

碳量子点@金纳米团簇比率荧光传感器的制备及应用

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摘 要: 比率荧光传感器与单波长荧光传感器相比抗干扰能力强、更加灵敏、可视化检测结果可靠。采用水热法合成氮、硫掺杂的碳量子点(N, S-CQDs)及稳定性良好的金纳米团簇(BSA-AuNCs), 构建了 N, S-CQDs@BSA-AuNCs 比率荧光传感器, 用于连续测定 Ce^{4+} 和 AA (维生素 C)。结果表明, 该比率荧光传感器基于“关-开-关”检测机理在最优条件下, $I_{\text{F}429}/I_{\text{F}692}$ 与 Ce^{4+} 浓度之间有良好的线性关系, 检测限为 $0.12 \mu\text{mol/L}$; N, S-CQDs@BSA-AuNCs/ Ce^{4+} 加入 AA 后, $I_{\text{F}429}/I_{\text{F}692}$ 与不同浓度的 AA 在 $1\sim 10 \mu\text{mol/L}$ 范围内也呈现良好的线性关系, 检测限为 $0.19 \mu\text{mol/L}$ 。探究了一些可能共存物质的干扰, 实现该比率荧光传感器对 Ce^{4+} 、AA 的选择性识别和快速检测。

关键词: 抗坏血酸; 铈离子; 比率荧光传感器; 碳量子点

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铈是地球上最丰富的稀土元素, 作为催化剂、磁体、荧光粉、抛光粉和陶瓷着色剂在工业中被广泛使用^[1]。在自然界的铈通常是以 Ce^{3+} 形式存在, 然而 Ce^{4+} 容易提取, 也更容易从氟碳酸铈中分离。因此, 在矿物开采和冶金工程中, 在后处理阶段经常将 Ce^{3+} 转换为 Ce^{4+} 。由于其广泛的开发和应用, Ce^{4+} 很可能在环境、食物链和生物体中产生累积, 从而对环境和生物系统造成严重损害; 因此 Ce^{4+} 的检测是非常必要的^[2]。AA 也可以称为维生素 C, 是维持人体正常活动最重要的维生素之一。大量研究表明, 人体内 AA 水平与许多疾病密切相关, 缺乏 AA 可能会患以下疾病: 口腔溃疡、脱发、老年痴呆症、坏血病等^[3-4]。摄取 AA 的简单健康的方法是多吃含有 AA 的水果和蔬菜。AA 浓度是测定水果新鲜度和营养质量的重要指标^[5-6]。因此, 正确、可靠地检查水果和蔬菜的 AA 对预防疾病和评价蔬菜的新鲜度具有重要意义。开发一种简单、可靠、高选择性的 AA 分析方法对分析应用和临床疾病诊断至关重要, 已经成为当前化学研究的一个关键课题。

在过去的几十年里, 已经报道了一系列检测 Ce^{4+} 或 AA 的方法, 目前常见检测方法有毛细管电泳法^[7]、色谱法^[8]、化学发光^[9]以及电化学法^[10]等。虽然这些方法是高灵敏度的, 但它们昂贵的设备、复杂和耗时的操作程序限制了进一步的应用。相比之下, 荧光法因其仪器简单、成本低、样品消耗少等优点近年来受到广泛关注^[11-13]。但是, 此类荧光分析方法仍存在一些技术上的挑战和不可避免的缺点。例如, 具有单发射荧光法检测结果往往受到背景信号、荧光物质浓度、周围环境等与检测环境无关因素的干扰。针对这一问题, 研发者提出了一种新的荧光分析策略, 即比率荧光分析法^[14]。比率荧光分析法通过双荧光信号实现了分析信号的自校准, 因而相比单发射信号的荧光法能更容易获得更准确的检测结果, 它为目标物体的分析和检测提供了一种新的思维方式。一般而言, 在比率荧光分析法检测过程中, 目标物会触发 2 个荧光发射信号的强度发生异步性的变化, 比如一个峰增强, 另一个峰保持恒定或减小^[15]。更为重要的是, 由于单个发射

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峰的猝灭或者增强而观察到的荧光亮度变化, 可以用“双发射”模式下的荧光颜色变化予以指示, 从而提高了分析检测的选择性和灵敏度。例如, 龙登莹^[16]利用近红外 CdS 和氧化钼纳米薄片(MoO_3 NSs)作为荧光探针, 成功地研制了一种基于 AND–NAND 逻辑对用于 AA 检测的比率荧光传感器。其主要传感原理是利用荧光探针与目标物相互作用导致的 2 个荧光探针的信号变化, 利用其信号强度比作为信号读出方式, 实现了对 AA 的高灵敏检测。

本工作中将氮硫掺杂的碳量子点(N, S-CQDs)和金纳米团簇(BSA-AuNCs)复合形成了一种灵敏的 N, S-CQDs@BSA-AuNCs 比率荧光传感器, 用于连续测定 Ce^{4+} 和 AA。以牛血清蛋白(BSA)为稳定剂合成了 BSA-AuNCs, 其具有优异的荧光性能, 良好的生物相容性, 高量子产率, 优良的胶体稳定性及超小尺寸的 BSA-AuNCs。发现 BSA-AuNCs 通过荧光增强机制选择性地识别 Ce^{4+} , 并建立了用于检测 Ce^{4+} 的“打开”型近红外荧光传感器平台。通过在 N, S-CQDs@BSA-AuNCs 系统的近红外荧光传感器平台中加入 AA, 开发了基于 Ce^{4+} 介导的 BSA-AuNCs“关闭”型近红外荧光传感策略检测 AA。

