Polyacrylic Acid-Ca(Eu) Nanoclusters as a Luminescence Sensor of Phosphate Ion

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Abstract: In this study, we synthesized polyacrylic acid (PAA)-Ca(Eu) nanoclusters as a luminescence sensor of phosphate ion by a complex method, and we aimed to achieve the quantitative detection of PO$_4^{3-}$ based on the sensitivity of the charge transfer band of Eu$^{3+}$ to anionic ligand. The resulting PAA-Ca(Eu) nanoclusters showed a well-dispersed and a dot-like morphology, with an ultra-small diameter (the average size of 2.17 nm) under high resolution transmission electron microscopy (HRTEM) observation. A dynamic light scattering particle size analyzer (DLS) showed a hydrodynamic size of 2.39 nm. The (PAA)-Ca(Eu) nanoclusters as a luminescence sensor showed a significantly higher sensitivity for PO$_4^{3-}$ than other anions (CO$_3^{2-}$, SiO$_3^{2-}$, SO$_4^{2-}$, SO$_3^{2-}$, Br$^-$, Cl$^-$, F$^-$). The luminescence intensity displayed a linear increase (y = 19.32x + 74.75, R$^2$ > 0.999) in a PO$_4^{3-}$ concentration range (0–10 mM) with the concentration of PO$_4^{3-}$ increase, and the limit of detection was 0.023 mM. The results showed good recovery rates and low relative standard deviations. These (PAA)-Ca(Eu) nanoclusters are hopeful to become a luminescence sensor for quantitatively detecting PO$_4^{3-}$.

Keywords: nanoclusters; Eu$^{3+}$ luminescence sensor; PO$_4^{3-}$ detection; charge transfer band

1. Introduction

Europium element with a unique 4f electron layer structure is a commonly used luminescent probe [1–3] due to its good optical stability, high thermal and chemical stability, narrow emission band, high resistance to photobleaching, and light quenching [4–6]. The excitation wavelength of Europium mainly includes the 350–475 nm band of energy levels transition and the charge transfer band (CTB) in the ultraviolet region [5,7]. The energy level transition excitation can obtain better near-infrared emission luminescence, which is mainly used in the biomedical field [6,8–12]. The CTB has unique properties, Eu$^{3+}$ binds to the anionic ligand to form a CTB. The position of the charge transfer transition band depends on the ligand [13–18]. Therefore, the CTB of Eu$^{3+}$ can be used for qualitative and quantitative analysis of the types and contents of anionic ligands. For example, CTB formed with phosphate in hydroxyapatite is at 254 nm, while CTB formed with anionic ligand in LaOF is at 285 nm [7,19].

Phosphorus plays an important role in organisms and the environment [20,21]. Excessive phosphate content in water can cause water pollution [22,23]. Phosphate in organisms participates in a variety of metabolism processes. Phosphate content is one of the important indicators of human health, and its quantitative detection is of great significance [24,25]. In this study, inspired by the biomineralization process of calcium phosphate, we used polyacrylic acid (PAA) to complex Ca$^{2+}$ and Eu$^{3+}$ ions to obtain PAA-Ca(Eu) nanoclusters as a sensor for the quantitative detection of PO$_4^{3-}$ based on the sensitivity of charge transfer band of Eu$^{3+}$ to anionic ligand. The morphology, size, ion selectivity and luminescence of...
PAA-Ca (Eu) nanoclusters were characterized, and the mechanism of quantitative phosphate radical detection was analyzed and explained by luminescence spectra and molecular dynamics simulation (MDS).

2. Materials and Methods
2.1. Synthesis of PAA-Ca (Eu) Nanoclusters

The PAA-Ca(Eu) nanoclusters were prepared by a complex method. An aqueous Ca(Eu) solution (20 mL) was prepared using CaCl$_2$·2H$_2$O (99.42 mg, Sinopharm, Beijing, China) and Eu(NO$_3$)$_3$·6H$_2$O (33.52 mg, Aladdin, Shanghai, China) with a concentration of 37.575 mM in which the Eu$^{3+}$/(Ca$^{2+}$ + Eu$^{3+}$) molar ratio was 10%. The solution was stirred vigorously to make it fully dissolved. An aqueous solution of PAA (average molecular weight of ~1800 g/mol, 216.43 mg, 20 mL, Sigma, St. Louis, USA) was quickly added to the aqueous Ca(Eu) solution, and the pH was adjusted to 7.5–8.0 using NH$_3$·H$_2$O (Sinopharm, Beijing, China) to yield the PAA-Ca(Eu) nanoclusters. The temperature of all the above solutions was room temperature (25 $^\circ$C).

