Approaching Fluorescence Sensors in Chemistry Education: Detection of Iron(III)-Ions Applying Carbon Quantum Dots

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Abstract

The determination of heavy metal ions can be used in a variety of contexts: For example, a relationship has been identified between various disease patterns and an oversupply or undersupply of iron(III) ions, which makes it important to examine the iron(III) content of samples. [1] In this context, carbon quantum dots are focused by current research: Some carbon quantum dots show a characteristic fluorescence in aqueous solutions which is extinguished in the presence of iron(III) ions. By using fluorescence spectroscopy and measuring fluorescence intensities in presence and absence of iron(III) ions, a linearized graph can be created determining very low iron(III) ion concentrations in aqueous solutions.[1] In this paper, we present new hands-on approaches and models to teach fluorescence sensing with carbon quantum dots augmenting previous work in chemistry education. [2-3] Referring to the principles of Green Chemistry, we apply naturally available and non-toxic precursors suitable for school purposes. Furthermore, the experiments presented in this paper give an opportunity to bridge the gap between schools and universities: They implement measurement methods like fluorescence spectroscopy and topics of current research which can be connected to curricular relevant contents.

Keywords: *carbon quantum dots, sensing, heavy metal detection*

1. Introduction

As a new topic in nanochemistry, carbon quantum dots (CQD) have emerged in the last few years since the discovery of small carbon nanoparticles in soot residues. In research, they are said to have properties such as easy synthesis, low toxicity compared to heavy metal nanoparticles and good biocompatibility. [4] They are therefore used, for example, for labeling cells, where they show colored fluorescence under UV light and thus make labeled cells more visible. In presence of heavy metal ions like iron(III) [1], cobalt(II) [3] or mercury(II) [5], the fluorescence of CQDs is selectively quenched, which can be applied to detect these metal ions. In this paper, we present first approaches on iron(III) sensing applying CQDs from sucrose as a precursor. Different models are shown which are discussed by researchers to explain why fluorescence of CQDs is quenched in presence of certain metal ions. In a qualitative experimental approach, different metal ions are added to a diluted dispersion of CQDs in order to show that only iron(III) ions are able to quench their fluorescence effectively. In an additional quantitative approach, iron(III) ions can be detected quantitatively by drawing a linear regression in a certain concentration range. Finally, educational aspects and curricular connections are outlined for this topic.

2. Theoretical Background

2.1 Bottom-Up-Synthesis of Carbon Quantum Dots

During a bottom-up synthesis of CQD, small organic precursor molecules like sugars are carbonized to larger nanoparticular structures which are mostly in a size range of 1-10 nm. [4] In detail, dehydration and carbonization processes are taking place leading to the formation of a partially graphitic structure inside (figure 1). [5]

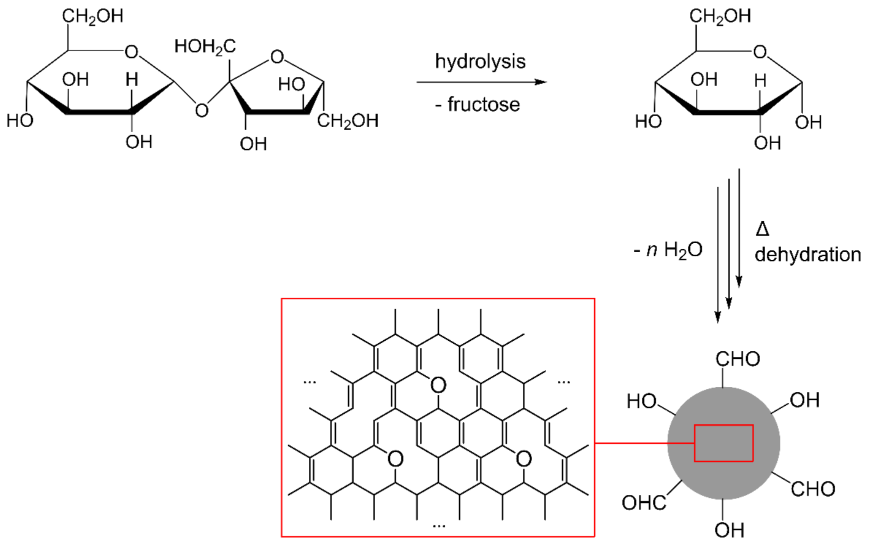

Fig. 1. Bottom-Up synthesis of CQDs made from sucrose as a precursor (adapted from [6]).