1 实验

1.1 试剂

硫脲(TC)、柠檬酸、牛血清蛋白(BSA)、氯金酸、氢氧化钠、抗坏血酸(AA)、硝酸铈、尿酸(UA)、葡萄糖(Glu)、多巴胺(DA)、半胱氨酸(Cys)、甘氨酸(Gly)、硝酸钠、硝酸铝、硝酸钴、硝酸钙、硝酸钾和硝酸铜, 分析纯。

1.2 N, S-CQDs 的制备

根据文献[17], 称取 0.25 g 硫脲和 0.25 g 柠檬酸溶于 20 mL 超纯水中, 超声处理 30 min, 在 80 °C 放置 8 h, 离心收集上清液, 用 1 000 Da 的透析袋透析 2 次, 得到的溶液为 N, S-CQDs 溶液。

1.3 BSA-AuNCs 的制备

以 BSA 为稳定剂的 AuNCs 是根据文献[18]报道的方法合成的。将 50 mg/mL BSA 溶液和 10 mol/L HAuCl_4 溶液混合搅拌 2 min, 逐滴加入 1 mL NaOH (1 mol/L), 100 °C 搅拌 7 min, 用 1 000 Da 的透析袋在超纯水中透析 12 h 后, 得到 BSA-AuNCs 溶液。

1.4 N, S-CQDs@BSA-AuNCs 的制备

将 BSA-AuNCs 和 N, S-CQDs 按 1 : 2 的体积比混合, 4 °C 储存备用。

1.5 Ce^{4+} 和 AA 的检测

取 5 mL N, S-CQDs@BSA-AuNCs 作为工作溶液, 然后与不同浓度的 Ce^{4+} 溶液混合, 加去离子水定容至 10 mL, 稳定一段时间, 在 345 nm 的激发波长下进行荧光测试。

将 5 mL N, S-CQDs@BSA-AuNCs 工作溶液和 100 μL 1 mmol/L Ce^{4+} 混合, 然后与不同浓度的 AA 溶液混合, 定容至 10 mL, 搅拌 2 min, 稳定一段时间后在 345 nm 激发波长处进行荧光测试。

2 结果与讨论

2.1 N, S-CQDs@BSA-AuNCs 检测机理分析

将发射蓝色荧光的 N, S-CQDs 和发射红色荧光的 BSA-AuNCs 混合构建比率荧光传感器。图 1 为该比率荧光传感器对 Ce^{4+} 及 AA 的检测机理模拟图。金属阳离子诱导的聚集是 BSA-AuNCs 最常见的聚集形式之一, 可以通过金属阳离子溶液中吸引 BSA-AuNCs 聚集, 并通过 AIE 效应获得更高的光致发光量子^[19]。该比率荧光传感器以 N, S-CQDs 为参考信号, BSA-AuNCs 为输出信号单元, 当在体系中加入 Ce^{4+} 时, Ce^{4+} 引起了 BSA-AuNCs 的显著聚集, N, S-CQDs@BSA-AuNCs 比率荧光探针检测 Ce^{4+} 主要归因于 Ce^{4+} 对 BSA-AuNCs 的聚集诱导荧光增强效应^[20]。当在体系中再加入 AA 时, 由于 Ce^{4+} 的强氧化性对 AA 具有很强的亲和力, 从而使 BSA-AuNCs 表面的 Ce^{4+} 脱附, 导致其荧光强度降低。