2.2. Characterization

High resolution transmission electron microscopy (HRTEM, Talos F200S, Waltham, MA, USA) was used to observe and to analyze the microstructure of the materials. Fourier transform infrared spectroscopy (FT-IR, Nicolet6700, Waltham, MA, USA) was used to record the spectra of the near infrared region (4000~400 cm$^{-1}$), analyze and study the vibration mode of the characteristic peak of the material, identify the substance, and determine the chemical composition or relative content of the substance. A dynamic light scattering particle size analyzer (DLS, Malvern, UK) was used to measure the particle size distribution and the dispersion coefficient of solution. Luminescence excitation and emission spectra of samples were measured by luminescence spectrophotometer (970CRT, Shanghai Sanco, Shanghai, China).

2.3. Detection of PO$_4^{3-}$

An aqueous solution of phosphate ion was prepared by Na$_2$HPO$_4$·12H$_2$O and added to the PAA-Ca(Eu) nanoclusters solution. Finally, NH$_3$·H$_2$O was used to adjust the pH to 9.0–9.5 for luminescence detection.

2.4. Preparation of Buffer Solution

A total of 1.07 g of NH$_4$Cl (Sinopharm, Beijing, China) was added to 100 mL of deionized water. After it was fully dissolved, ammonia was added to adjust the pH of the aqueous solution to 8.0 to obtain the buffer solution.

2.5. Molecular Dynamics Simulation

All MDS employed the AMBER/general AMBER force field. In the cubic simulation unit with an initial size of 10 nm, the step change was set to 1 fs, and all simulations were run for 50 ns in real time using Gromacs 2018 software package [26,27].

3. Results and Discussion
3.1. Structure Characterization

First, the microstructure and the particle size of PAA-Ca (Eu) nanoclusters were characterized (Figure 1). Through HRTEM, it can be seen that the nanoclusters present dot-like particles, and the nanoclusters do not gather directly. The particle size also presents a relatively uniform distribution. Through the statistics of the nanoclusters in the HRTEM image, their particle size is concentrated in the range of 1.8–2.4 nm (this particle size range accounts for 88% of the total particle size), with an average particle size of 2.17 nm. DLS test results also showed a similar hydrodynamic size (2.39 nm).
Figure 1. (a) High-resolution transmission electron microscopy image of PAA-Ca (Eu) nanoclusters; (b) Particle size statistics of (a); (c) Hydrodynamic size of PAA-Ca(Eu) nanoclusters.

In addition, FT-IR spectra of PAA-Ca (Eu) nanoclusters and samples with different PO$_4^{3−}$ additions are shown in Figure 2. The absorption peak at 3478 cm$^{-1}$ is the O-H stretching vibration peak in PAA molecule [28]. The absorption peaks at 1556 cm$^{-1}$ and 1401 cm$^{-1}$ are the asymmetric stretching vibration peak ($\nu_{as}$) and the symmetric stretching vibration peak ($\nu_{s}$) of COO$^-$ in the PAA molecule, respectively. Compared with pure PAA, the C=O absorption peak shifts to a low frequency and the C-O absorption peak shifts to a high frequency, which indicates that the coordination between carboxylic acid and the metal ions in PAA is a bridge coordination compound [29,30]. After adding PO$_4^{3−}$, the absorption peak of the phosphate ion appeared obviously in the infrared spectrum, which was located at 1104 cm$^{-1}$, 1072 cm$^{-1}$ and 536 cm$^{-1}$, belonging to the asymmetric stretching ($\nu_{as}$) and the asymmetric angle change ($\nu_{4}$) of PO$_4^{3−}$ [31,32].

Figure 2. Fourier transform infrared spectroscopy spectra of PAA-Ca (Eu) nanoclusters with different PO$_4^{3−}$ concentration. I–III are 0 mM, 2 mM, and 7.5 mM.