Due to oxidative processes, the particles have numerous polar functional groups on the outside, which make them easily dispersible in water. To induce particle formation in a bottom-up process, several methods can be applied like microwave irradiation or hydrothermal treatment with an autoclave.[4]

As Tang et al. describe in their paper [6], the simple application of heat can also be used to synthesize CQDs from different sugars. We use a slightly adapted experimental procedure (experiment 1) that ensures a fast and reliable bottom-up synthesis of CQD.

2.2 Iron(III)-Sensing with Carbon Quantum Dots: Relevance and Models of Fluorescence Quenching

For some types of CQDs synthesized by bottom-up approaches, it is reported that their fluorescence is quenched in presence of heavy metal ions. Especially for iron(III) ions, numerous references can be found in scientific literature. [1, 7-8] Their detection can be relevant in medicinal or environmental contexts. For example, an excess of iron(III) is associated with the increased incidence of diseases such as Alzheimer's [9] or Parkinson's [10], which makes it necessary to monitor iron(III) concentrations in water. Limit values that have been set for drinking water are 5.4 μmolL^{-1} (USA) [11] and 3.57 μmolL^{-1} (EU) [1], respectively.

To explain why the fluorescence of CQDs is quenched in presence of iron(III) ions, a simplified excitation-emission model can be applied: Electrons are excited from the highest occupied energetic state (HOE) to the lowest unoccupied energetic state (LUE) when the CQDs are illuminated with UV light. Due to non-radiative transitions, the emitted light has a longer wavelength, i. e. lower energy, than the light used for excitation. [12] In presence of iron(III) ions, it is assumed that a complex-like bonding between oxygen atoms at the hydroxy surface groups of CQDs and iron(III) ions having 3d orbitals is formed. [13] Figure 2 summarizes the quenching mechanism described above.

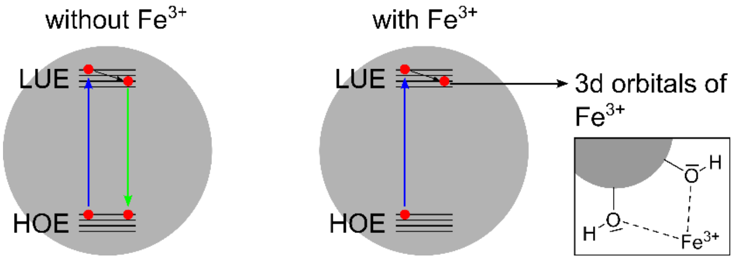

Fig. 2. Fluorescence mechanism of CQDs in absence and presence of iron(III) ions, format ion of a complex-like bonding [13] between hydroxy surface groups and iron(III) ions (black box).

2.3 Quantitative Approach to Calculate Quencher Concentrations by Linear Extrapolation

Generally, the quenching process described above consists of a fluorescent substance (CQDs) and a quencher (iron(III) ions) forming a complex which is non-fluorescent. This quenching mechanism is called **static quenching**. [14]

Under the assumption that only static quenching is relevant in presence of iron(III) ions, equation 1 displays the ideal linear relationship between the fluorescence intensity in absence related to the fluorescence intensity in presence of iron(III) ions ($I_{0,\text{fl.}}/I_{\text{fl.}}$) and the iron(III) concentration ($c_{\text{Fe}^{3+}}$). K represents the Stern-Volmer quenching constant (the slope) and b the intercept (**Stern-Volmer plot**). [14, p. 11]

$$\frac{I_{0,\text{fl.}}}{I_{\text{fl.}}} = K \cdot \frac{c_{\text{Fe}^{3+},\text{calc.}}}{c^0} + \text{b} \tag{1}$$

Due to the fact that $I_{0,\text{fl.}}/I_{\text{fl.}}$ is 0 if there are no iron(III) ions in the solution, the intercept b always takes the value 1.

Calculating $I_{0,\text{fl.}}/I_{\text{fl.}}$ for different values of $c_{\text{Fe}^{3+}}$, a linear regression can be used to determine unknown iron(III) concentrations in solutions. Figure 3 displays the linear relationship in equation 1.

