fluorescence Response of Carbon Dots/Al\(^{3+}\)-CuNCs to Sodium Hexametaphosphate

In order to eliminate potential interferences in practical sample analysis and enhance the performance of the Carbon Dots/Al\(^{3+}\)-CuNCs detection method, the factors of pH value and reaction time were investigated and optimized. The impact of changing pH values within the range of 3-10 on the fluorescence sensing system was examined. The optimal sensing performance was observed at pH 6, which may be attributed to the strongest complexation between sodium hexametaphosphate and Al\(^{3+}\)-CuNCs at this pH value (Figure 2A). With pH 6 determined as the optimal value, the incubation time between Carbon Dots/Al\(^{3+}\)-CuNCs and sodium hexametaphosphate was measured. As shown in the graph, a stable fluorescence response intensity was achieved within a reaction time of less than 2 minutes, indicating a fast response time (Figure 2B).

![Figure 2](image)

In addition, the ratio between carbon dots and Al\(^{3+}\)-CuNCs had been optimized. Carbon dots and Al\(^{3+}\)-CuNCs were mixed in ratios of 2:1, 1:1, 1:2, 1:4, and 1:6 to explore the optimal ratio for the fluorescence response measured using hexametaphosphate sodium (Figure 3). The optimal ratio of carbon dots to Al\(^{3+}\)-CuNCs for the best fluorescence color was optimized, showing significant color changes under UV excitation (365 nm). When different ratios of Al\(^{3+}\)-CuNCs were added, the blue emission from the carbon dots shifted to red. When the ratio of carbon dots to Al\(^{3+}\)-CuNCs was 2:1, two emission peaks appeared at 444 nm and 610 nm under single-wavelength excitation (\(\lambda_{ex}=320\) nm), with pronounced red fluorescence under 365 nm UV light. Changes in emission intensity and fluorescence color were recorded when the carbon dots/Al\(^{3+}\)-CuNCs nanoprobe was added to the solution. The change in fluorescence intensity (I(610)/I(450)) represented the variation in fluorescence intensity ratio before and after adding hexametaphosphate sodium. The most significant change in fluorescence intensity was observed when carbon dots and Al\(^{3+}\)-CuNCs were mixed in a 1:1 ratio.

![Figure 3](image)
(Figure 3A). The colors of different ratios of nanoprobe are shown in Figure 3B, with a blue color observed at a ratio of 1:6. In conclusion, we chose a 1:1 ratio to prepare the carbon dots/Al\textsuperscript{3+}-CuNCs.

Figure 3. (A) shows the fluorescence intensity changes of Carbon Dots/Al\textsuperscript{3+}-CuNCs at 444 nm and 610 nm, as well as the ratio of adding Sodium Hexametaphosphate to not adding Sodium Hexametaphosphate at different composition ratios. (B) displays the photographs under 365 nm excitation and their corresponding color patches. The concentration of Sodium Hexametaphosphate is 4 μM.

3.3. Carbon point / Al\textsuperscript{3+}-CuNCs

To determine the sensitivity of the carbon point / Al\textsuperscript{3+}-Cu NCs nanoprobe to sodium hexametaphosphate, different concentrations of sodium hexametaphosphate samples (0-6 μM) were added to the carbon point / Al\textsuperscript{3+}-Cu NCs solution to obtain all fluorescence spectra. As shown in Figure 4A, the fluorescence at 610 nm in carbon point / Al\textsuperscript{3+}-Cu NCs decreased enhanced at 444 nm. The relative proportion between nanoprobe sensitivity and fluorescence intensity was investigated, and the relationship between fluorescence intensity and nanoprobe sensitivity was discussed. A strong linear correlation between the fluorescence intensity ratio (I\textsubscript{610}/I\textsubscript{444}) and the sodium hexametaphosphate concentration was observed between 0.1-6 μM (Figure 4B). The regression equation can be expressed as \( \frac{I_{610}}{I_{444}} = -0.1848x + 1.2586 \), where \( x \) represents the sodium hexametaphosphate concentration. The regression equation has a correlation coefficient of 0.997, indicating a strong linear relationship. The limit of detection (LOD) was estimated to be 0.023 μM. In addition, field fluorescence measurements were recorded for the smartphone, and RGB values were obtained. This method was used to obtain standard images of fluorescence at different concentrations of sodium THP (0-6 μM), including RGB values (Figure 4C). With increasing sodium hexametaphosphate, the fluorescence color of carbon dots / Al\textsuperscript{3+}-CuNCs changes regularly and could be distinguished by the naked eye. This method could be used for visual detection of NaHP based on fluorescence color changes. The ratio of RGB R and B values (which can show changes in fluorescence color) varies linearly in the range of 0.1-6 μM, with a LOD of 0.036 μM. The regression equation can be expressed as \( \frac{R}{B} = -0.1615x + 1.0536 \), where \( x \) represents the sodium hexametaphosphate concentration with a correlation coefficient of 0.994.

Figure 4. (A) Chart of fluorescence intensity ratio (I\textsubscript{610}/I\textsubscript{444}) of different concentrations of Sodium hexametaphosphate (0-6 μM). (B) The corresponding linear relationship. (C) Linearity between different concentrations of sodium hexametaphosphate and visualized G / B values. (D) Fluorescence images of systems containing different concentrations of sodium hexametaphosphate under UV light. The carbon-point / Al\textsuperscript{3+}-CuNCs ratio is 1:1.
3.4. Effects of coexisting matter

The complexity of real samples, not only in sensitivity but more fundamentally in specificity, poses a great challenge for its detection by carbon point / Al$^{3+}$-CuNCs. Therefore, this study discussed the effects of common metal ions, anions and small molecule interference in water on ratiometric nanoprobes.