3.2. Luminescent Characterization

3.2.1. Ion Selectivity

PAA-Ca(Eu) nanoclusters were used as sensors to detect common anions (the anion concentration was 10 mM). As shown in Figure 3a, PO$_4^{3−}$ is the most sensitive to the sensor, and it has the highest luminescence intensity. The luminescence emission peak with the maximum luminescence intensity (617 nm) was selected for comparison, as shown in Figure 3b. It can be more intuitively observed that the sensor is sensitive to PO$_4^{3−}$. Figure 3c shows that CTB positions and intensities are different for different anionic ligands.
The CTB of PO$_4^{3-}$ position is unique, and it is the strongest. All of the above indicated that PAA-Ca (Eu) nanoclusters could be used for the detection of PO$_4^{3-}$ concentration.

\[ \text{LOD} = 3\sigma/K \]  

(1)

Figure 3. (a) Emission spectra ($\lambda_{\text{ex}} = 254$ nm) of different anions at the excitation wavelength of 254 nm; (b) Luminescence intensity of the characteristic emission peak at 617 nm was selected for comparison; (c) Excitation spectra ($\lambda_{\text{em}} = 617$ nm) of different anions at emission wavelengths of 617 nm.

3.2.2. Detection of PO$_4^{3-}$ Concentration

In the emission spectrum excited at 254 nm, Eu$^{3+}$ showed characteristic emission at 594 ($5\text{D}_0 \rightarrow 7\text{F}_4$), 617 ($5\text{D}_0 \rightarrow 7\text{F}_3$), 654 ($5\text{D}_0 \rightarrow 7\text{F}_3$), and 699 nm ($5\text{D}_0 \rightarrow 7\text{F}_3$) (Figure 4a). Figure 4b shows that with the increase of PO$_4^{3-}$ concentration, the increase of luminescence first increased and then remained basically unchanged. The linear fitting of PO$_4^{3-}$ concentration in the range of 0–10 mM showed that the linear equation was $y = 19.32x + 74.75$, and its $R^2$ was 0.999, indicating that PAA-Ca(Eu) nanoclusters can quantitatively detect PO$_4^{3-}$ in this concentration range. In the excitation spectrum, Eu-O CTB gradually moved to the left from 273.7 nm to 258.6 nm with the increase of PO$_4^{3-}$ concentration, indicating that the anion ligand connected to Eu$^{3+}$ changed during this process.

The detection limit of the fluorescent sensor is calculated using Formula (1), where LOD is limit of detection, $\sigma$ is the standard deviation of the blank, and $K$ is the slope of the linear relationship. We tested six groups of blank samples, obtained their standard deviation, and calculated that the detection limit of the luminescence sensor for PO$_4^{3-}$ was 0.023 mM. It shows that the sensor can be used to detect PO$_4^{3-}$ in serum and other
samples [33]. We added a known concentration of PO$_4^{3-}$ to the sample, which reacted with PAA-Ca(Eu) nanoclusters, and then tested its luminescence at 254 nm excitation wavelength. According to the emission peak intensity at 617 nm and the linear equation in Figure 4b, the spiked recovery rate of PO$_4^{3-}$ in the sample was calculated. The results are shown in Table 1. Overall, all samples showed good recovery rates and low relative standard deviations (RSD) within the linear range, making PAA-Ca(Eu) nanoclusters a sensor for PO$_4^{3-}$ quantitative detection.

Table 1. Results and recovery of samples ($n = 3$).

| PO$_4^{3-}$ Spiked (mM) | PO$_4^{3-}$ Found (mM) | Recovery (%) | RSD (%) |
|-------------------------|------------------------|--------------|---------|
| 1                       | 1.060                  | 106.0        |         |
| 4                       | 4.200                  | 105.0        |         |
| 5                       | 4.793                  | 95.9         | 4.2     |
| 8                       | 7.951                  | 99.4         |         |
| 10                      | 9.914                  | 99.1         |         |

3.2.3. Buffer Solution

It can be seen from Figure 5 that in an aqueous solution and a buffer solution, the luminescence intensity of the PAA-Ca(Eu) nanoclusters is basically the same after reacting with PO$_4^{3-}$ of the same concentration. It proved that the luminescence sensor also has a good sensing function in the buffer solution.