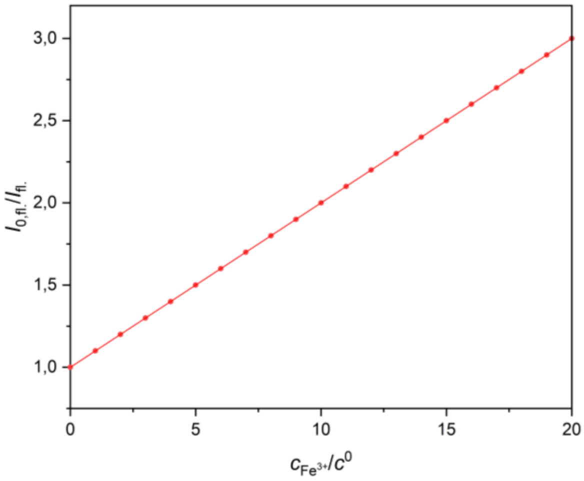

Fig. 3. Ideal Stern-Volmer plot for static quenching.

The literature primarily finds a linear relationship for very low iron(III) concentrations (0-100 µmolL^{-1}) [15], which is advantageous in the context of the limit values of drinking water described above.

3. Experimental Section

3.1 Experiment 1: Synthesis and Detection of Carbon Quantum Dots with Sucrose

CQDs are synthesized by an adapted bottom-up approach which can be found in [9].

Equipment: beaker (50 mL, 2x), pipette, spatula, tripod with clamp, heating plate (with contact thermometer), silicon oil bath, UV lamp ($\lambda \approx 400$ nm, e.g., Lunartec$^{\text{TM}}$ NC-5997-675)

Chemicals: water (demineralized), sucrose

Procedure: The silicon oil bath is pre-heated to a temperature of approx. 250 °C. Subsequently, sucrose (4 g) is added to the beaker and heated in the oil bath for 6 minutes. The residue is dissolved in demineralized water (50 mL). After diluting 1 mL of the resulting solution to 10 mL with demineralized water, it is observed in a dark room under the UV lamp.

Results: During the heating process, a dark-brown residue is formed, which can be completely dissolved in water. The solution likewise appears dark-brown and yellow after dilution. Under illumination with the UV lamp, a green fluorescence can be observed (figure 4).

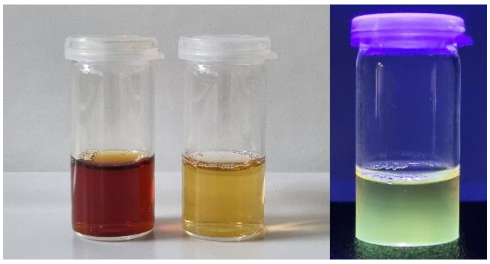

Fig. 4. CQD solution and diluted CQD solution under daylight (left), fluorescence of diluted CQD solution under UV light ($\lambda \approx 400$ nm, right).

Interpretation: Due to condensation and dehydration processes, CQDs are formed. Under UV light, electrons are excited from lower to higher energetic states, and after non-radiative ablation, lower-energetic light is emitted (see figure 2 left side).

3.2 Experiment 2: Qualitative Detection of Iron(III) Ions with Carbon Quantum Dots

To assess whether CQDs can qualitatively detect iron(III) ions, the influence of different metal ions on fluorescence was assessed.

Equipment: beaker (50 mL), snap-cap vial (6x), Eppendorf pipette (with tips), fluorescence spectrometer (Jasco Spectrophotometer), quartz cuvette, UV lamp ($\lambda \approx 400$ nm, e.g., Lunartec^{TM} NC-5997-675)

Chemicals: reaction product (undiluted) of experiment 1, iron(III)-nitrate-nonahydrate (GHS03, 07), copper(II)-sulfate-hydrate (GHS 05, 07, 09), magnesium(II)-sulfate, zinc(II)-sulfate-heptahydrate (GHS 05, 07, 09), aluminium(III)-sulfate-octadecahydrate (GHS 05)

Procedure: The reaction product of experiment 1 (3 mL) is diluted to a total volume of 30 mL using demineralized water. Then, metal salt solutions with a molar concentration *c* of 0.1 molL^{-1} are prepared. Afterwards, the metal salt solutions (0.9 mL) are added to each 5 mL of the diluted CQD solution. One sample is diluted with demineralized water (0.9 mL). The resulting solutions are observed under the UV lamp in a dark room. It is recommended that the liquid level in the snap cap jar is relatively low.

Results: Under UV light, it can be seen that only CQD solution with added iron(III) ions appears to fluoresce significantly less (figure 5).