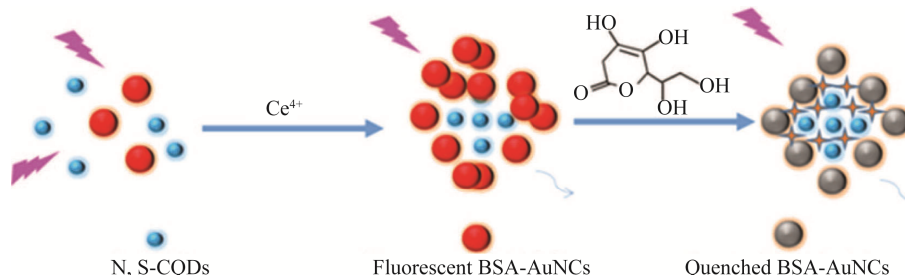


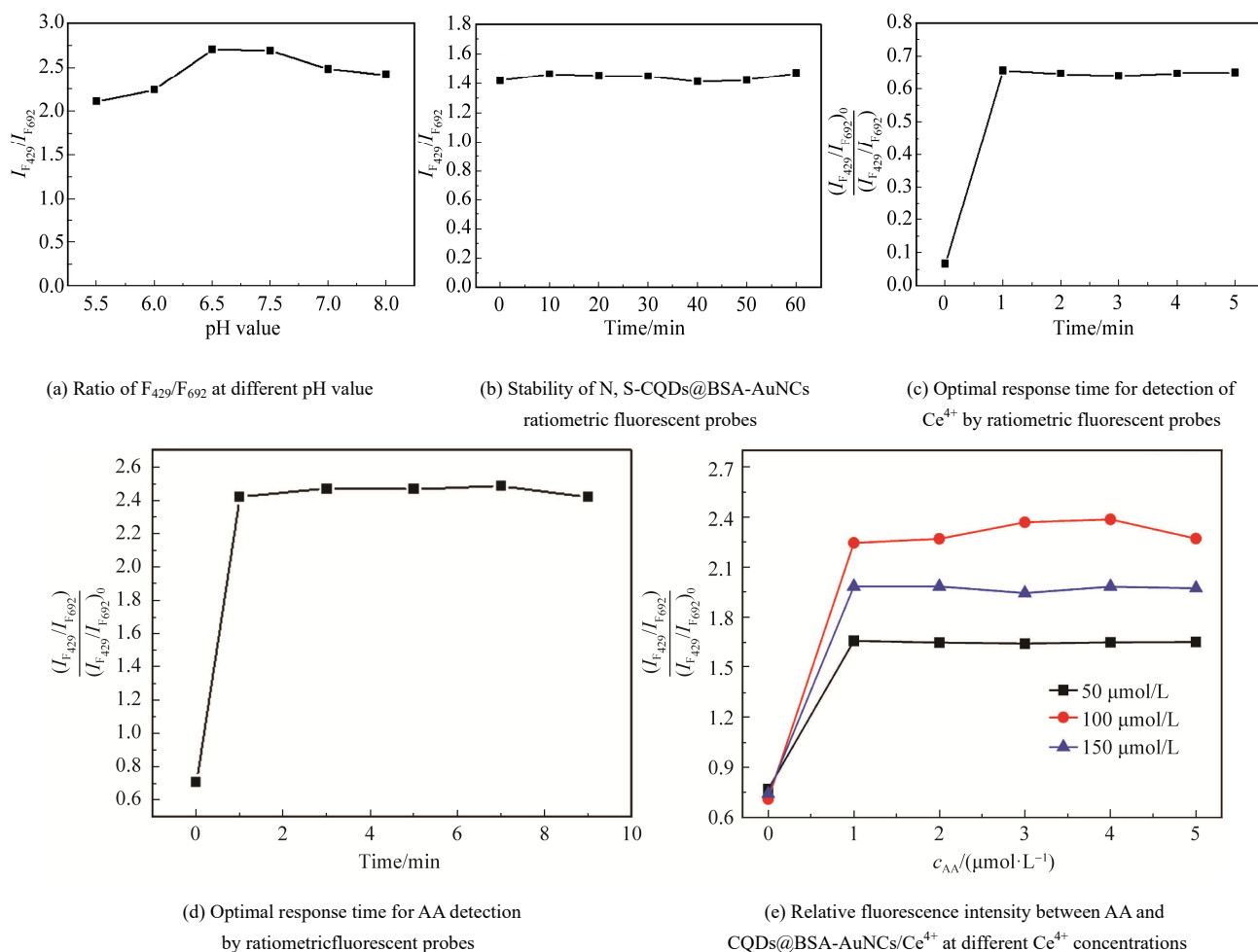
图 1 N, S-CQDs@BSA-AuNCs 体系比率荧光传感器的检测机理图

Fig. 1 Mechanism diagram of ratio fluorescence probe in N, S-CQDs@BSA-AuNCs system

2.2 实验条件优化

为了获得最佳实验结果, 对影响 Ce^{4+} 和 AA 检测的因素包括反应时间、稳定性和 pH 值进行了考查 (见图 2)。首先研究了 N, S-CQDs@BSA-AuNCs 比率荧光传感器溶液的 pH 值与荧光强度比值之间的关系。由图 2a 可见, 不同 pH 值的 CQDs@BSA-AuNCs 与一定浓度的 Ce^{4+} 混合后荧光强度比值 F_{429}/F_{692} 会发生变化。N, S-CQDs 表面带有羟基、氨基等官能团^[17], 当 pH 值处于弱酸性区域时, N, S-CQDs 表面带有羟基官能团与 H^+ 反应^[21]; 随着 pH 值增大, 溶液接近中性或为中性时, 荧光强度 F_{429}/F_{692} 随着 pH 值增加而增强; 当 pH 值进一步增加, N, S-CQDs 表面氨基官能团受表面电子云密度的影响; 当 pH 值处于碱性时, 与 OH^- 结合产生聚集现象, 导致 $\pi-\pi$ 相互作用增强, 电子-空穴辐射复合减弱, 荧光强度减弱^[22]。根据测试结果选择 pH=6.5 为后续溶液测试的 pH 值。由图 2b 可见, 检测时间对 CQDs@BSA-AuNCs 比率荧光传感器荧光强度产生

影响。在 60 min 内, 该比率传感器的荧光强度比值 $I_{F_{429}}/I_{F_{692}}$ 保持稳定, 说明该比率荧光传感器有良好的稳定性。由图 2c 可见, N, S-CQDs@BSA-AuNCs 比率荧光传感器与 Ce^{4+} 混合后, 不同反应时间的荧光强度发生了变化。1 min 后, 荧光强度趋于稳定, 所以选择 1 min 为检测时间。由图 2d 可见, N, S-CQDs@BSA-AuNCs/ Ce^{4+} 比率荧光传感器与 AA 混合后, 不同反应时间荧光强度发生了变化, 因此也选择 1 min 作为检测时间。由图 2e 可见, 在 N, S-CQDs@BSA-AuNCs 传感器体系中, Ce^{4+} 浓度为 100 $\mu\text{mol/L}$ 时可获得好的分析灵敏度和最佳的增强幅度, 为 50 $\mu\text{mol/L}$ 或 150 $\mu\text{mol/L}$ 均会影响检测效果。这可能是由于 Ce^{4+} 浓度太高时, 激发能量会以由分子间作用力形成超分子结构的形式耗散, 荧光的恢复效果会被降低; 浓度太低时, 则 N, S-CQDs@BSA-AuNCs 荧光传感器的荧光强度太低, 使得 AA 检测的线性范围变窄, 因此将 Ce^{4+} 浓度选择为 100 $\mu\text{mol/L}$ 对 AA 进行定量检测。



AA is vitamin C.