![Figure 5](image.png)

**Figure 5.** (a) The emission spectrum of PAA-Ca(Eu) nanoclusters in aqueous solution and buffer solution after reacting with different concentrations of PO$_4^{3-}$; (b) luminescence intensity at 617 nm.

3.3. Mechanism of PO$_4^{3-}$ Concentration Detection

After adding PO$_4^{3-}$ to PAA-Ca(Eu) nanoclusters, the vibrational peak of PO$_4^{3-}$ appeared in FT-IR, and the peak position and intensity of CTB changed in the excitation spectra ($\lambda_{em} = 617$ nm), indicating that the anions bonded with Eu changed in this process. In addition, MDS showed that Eu$^{3+}$ combines with the oxygen anion of the PAA carboxyl group in PAA-Ca(Eu) nanoclusters, showing Eu–O$_1$ CTB (Figure 6a). When PO$_4^{3-}$ was added to the PAA-Ca(Eu) nanoclusters, the COO$^-$ bonded Eu$^{3+}$ was bound by the oxygen anion of PO$_4^{3-}$, displaying a new Eu–O$_2$ CTB (Figure 6b). This change in the bonding state of Eu$^{3+}$ caused an increased energy state, corresponding to the shift to a low wavelength and an increased luminescence intensity. Based on this mechanism, the quantitative detection of PO$_4^{3-}$ can be realized.
4. Conclusions

In conclusion, we synthesized ultra-small PAA-Ca(Eu) nanoclusters with an average particle size of 2.17 nm under HRTEM observation. The nanoclusters are sensitive to PO$_4^{3-}$, and they can be used for quantitative detection of PO$_4^{3-}$ in a certain concentration range (0–10 mM), with good linear correlation. The LOD is 0.023 mM. Based on the sensitivity of CTB of Eu$^{3+}$ to anionic ligand, the quantitative detection of PO$_4^{3-}$ can be carried out. In addition, the detected concentration range by the PAA-Ca(Eu) nanoclusters sensor covers the content of PO$_4^{3-}$ in serum, urine, and sewage. So, it is hoped that it can detect PO$_4^{3-}$ in physiological conditions and a natural environment.

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**References**

1. Chen, L.; Cheng, P.; Zhang, Z.; He, L.; Oeckler, O. Reduced Local Symmetry in Lithium Compound Li$_2$SrSiO$_4$ Distinguished by an Eu$^{3+}$ Spectroscopy Probe. *Adv. Sci.* 2019, 6, 1802126. [CrossRef] [PubMed]
2. Ma, H.; Song, B.; Wang, Y.X.; Cong, D.Y.; Jiang, Y.F.; Yuan, J.L. Dual-emissive nanoarchitecture of lanthanide-complex-modified silica particles for in vivo ratiometric time-gated luminescence imaging of hypochlorous acid. *Chem. Sci.* 2017, 8, 150–159. [CrossRef] [PubMed]
3. Pan, H.; Xu, S.; Ni, Y.H. Rare-earth post-modified Zn-based coordination polymer microspheres: Simple room-temperature preparation, fluorescent performances and application for detection of tryphosphate. *Sens. Actuators B-Chem.* 2019, 283, 731–739. [CrossRef]
4. Gupta, S.K.; Kadam, R.M.; Pujari, P.K. Lanthanide spectroscopy in probing structure-property correlation in multi-site photoluminescent phosphors. *Coord. Chem. Rev.* 2020, 420, 213–405. [CrossRef]
5. Binnemans, K. Interpretation of europium(III) spectra. *Coord. Chem. Rev.* 2015, 295, 1–45. [CrossRef]
6. Syamchand, S.S.; Sony, G. Europium enabled luminescent nanoparticles for biomedical applications. *J. Lumin.* 2015, 165, 190–215. [CrossRef]
7. Xing, Q.; Zhang, X.; Wu, D.; Han, Y.; Nirmali Wickramaratne, M.; Dai, H.; Wang, X. Ultrasound-Assisted Synthesis and Characterization of Heparin-Coated Eu$^{3+}$ Doped Hydroxyapatite Luminescent Nanoparticles. *Colloid Interface Sci. Commun.* 2019, 29, 17–25. [CrossRef]
8. Ma, B.J.; Zhang, S.; Qiu, J.C.; Li, J.H.; Sang, Y.H.; Xia, H.B.; Jiang, H.D.; Claverie, J.; Liu, H. Eu/Tb codoped spindle-shaped fluorinated hydroxyapatite nanofibers for dual-color cell imaging. *Nanoscale* 2016, 8, 11580–11587. [CrossRef]