As shown in Figure 5A, compared to metal ions such as K$^+$, Na$^+$, Ca$^{2+}$, Mg$^{2+}$, Mn$^{2+}$, Zn$^{2+}$, anions such as Cl$^-$, NO$_3^-$, HCO$_3^-$, SO$_4^{2-}$, Ac$^-$, ascorbate (AA), glucose (GLU), hydrogen peroxide (H$_2$O$_2$) did not interfere with the ratio of fluorescence intensity (I$_{610}$ / I$_{444}$), only sodium hexadetaphate achieved the double fluorescence response. Some species exhibited disturbances such as HPO$_4^{2-}$ and PO$_4^{3-}$, probably because of the complexation among these ions and Al ions. However, compared with sodium hexametaphosphate, the fluorescence changes caused by these interfering substances were very weak (Figure 5B). The prepared carbon point / Al$^{3+}$-CuNCs showed a high selective response to sodium hexametaphosphate as a ratiometric type nanoprobe. Under the UV lamp, the fluorescence of carbon point / Al$^{3+}$-CuNCs did not change after the addition of other disruptor, while sodium hexametaphosphate was significantly turned blue, demonstrating its high selectivity for visual detection of sodium hexametaphosphate (Figure 5C).

![Figure 5](image)

3.5. The content of sodium hexametaphosphate in green tea drinks was tested

To verify the feasibility of the carbon Point / Al$^{3+}$-CuNCs method, we determined sodium hexametaphosphate in green tea samples. Actual samples were diluted with deionized water. In a typical experiment, 200μL carbon point / Al$^{3+}$-CuNCs, 200μL of diluted actual samples were mixed with 600μL 2-morpholine ethylsulfonic acid buffer (pH=6) and mixed for 30s. The mixture was equilibrated under ambient conditions for several minutes and studied by the proportions of preparation. It was also determined by the standard addition method. Pretreated samples were mixed with different concentrations of sodium hexametaphosphate. After the samples reached equilibrium, the RGB values of the sample fluorescence images were recorded. The results in table 1 show that the recovery of these samples was between 97.5% and 102.5%, with a relative standard deviation within 3.1%. The test results of the real samples showed that the ratio carbon point / Al$^{3+}$-CuNCs have good utility for the monitoring of sodium hexametaphosphate in the real system. In contrast to previous literature reports listed in Table 2, we found that all of these chemical sensors were able to detect sodium hexametaphosphate, but they still had cumbersome synthesis processes. The carbon point / Al$^{3+}$-CuNCs presented had good performance in terms of detection limit and detection of concentration range without the use of cumbersome methods. In addition, this method presents more color changes under the ultraviolet light, so it is more convenient and sensitive in visualization, which can improve the efficiency for field analysis.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Added/(μmol/L)</th>
<th>Found/(μmol/L)</th>
<th>Recovery/%</th>
<th>RSD/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tea Drink I</td>
<td>0</td>
<td>2.45</td>
<td>-</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4.50</td>
<td>102.5</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1.72</td>
<td>-</td>
<td>2.2</td>
</tr>
<tr>
<td>Tea Drink II</td>
<td>2</td>
<td>3.67</td>
<td>97.5</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>3.19</td>
<td>-</td>
<td>2.9</td>
</tr>
<tr>
<td>Tea Drink III</td>
<td>0</td>
<td>5.31</td>
<td>101.0</td>
<td>2.4</td>
</tr>
</tbody>
</table>

Table 1. Content of sodium hexametaphosphate in real samples tested using the smartphone application
4. Conclusion

A direct, highly sensitive ratiometric fluorescence probe was prepared on the basis of carbon points and copper nanoclusters. This probe could detect trace amounts of sodium hexametaphosphate in tea beverage samples. Due to the AIE properties of CuNCs, carbon point and copper nanocluster mixture had double emission peaks at 444 nm and 610 nm, which showed a ratiometric fluorescence response in the presence of Al\(^{3+}\). Under optimal conditions, carbon point / Al\(^{3+}\)-Cu NCs showed good sensitivity to sodium hexametaphosphate in the range of 0.1–6μM with a limited limit of detection of 0.023 μM. Since the dual-emission fluorescent probes had a self-calibrated function, carbon point / Al\(^{3+}\)-Cu NCs were found to have higher sensitivity and accuracy than single-emitted CuNCs probes. Finally, the selectivity and sensitivity of sodium hexametaphosphate in tea beverage samples was achieved by using a fluorescence spectrometer and a smartphone.

References


Table 2. Comparison of the determination methods of sodium hexametaphosphate

<table>
<thead>
<tr>
<th>Sensing system</th>
<th>Linear range (μM)</th>
<th>LOD (μM)</th>
<th>Reference</th>
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<tbody>
<tr>
<td>FPEI/EY(^a)</td>
<td>0.1-4.2</td>
<td>0.038</td>
<td>[15]</td>
</tr>
<tr>
<td>Terbium (III) complex</td>
<td>0.5-100</td>
<td>0.38</td>
<td>[2]</td>
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<tr>
<td>Thioflavin T</td>
<td>0.5-6</td>
<td>0.116</td>
<td>[16]</td>
</tr>
<tr>
<td>Acridine orange</td>
<td>0.8-11</td>
<td>0.53</td>
<td>[17]</td>
</tr>
<tr>
<td>Azure B</td>
<td>0.6-10</td>
<td>0.56</td>
<td>[18]</td>
</tr>
<tr>
<td>CDs/Al(^{3+})-CuNCs</td>
<td>0.1-6</td>
<td>0.036</td>
<td>This Method</td>
</tr>
</tbody>
</table>

\(^a\): formaldehyde functionalized polyethyleneimine/eosin Y