图 2 比率荧光探针的稳定性以及检测 Ce^{4+} 、AA 的最佳响应时间、pH 值、浓度

Fig. 2 Stability of fluorescent probe with ratio and optimum response time, pH value, concentration for Ce^{4+} and AA detection

2.3 通过打开模式检测 Ce^{4+}

在最优的实验条件下,研究了 N, S-CQDs@BSA-AuNCs 比率荧光传感器对 Ce^{4+} 的定量检测分析, Ce^{4+} 的浓度为 1、2、3、4、5、6、7 $\mu\text{mol/L}$ (见图 3)。由图 3a 可见,随着 Ce^{4+} 浓度增加,该体系中 692 nm 处的荧光逐渐增强,而 N, S-CQDs 的化学惰性和良好的光稳定性使其扮演背景信号的角色,429 nm 处的荧光基本保持不变。如图 3b 可见,该比率荧光传感的荧光强度比 I_{429}/I_{692} 与 Ce^{4+} 浓度

之间存在良好的线性关系,公式为:

$$I_{F_{429}}/I_{F_{692}} = -0.1633x + 1.21958 \quad (1)$$

其中: x 代表 Ce^{4+} 浓度, ($R^2=0.95209$), 计算出最低检测限 LOD 为 0.12 $\mu\text{mol/L}$ 。

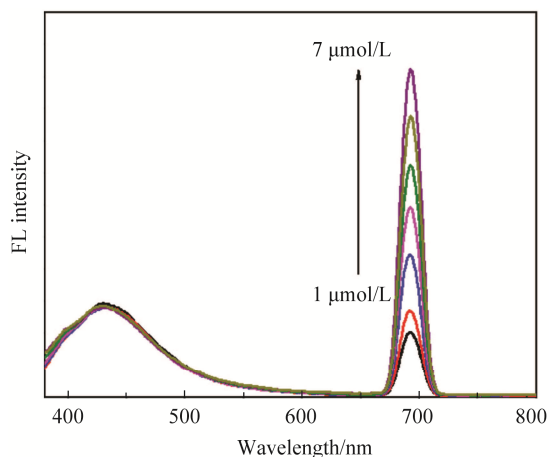
表 1 显示了各种荧光和电化学传感器对 Ce^{4+} 的检测结果。从表 1 可以看出,这项工作为 Ce^{4+} 检测提供了较低的检测限和较大的检测范围,检测限值为 0.12 $\mu\text{mol/L}$, 优于其它报告中的数据。综上所述,该方法具有相当甚至更高的检测灵敏度。

表 1 与其它报告的结果相比当前传感器的 Ce^{4+} 检测限值

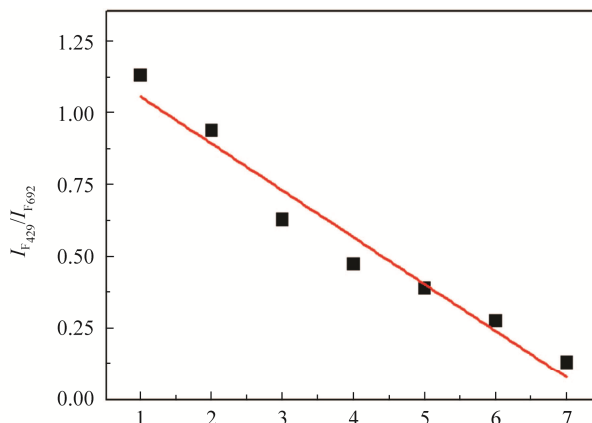
Table 1 Comparison of Ce^{4+} detection limit values of current sensors with other reported results

Sensing method	Sensing probe	Linear range/ $(\mu\text{mol}\cdot\text{L}^{-1})$	LOD/ $(\mu\text{mol}\cdot\text{L}^{-1})$	Reference
Fluorescence	NB-CDs	0.14–180.00	0.14	[23]
Fluorescence	C-dots/Bphen- Fe^{2+}	0.5–100.0	0.50	[24]
Fluorescence	GQDs	400–1 400	10.0	[25]
Fluorescence	N-GQDs	1–44	0.84	[26]
Fluorescence	N, S-CQDs@BSA-AuNCs	1–7	0.12	This work

LOD is limit of detection.



(a) N, S-CQDs@BSA-AuNCs ratimetric fluorescent probes for Ce^{4+} fluorescence response spectra with different concentrations



(b) Linear relationship between fluorescence intensity ratio ($I_{F_{429}}/I_{F_{692}}$) and Ce^{4+} with different concentrations

图 3 1~7 $\mu\text{mol/L}$ Ce^{4+} 的荧光响应图谱及 Ce^{4+} 与比例荧光强度 $I_{F_{429}}/I_{F_{692}}$ 之间的线性关系

Fig. 3 Fluorescence response profile of 1–7 $\mu\text{mol/L}$ Ce^{4+} and linear relationship between Ce^{4+} and proportional fluorescence intensity $I_{F_{429}}/I_{F_{692}}$