9. Zhang, T.T.; Wang, Z.J.; Xiang, H.J.; Xu, X.; Zou, J.; Lu, C.C. Biocompatible Superparamagnetic Europium-Doped Iron Oxide Nanoparticle Clusters as Multifunctional Nanoprobes for Multimodal In Vivo Imaging. *ACS Appl. Mater. Interfaces* 2021, 13, 33880–33886. [CrossRef]

10. Liu, Y.T.; Zhou, S.X.; Fan, L.Z.; Fan, H. Synthesis of red fluorescent graphene quantum dot-europium complex composites as a viable imaging platform. *Microchim. Acta* 2016, 183, 2605–2613. [CrossRef]

11. Podyachev, S.N.; Zairov, R.R.; Mustafina, A.R. 1,3-Diketone Calix 4 arene Derivatives-A New Type of Versatile Ligands for Metal Complexes and Nanoparticles. *Molecules* 2021, 26, 1214. [CrossRef] [PubMed]

12. Zairov, R.R.; Dovzhenko, A.P.; Sapunova, A.S.; Voloshina, A.D.; Tatarinov, D.A.; Nizameev, I.R.; Gubaidullin, A.T.; Petrov, K.A.; Enrichi, F.; Vomiero, A.; et al. Dual red-NIR luminescent Eu-Yb heterolanthanide nanoparticles as promising basis for cellular imaging and sensing. *Mater. Sci. Eng. C-Mater. Biol. Appl.* 2019, 105, 110057. [CrossRef]

13. Dorenbos, P. Systematic behaviour in trivalent lanthanide charge transfer energies. *J. Phys.-Condens. Matter* 2003, 15, 8417–8434. [CrossRef]

14. Dorenbos, P. The Eu $^{3+}$ charge transfer energy and the relation with the band gap of compounds. *J. Lumin.* 2005, 111, 89–104. [CrossRef]

15. Zhou, B.Y.; Du, H.; Luo, P.L.; Ye, J.Y. Structural and luminescent properties of YOF:Eu$^{3+}$ nanocrystals embedded glass-ceramics derived by Spark Plasma Sintering. *Opt. Mater.* 2021, 118, 111247. [CrossRef]

16. Li, L.; Zhang, S.Y. Dependence of charge transfer energy on crystal structure and composition in Eu$^{3+}$-doped compounds. *J. Phys. Chem. B* 2006, 110, 21438–21443. [CrossRef]

17. Kitagawa, Y.; Ueda, J.; Fuji, K.; Yashima, M.; Funahashi, S.; Nakanishi, T.; Takeda, T.; Hiroski, N.; Hongo, K.; Maezono, R.; et al. Site-Selective Eu$^{3+}$ Luminescence in the Monoclinic Phase of YSiO$_2$N. *Chem. Mater.* 2021, 33, 8873–8885. [CrossRef]

18. Zairov, R.R.; Dovzhenko, A.P.; Podyachev, S.N.; Sudakova, S.N.; Kornev, T.A.; Shvedova, A.E.; Maslyi, A.N.; Syakaev, V.V.; Alekseev, I.S.; Vatsouro, I.M.; et al. Role of PSS-based assemblies in stabilization of Eu and Sm luminescent complexes and their thermostressive luminescence. *Colloids Surf. B Biointerfaces* 2022, 217, 112664. [CrossRef]

19. Zhu, B.; Chen, N.; Zhu, D.H.; Li, Y.S.; Sun, W.; Liu, G.H.; Du, G.P. Thermal annealing of LaF$_3$:Eu$^{3+}$ nanocrystals synthesized by a solvothermal method and their thermoresponsive luminescence. *J. Sol-Gel Sci. Technol.* 2013, 66, 126–132. [CrossRef]