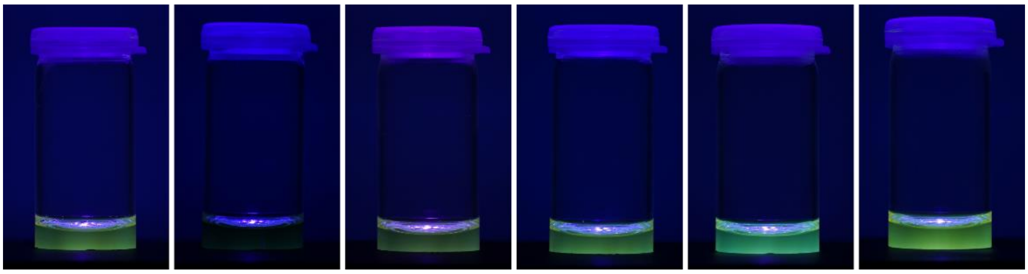

Fig. 5. Fluorescence of CQD solution under UV light ($\lambda \approx 400$ nm). From left to right: without metal ions, Fe^{3+}, Cu^{2+}, Mg^{2+}, Al^{3+}, Zn^{2+}.

To quantify this phenomenon, fluorescence spectra with an excitation wavelength $\lambda_{exc.}$ of 400 nm (this corresponds to the maximum emission of the UV lamp, approximately) were recorded in order to measure the fluorescence intensity $I_{fl.}$ (figure 6). Additionally, the relative fluorescence decrease $I_{fl.}/I_{0,fl.}$ was calculated for each sample (table 1).

Table 1. Measured Values for Experiment 2.

Sample No.	Metal Salt	$I_{fl}/\mathrm{a.u.}$	$\frac{I_{fl.}}{I_{0,fl.}}$
Ref	-	1600	1
1	Fe^{3+}	450	0.28
2	Mg^{2+}	1560	0.98
3	Zn^{2+}	2130	1.33
4	Al^{3+}	2530	1.58
5	Cu^{2+}	1170	0.73

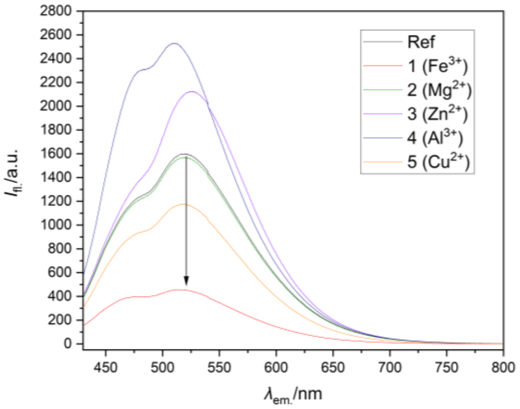

Fig. 6. Plot of fluorescence intensities $I_{fl.}$ against the emission wavelength $\lambda_{em.}$ ($\lambda_{exc.}$ = 400 nm).

Interpretation: According to the literature [8], it can be assumed that excited electrons from the CQDs are transferred to empty 3d orbitals of iron(III)-aqua complexes leading to a quenched fluorescence. The value of $I_{fl.}/I_{0,fl.}$ for sample 1 supports the observation that the fluorescence is quenched in presence of iron(III) ions. Surprisingly, there was an increase in the measured fluorescence intensity $I_{fl.}$ for samples 3 and 4, which has also qualitatively been noted in the literature. [7, 15]

To assess whether the absorption of iron(III)-aqua complexes can play a role in the quenching process, an absorption spectrum of the iron(III)-nitrate stock solution (c = 0.1 $\mathrm{molL^{-1}}$, 0.9 mL) diluted with demineralized water to a total volume of 5.9 mL ($c \approx 0.015$ $\mathrm{molL^{-1}}$) was recorded in order to have comparable concentrations (Jasco V-750 Spectrophotometer). The spectrum reveals that the absorption of the diluted iron(III)-nitrate solution at λ = 400 nm) is relatively low, which makes it reasonable to assume that the absorption of iron(III) ions does not play a significant role (figure 7).