2.4 通过关闭模式检测 AA

图 4 为 1~10 $\mu\text{mol/L}$ AA 的荧光响应图谱及 AA 与比例荧光强度 $I_{F_{429}}/I_{F_{692}}$ 之间的线性关系。由图 4a 可见,在 N, S-CQDs@BSA-AuNCs/ Ce^{4+} 体系中加入 AA 后,BSA-AuNCs 的荧光强度有规律的降低, N, S-CQDs 荧光峰强度也有一定的降低,这可能是溶液测试背景的影响。由图 4b 可见, N, S-CQDs@BSA-AuNCs/ Ce^{4+} 与不同浓度的 AA 在 1~10 $\mu\text{mol/L}$ 范围内呈现良好的线性关系,公式为:

$$I_{F_{429}}/I_{F_{692}} = 0.02116x + 0.18153 \quad (2)$$

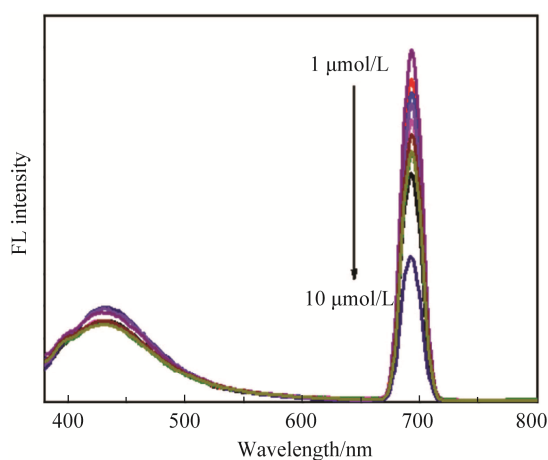
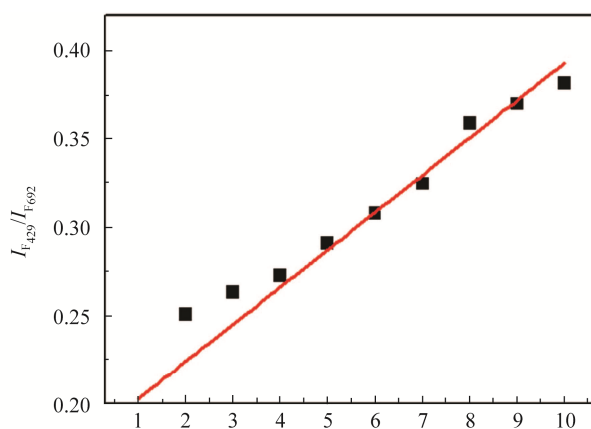
其中: x 代表 AA 浓度, ($R^2=0.91048$), 计算出最低检测限 LOD 为 0.19 $\mu\text{mol/L}$ 。

关闭再开启的原因可能是由于 Ce^{4+} 的强氧化性对 AA 具有很强的亲和力,从而使 BSA-AuNCs 表面的 Ce^{4+} 脱附,导致其荧光恢复。与之前报道的一些检测 AA 的比色法或荧光法(见表 2)相比,该方法具有相当甚至更高的检测灵敏度。

表 2 与其它报告的结果相比当前传感器的 AA 检测限值

Table 2 Limit of detection AA of the current sensor in relation to other reported result

Sensing method	Sensing probe	Linear range/($\mu\text{mol}\cdot\text{L}^{-1}$)	LOD/($\mu\text{mol}\cdot\text{L}^{-1}$)	Reference
Colorimetry	Co-POP	20–400	1.60	[27]
Colorimetry	Cu-Ag/r GO	5–30	3.6	[28]
Electrochemistry	GO/P(ANI-co-THI)	500–5 000	242	[29]
Electrochemistry	GO-CQDs	800–9 000	31.57	[30]
Electrochemistry	HNGA/GCE	50–4 000	16.7	[31]
Fluorescence	GQDs	5–75	1.0	[32]
Fluorescence	CuNCs	50–1 000	41.94	[33]
Fluorescence	N, S-CQDs@BSA-AuNCs/Ce ⁴⁺	1–10	0.19	This work

(a) N, S-CQDs@BSA-AuNCs/Ce⁴⁺ fluorescence response spectra of different concentrations in AA fluorescence control group(b) Linear relationship between fluorescence intensity ratio (I_{F429}/I_{F692}) and AA with different concentrations图 4 1~10 $\mu\text{mol/L}$ AA 的荧光响应图谱及 AA 与比例荧光强度 I_{F429}/I_{F692} 之间的线性关系Fig. 4 Fluorescence response profile of 1–10 $\mu\text{mol/L}$ AA and linear relationship between AA and proportional fluorescence intensity I_{F429}/I_{F692}