20. Cieslik, B.; Konieczka, P. A review of phosphorus recovery methods at various steps of wastewater treatment and sewage sludge management. The concept of “no solid waste generation” and analytical methods. *J. Clean. Prod.* 2017, 142, 1728–1740. [CrossRef]

21. Whyte, M.P. Hypophosphatasia-etiology, nosology, pathogenesis, diagnosis and treatment. *Nat. Rev. Endocrinol.* 2016, 12, 233–246. [CrossRef] [PubMed]

22. Yang, Q.; Wang, X.L.; Luo, W.; Sun, J.; Xu, Q.X.; Chen, F.; Zhao, J.W.; Wang, S.N.; Yao, F.B.; Wang, D.B.; et al. Effectiveness and mechanisms of phosphate adsorption on iron-modified biocorns derived from waste activated sludge. *Bioreour. Technol.* 2018, 247, 537–544. [CrossRef] [PubMed]

23. Zhang, M.; Song, G.; Gelardi, D.L.; Huang, L.B.; Khan, E.; Masek, O.; Parikh, S.J.; Ok, Y.S. Evaluating biochar and its modifications for the removal of ammonium, nitrate, and phosphate in water. *Water Res.* 2020, 186, 116303. [CrossRef] [PubMed]

24. Hruska, K.A.; Mathew, S.; Lund, R.; Qiu, P.; Pratt, R. Hyperphosphatemia of chronic kidney disease. *Kidney Int.* 2008, 74, 148–157. [CrossRef] [PubMed]

25. Virkki, L.V.; Biber, J.; Murer, H.; Forster, I.C. Phosphate transporters: A tale of two solute carrier families. *Am. J. Physiol.-Ren. Physiol.* 2007, 293, F643–F654. [CrossRef]

26. Abraham, M.J.; Murto, T.; Schulz, R.; Pall, S.; Smith, J.C.; Hess, B.; Lindahl, E. GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX* 2015, 1–2, 19–25. [CrossRef]

27. Kutzner, C.; Pall, S.; Fechner, M.; Eszternann, A.; de Groot, B.L.; Grubmuller, H. More bang for your buck: Improved use of GPU simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX* 2015, 1–2, 2139–2143. [CrossRef]

28. Li, F.; Xing, Q.G.; Han, Y.C.; Li, Y.; Wang, Y.; Perera, T.S.H.; Dai, H.L. Ultrasonically assisted preparation of poly(acrylic acid)/calcium phosphate hybrid nanogels as pH-responsive drug carriers. *Mater. Sci. Eng. C-Mater. Biol. Appl.* 2014, 40, 2418–2431. [CrossRef]

29. Escudero, A.; Calvo, M.E.; Rivera-Fernandez, S.; de la Fuente, J.M.; Ocaña, M. Microwave-Assisted Synthesis of Biocompatible Europium-Doped Calcium Hydroxyapatite and Fluoroapatite Luminescent Nanospindles Functionalized with Poly(acrylic acid). *Langmuir* 2013, 29, 1985–1994. [CrossRef]

30. Kirwan, I.J.; Fawell, P.D.; Van Bronswijk, W. In situ FTIR-ATR Examination of Poly (acrylic acid) Adsorbed onto Hematite at Low pH. *Langmuir* 2003, 19, 5802–5807. [CrossRef]

31. Ding, H.C.; Pan, H.H.; Xu, X.R.; Tang, R.K. Toward a Detailed Understanding of Magnesium Ions on Hydroxyapatite Crystallization Inhibition. *Cryst. Growth Des.* 2014, 14, 763–769. [CrossRef]

32. Qin, J.L.; Zhong, Z.Y.; Ma, J. Biomimetic synthesis of hybrid hydroxyapatite nanoparticles using nanogel template for controlled release of bovine serum albumin. *Mater. Sci. Eng. C* 2016, 62, 377–383. [CrossRef] [PubMed]

33. Khoshnati, S.; Bourgine, A.; Julien, M.; Weiss, P.; Guichieux, J.; Beck, L. The emergence of phosphate as a specific signaling molecule in bone and other cell types in mammals. *Cell. Mol. Life Sci.* 2011, 68, 205–218. [CrossRef] [PubMed]