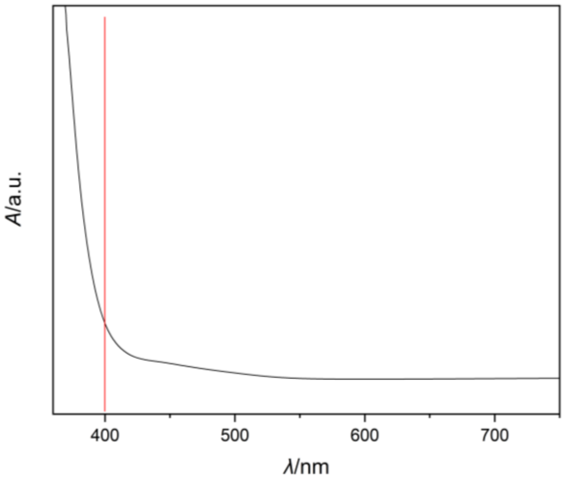

Fig. 7. Absorbance A of a diluted iron(III)-nitrate solution ($c \approx 0.015$ $\mathrm{molL^{-1}}$). The red line marks the excitation wavelength λ applied in experiment 2 and 3.

3.3 Experiment 3: Quantitative Detection of Iron(III) Ions with Carbon Quantum Dots

To assess whether CQDs can quantitatively detect iron(III) ions in very low concentrations, a linearized Stern-Volmer plot is created to determine an unknown iron(III) concentration.

Equipment: beaker (50 mL), Eppendorf pipette (with tips)

Chemicals: CQD solution (see experiment 1), iron(III)-nitrate solution (c = 1 mmolL^{-1}), demineralized water

Procedure: The reaction product of experiment 1 (0.5 mL) is diluted to a total volume of 5 mL using demineralized water. Then, analyte solutions are prepared according to table 1. Afterwards, emission spectra are recorded using the fluorometer ($\lambda_{ex.}$ = 400 nm).

Table 1. Analyte Solutions for Experiment 3.

Sample No.	$V_{\mathrm{CQD}}\,/\mathrm{\mu L}$	$V_{\mathrm{H_2O}}/\mathrm{\mu L}$	$V_{\mathrm{Fe^{3+}}}/\mathrm{\mu L}$	$V_{\mathrm{ges}}/\mathrm{\mu L}$	$c_{\mathrm{Fe^{3+}}}/\mathrm{\mu molL^{-1}}$	$I_{\mathrm{fl}}/\mathrm{a.u.}$	$\frac{I_{0,\mathrm{fl}}}{I_{\mathrm{fl}}}$
Ref	500	500	0	1000	0	1513	1.000
1	500	495	5	1000	5	1446	1.046
2	500	490	10	1000	10	1357	1.115
3	500	485	15	1000	15	1282	1.181
4	500	480	20	1000	20	1235	1.226

Results: The intensity of the fluorescence decreases as the concentration of iron(III) ions increases (table 2, figure 3).

Table 2. Measured Values for Experiment 3.

Sample No.	$c_{\mathrm{Fe^{3+}}}/\mathrm{\mu molL^{-1}}$	$I_{\mathrm{fl}}/\mathrm{a.u.}$	$\frac{I_{0,\mathrm{fl.}}}{I_{\mathrm{fl.}}}$
Ref	0	1513	1.000
1	5	1446	1.046
2	10	1357	1.115
3	15	1282	1.181
4	20	1235	1.226

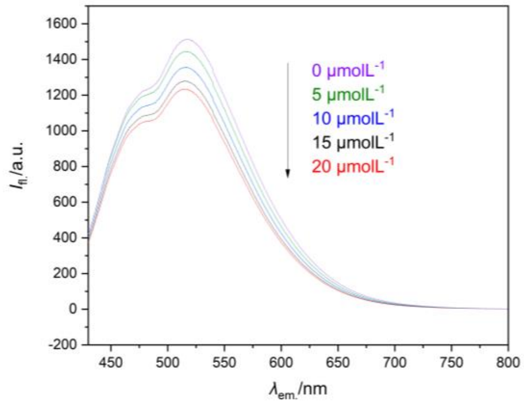

Fig. 8. Plot of fluorescence intensities $I_{fl.}$ against the emission wavelength $\lambda_{em.}$ ($\lambda_{exc.}$ = 400 nm) for different concentrations of iron(III) ions.

Plotting the relative fluorescence intensity $I_{0,fl.}/I_{fl.}$ /(table 2) against the concentration of iron(III) ions $c_{\mathrm{Fe^{3+}}}$ according to equation 1, a linear correlation can be found with a correlation coefficient R^2 of approx. 0.994 (figure 9).