2.5 选择性分析以及抗干扰实验

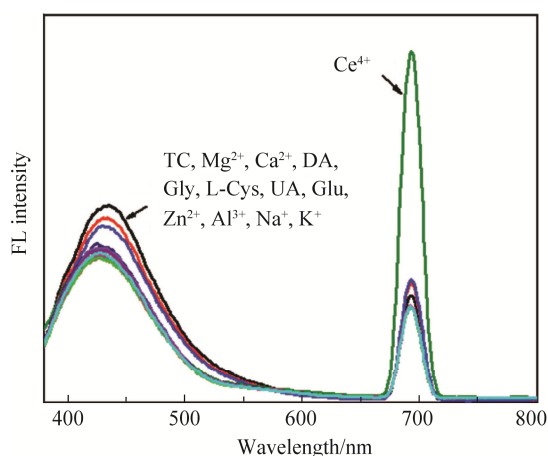
选择性是确保传感器准确性和可靠性的先决条件，为了证明比率荧光传感器对 Ce^{4+} 的高选择性，以及在该反应体系中其他物质是否会对荧光强度产生变化，所以选择了几种常用的抗生素或常见离子作为测试物质代替 Ce^{4+} 研究该传感系统对 Ce^{4+} 的选择性。图 5 是用于检测 Ce^{4+} 的选择性和抗干扰性的比率荧光传感器荧光变化和 I_{F429}/I_{F692} 比值的变化图。图 5a 为比率荧光传感器与不同抗生素之间的荧光图谱。由图 5a 可见，响应信号传感器只只对 5 $\mu\text{mol/L}$ Ce^{4+} 产生了明显的响应信号，而对 5 $\mu\text{mol/L}$ 的半胱氨酸(Cys)、葡萄糖(Glu)等抗生素，以及浓度为 1 mmol/L 的常见金属离子，如 Mg^{2+} 、 Cu^{2+} 等，

该传感器的响应比较弱。图 5b 为比率荧光传感器对不同抗生素的荧光响应强度比 I_{F429}/I_{F692} 柱状图，通过研究混合物的荧光强度比 I_{F429}/I_{F692} 。由图 5b 可见，荧光值 I_{F429}/I_{F692} 的比值为 0.4，而对于其他抗生素或普通离子则达到 1.81，这表明该传感器对 Ce^{4+} 具有很强的选择性。为了进一步研究该方法的抗干扰能力和可靠性，将干扰物质和 Ce^{4+} 与比率荧光传感器同时混合。由图 5c 可见，干扰物均不会影响该比率荧光传感器的荧光强度。由图 5d 可见，当存在干扰物时荧光强度比有微小幅度变化，但总体来说，该比率荧光传感器具有较高的抗干扰率。

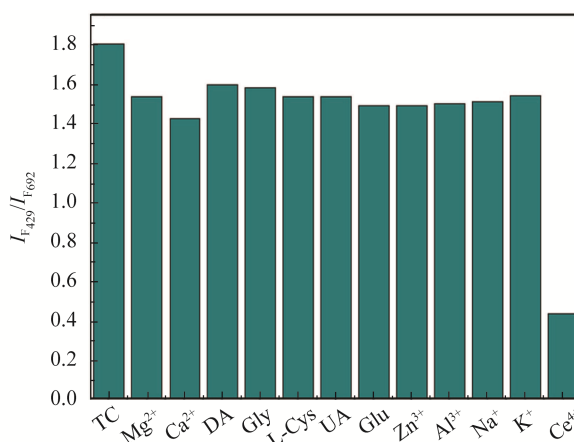
同样，“关闭”传感器用于评估 AA 识别的选择性。研究了十几种干扰物质对复合传感器荧光的影响。

响。图 6 为荧光探针与各种抗生素和干扰素之间的荧光光谱。由图 6 可见, 添加 AA 后, I_{F429}/I_{F692} 明显恢复, 而其它几种干扰物质对荧光强度比

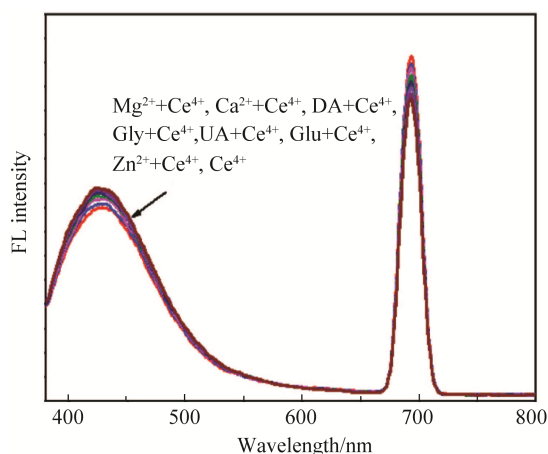
I_{F429}/I_{F692} 的影响很小。总而言之, 该比例荧光传感器具有更高的抗干扰率, 可在多种实际样本中成功检测出 AA, 灵敏度高。



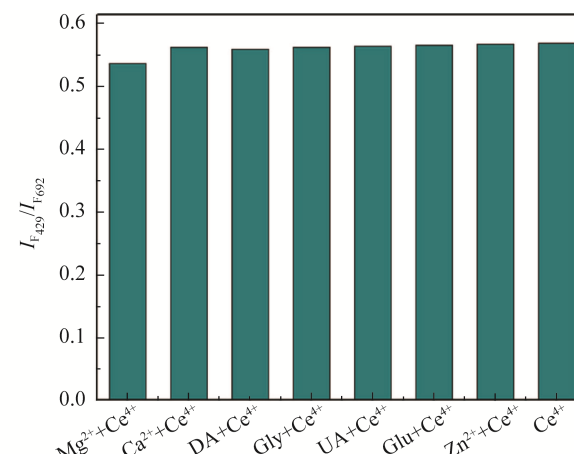
(a) Fluorescence spectra between ratiometric fluorescent probes and different interferents



(b) Determine the fluorescence intensity of different antibiotics



(c) Fluorescence spectra between ratiometric fluorescent probes and different interferents



(d) Detection of fluorescence intensity ratio of different antibiotics

TC is thiocarbanidin. DA is dopamine. Gly is glycocoll. Cys is cysteine. Glu is glucose. UA is uric acid.