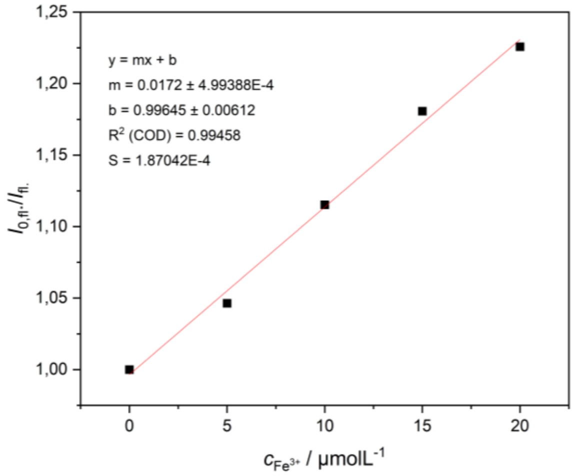

Fig. 9. Linear Regression of the relative fluorescence intensity $I_{0,\mathrm{fl.}}/I_{\mathrm{fl.}}$ against the concentration of iron(III) ions $c_{\mathrm{Fe}^{3+}}$.

Interpretation: Due to the formation of a complex-like bonding between the CQD as a fluorophore and iron(III) ions as a quencher which is described in the literature, It can be assumed that static quenching is involved in this process. Zhang et al. report static quenching for the detection of iron(III) ions with CQDs synthesized from mustard seeds in a bottom-up approach. [16]

In order to use the plot in Figure 9 as a model for the determination of an unknown iron(III) concentration, a sample with an iron(III) concentration of 7 $\mu\mathrm{molL}^{-1}$ (sample 5Test) was prepared and the fluorescence intensity $I_{\mathrm{fl.}}$ was measured according to samples 1-4. In relation to $I_{0,\mathrm{fl.}}$, the concentration of iron(III) ions can be calculated using the linear regression (equation 2).

$$\frac{I_{0,\mathrm{fl.}}}{I_{\mathrm{fl.}}} = 0.0172 \cdot \frac{c_{\mathrm{Fe}^{3+},\mathrm{calc.}}}{c^0} + 0.99645 \qquad (2)$$

$$c_{\mathrm{Fe}^{3+},\mathrm{calc.}} = \frac{\frac{I_{0,\mathrm{fl.}}}{I_{\mathrm{fl.}}} - 0.99645 \cdot c^0}{0.0172} \approx 5.7\ \mu\mathrm{molL}^{-1}$$

Table 3. Calculation of an iron(III) concentration $c_{\mathrm{Fe}^{3+},\mathrm{calc.}}$ in sample 5Test.

Sample No.	$c_{\mathrm{Fe}^{3+}}/\ \mu\mathrm{molL}^{-1}$	$I_{\mathrm{fl}}/\mathrm{a.u.}$	$\frac{I_{0,\mathrm{fl.}}}{I_{\mathrm{fl.}}}$	$c_{\mathrm{Fe}^{3+},\mathrm{calc.}}/\mu\mathrm{molL}^{-1}$	Δabs./µmolL^{-1}	Δrel.
5Test	7	1383	1.094	5.7	1.3	0.186

Hence, the concentration in sample 5Test can be calculated with a relative error Δrel. of approx. 18.6%, what can be considered sufficient in the school context. Possible errors can result from the preparation of solutions and the deviation of the linear regression itself.

4. Educational Aspects

The experiments presented can be used to create numerous links to sustainability-related topics in chemistry class. First and foremost, the bottom-up-synthesis of CQDs applies natural resources like sugars and can therefore be considered a sustainable process. As a possible discussion in class, students can assess under which conditions the synthesis is sustainable or not (e. g., application of renewable energies). The subsequent detection of iron(III) ions might be considered in environmental contexts (iron(III) concentration in drinking water) or medical contexts (relevance of an excess or deficiency of iron(III) in the body) serving as a motivation for students. By preparing different reference solutions according to experiment 3, a quantitative determination of a solution with an unknown iron(III) concentration can be conducted. In the context of Sustainable Development Goals (SDGs), it is possible to identify relationships to SDG 3 (Good Health and Well-Being) and SDG 6 (Clean Water and Sanitation). [17]

Due to the fact that a spectrofluorometer is required for experiment 3, it is primarily located in a university setting or can be carried out in cooperation with a university. This experiment can also be used to practise and consolidate common methods used in scientific laboratories, such as the application of Eppendorf pipettes. In schools, a qualitative approach according to experiment 2 can be used instead.