图 5 传感器对 Ce^{4+} 的选择性和灵敏性

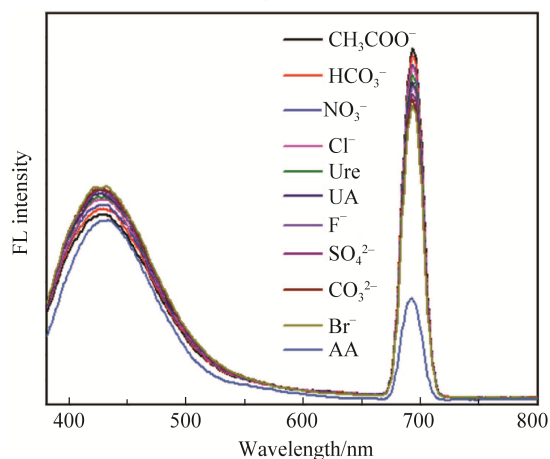
Fig. 5 Selectivity and sensitivity of sensors to Ce^{4+}

2.6 实际样本中的应用

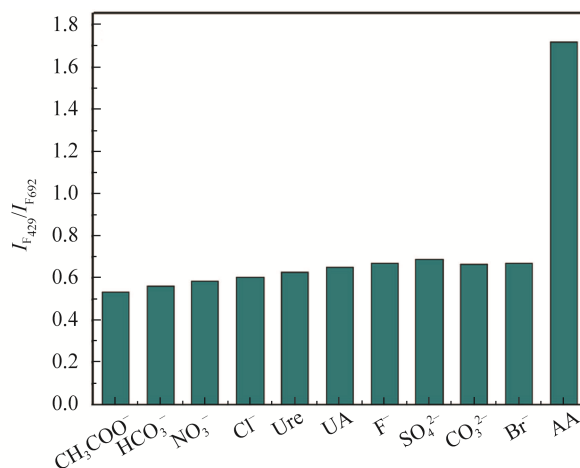
为了进一步验证 N, S-CQDs@BSA-AuNCs 比率荧光传感器在实际水样中的应用, 选取了自来水作为待检测水样。然后利用比率荧光传感器测定自来水样本中的 Ce^{4+} 含量。测试结果如表 3 所示, 3 个样本的 Ce^{4+} 检测回收率范围为 101.2%~104.2%, 相对标准偏差范围为 0.25%~0.44%。

为了进一步验证 N, S-CQDs@BSA-AuNCs/ Ce^{4+}

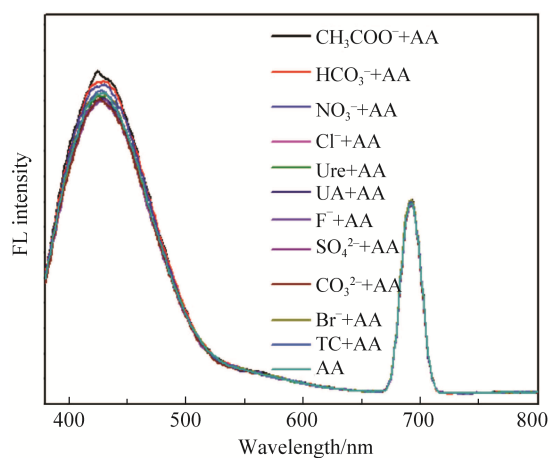
比率荧光传感器在实际样本中检测 AA 的应用, 对市售桃汁和 AA 片(维生素 C 片)进行预处理, 取上清液。将前处理得到的果汁饮料及维生素 C 片稀释液分别加入 1、3、5 $\mu\text{mol/L}$ AA, 使用比率荧光传感器进行检测。由表 4 可知, 回收率结果表明 2 种实际样本的 AA 检测回收率均在 96.31%~100.93%之间, 相对标准偏差在 0.14%~0.44%之间。表明该传感器具有较强的可靠性, 可用于实际样本中 Ce^{4+} 和 AA 的检测。



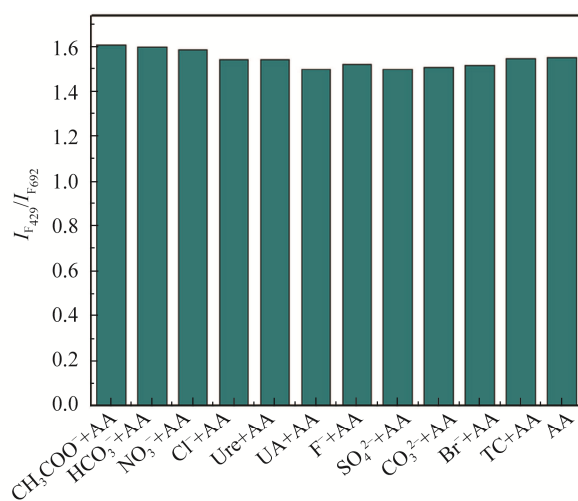
(a) Fluorescence spectra between ratiometric fluorescent probes and different interferents



(b) Measurement of fluorescence intensity of different antibiotics



(c) Fluorescence spectra between ratiometric fluorescent probes and different interferents



(d) Detection of fluorescence intensity ratio of different antibiotics

图 6 传感器对 AA 的选择性和灵敏性
Fig. 6 Selectivity and sensitivity of sensors to AA

表 3 实际样品中 Ce^{4+} 检测
Table 3 Determination of Ce^{4+} in real samples

Samples	Spkied/ $(\mu\text{mol}\cdot\text{L}^{-1})$	Found/ $(\mu\text{mol}\cdot\text{L}^{-1})$	Recovery/%	RSD/%
Water 1	1	1.01 ± 0.02	101.2 ± 0.02	0.44
Water 2	3	3.12 ± 0.01	104.2 ± 0.03	0.27
Water 3	5	5.15 ± 0.02	103.7 ± 0.04	0.25

RSD is relative standard deviation.