Concerning the mechanism behind the iron(III) detection by fluorescence sensing, a link can be created to the orbital model (3d orbitals of iron(III) ions) and to the subject area of light and color (HOE/NUE, excitation/emission-model, fluorescence). As a didactical reduction, further possible mechanisms discussed in the literature are neglected.

5. Conclusion

In this paper, we presented experimental approaches for the detection of iron(III) ions in order to investigate a possible application of CQDs for chemistry lessons. With sucrose, a non-toxic, renewable and well-known precursor is used to produce CQDs with the bottom-up method. Possible contexts in chemistry lessons are the medical relevance of iron(III) ions or the determination of concentrations in water samples. The explication of the quenching phenomenon can be linked to the topics light/color, and the orbital model. Especially the quantitative approach presented in experiment 3 is suitable for university settings creating a link between school and research.

REFERENCES

[1] Xiang, Z., Jiang, Y., Cui, C., Luo, Y. & Peng, Z. (2022). *Molecules, 27*(19). https://doi.org/10.3390/molecules27196749.
[2] Schneider, E. M., Bärtsch, A., Stark, W. J. & Grass, R. N. (2019). *J. Chem. Educ., 96*(3), 540–545. https://doi.org/10.1021/acs.jchemed.8b00114.
[3] Wu, F., Zhang, R. & Zhou, J. (2024). *J. Chem. Educ., 101*(7), 2784–2789. https://doi.org/10.1021/acs.jchemed.3c00329.
[4] Haufe, H. & Böttcher, H. (2018). *Chem. Unserer Zeit, 52*(6), 398–409. https://doi.org/10.1002/ciuz.201800772.
[5] Nair, V. R., Shanthil, M., Sandeep, K., Savitha, K. U., Archana, A., Deepamol, V., Swetha, C. & Vaishag, P. V. (2023). *ACS Omega, 8*(32), 29468–29474. https://doi.org/10.1021/acsomega.3c03125.
[6] Tang, Q., Zhu, W., He, B. & Yang, P. (2017). *ACS Nano, 11*(2), 1540–1547. https://doi.org/10.1021/acsnano.6b06867.
[7] Chen, Y., Sun, X., Pan, W., Yu, G. & Wang, J. (2019). *Front. Chem., 7*, 911. https://doi.org/10.3389/fchem.2019.00911.
[8] Zhang, Y.-L., Wang, L., Zhang, H.-C., Liu, Y., Wang, H.-Y., Kang, Z.-H. & Lee, S.-T. (2013). *RSC Adv., 3*(11), 3733. https://doi.org/10.1039/C3RA23410J.
[9] Connor, J. R., Menzies, S. L., St Martin, S. M. & Mufson, E. J. (1992). *J. Neurosci. Res., 31*(1), 75–83. https://doi.org/10.1002/jnr.490310111.
[10] Berg, D., Gerlach, M., Youdim, M. B., Double, K. L., Zecca, L., Riederer, P. & Becker, G. (2001). *J. Neurochem., 79*(2), 225–236. https://doi.org/10.1046/j.1471-4159.2001.00608.x.
[11] Ji, W., Zhu, Z., Dong, S., Nie, J. & Du, B. (2019). *Sensors, 19*(19). https://doi.org/10.3390/s19194223.
[12] Tausch, M. (2019). *Chemie mit Licht [Chemistry with Light].* Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-662-60376-5.
[13] Zu, F., Yan, F., Bai, Z., Xu, J., Wang, Y., Huang, Y. & Zhou, X. (2017). *Microchim. Acta, 184*(7), 1899–1914. https://doi.org/10.1007/s00604-017-2318-9.
[14] Lakowicz, J. R. (2006). *Principles of fluorescence spectroscopy* (3rd Edition). Springer.
[15] Eskalen, H., Uruş, S., Kavgacı, M., Kalmış, H. V. & Tahta, B. (2024). *Biomass Convers. Biorefin., 14*(1), 1201–1214. https://doi.org/10.1007/s13399-023-04048-5.
[16] Zhang, Q., He, S., Zheng, K., Zhang, L., Lin, L., Chen, F., Du, X. & Li, B. (2022). *Inorg. Chem. Commun., 146*, 110034. https://doi.org/10.1016/j.inoche.2022.110034.
[17] United Nations (Ed.). *The 17 Goals*. https://sdgs.un.org/goals.