表 4 实际样品中 AA 检测
Table 4 Determination of AA in real sample

Samples	Determination/ $(\mu\text{mol}\cdot\text{L}^{-1})$	Added/ $(\mu\text{mol}\cdot\text{L}^{-1})$	Found/ $(\mu\text{mol}\cdot\text{L}^{-1})$	Recovery/%	Relative standard deviation/%
Peach juice drink	0.95	1	1.92 ± 0.01	98.46 ± 0.01	0.44
		3	3.97 ± 0.02	100.50 ± 0.06	0.28
		5	5.80 ± 0.02	98.80 ± 0.08	0.14
Vitamin c tablet	2.35	1	3.31 ± 0.02	96.31 ± 0.05	0.27
		3	5.40 ± 0.04	100.93 ± 0.04	0.36
		5	7.32 ± 0.06	99.59 ± 0.01	0.18

3 结论

通过 N, S-CQDs 和 BSA-AuNCs 构建了 N, S-CQDs@BSA-AuNCs 比率荧光传感器, 用于连续测定 Ce^{4+} 和 AA。当在体系中加入 Ce^{4+} 时, BSA-AuNCs 被 Ce^{4+} 氧化, 聚集成更大的颗粒, 荧光增强。因此构建了一个用于 Ce^{4+} 检测的“打开”型近红外荧光传感平台。传感器的荧光强度比 $I_{\text{F}429}/I_{\text{F}692}$ 与 Ce^{4+} 浓度之间存在良好的线性检测关系, 检出限为 $0.12 \mu\text{mol/L}$ 。随后, 通过添加 AA 猝灭 N, S-CQDs@BSA-AuNCs/ Ce^{4+} 系统的近红外荧光强度, 开发了基于 Ce^{4+} 介导的 N, S-CQDs@BSA-AuNCs “关闭”型近红外荧光传感策略检测 AA, 检出限为 $0.19 \mu\text{mol/L}$ 。有较好的抗干扰能力, 该比率荧光传感器成功应用于实际自来水中 Ce^{4+} 浓度的检测, 市售桃汁和维生素 C 片中的 AA 浓度检测。

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Preparation and Application of Carbon Quantum Dots@Gold Nanoclusters Ratio Fluorescence Sensor

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Extended Abstract

Introduction Cerium is the most abundant rare-earth element and is widely used in industry as catalyst, magnet, phosphor, polishing powder and ceramic colorant. Cerium occurs naturally in the form of Ce^{3+} . Ce^{4+} is easily extracted and separated from cerium fluocarbonate. Therefore, Ce^{3+} is often converted to Ce^{4+} during the reprocessing stage in mineral extraction and metallurgical engineering. Ce^{4+} is likely to accumulate in the environment, food chain and organisms due to its extensive development and application, causing serious damage to the environment and biological systems. It is thus necessary to detect Ce^{4+} . AA can be also called vitamin C as one of the most important vitamins to maintain the normal activities of the human body. Previous studies indicate that the level of AA in the human body is closely related to many diseases, lack of AA may suffer from the following diseases like mouth ulcer, hair loss, Alzheimer's disease, scurvy and so on. A simple and healthy way to consume AA is to eat more fruits and vegetables that contain AA. AA concentration is an important index to determine fruit freshness and nutritional quality. It is thus of great significance to check the AA of fruits and vegetables correctly and reliably to prevent diseases and evaluate the freshness of vegetables. The development of a simple, reliable and highly selective AA assay is crucial for analytical applications and clinical disease diagnosis, becoming a key topic in current chemistry research.

In the past few decades, a series of methods for the detection of Ce^{4+} or AA are reported, and a fluorescence method has attracted recent attention due to its advantages such as simple instrument, low cost and low sample consumption. However, such fluorescence analysis methods still have some technical challenges and inevitable shortcomings. For instance, the detection results with single emission fluorescence method are often interfered by some factors unrelated to the detection environment, such as background signal, fluorescence substance concentration, and surrounding environment. To solve this problem, a fluorescence analysis strategy, namely ratio fluorescence analysis, was proposed. The ratio fluorescence analysis method realized the self-calibration of the analysis signal through the double fluorescence signal, easily obtaining the more accurate detection results rather than the fluorescence method with single emission signal. It provides an effective way for the analysis and detection of target objects.

Methods According to the method described in the literature, 0.25 g thiourea and 0.25 g citric acid were dissolved in 20 mL ultra-pure water, treated with ultrasound for 30 min, then placed at 80 °C for 8 h, centrifuged to collect the supernatant, and dialyzed twice with 1 000 Da dialysis bag. The solution obtained was N, S-CQDs solution.

BSA-AuNCs were prepared according to the method reported in the literature. After 50 mg/mL BSA solution and 10 mmol/L HAuCl_4 solution were mixed under stirring for 2 min, 1 mL NaOH (1 mol/L) was added drop by drop and stirred at 100 °C for 7 min. After dialysis in ultra-pure water with 1 000 Da bag for 12 h, BSA-AUNCS solution was obtained.

BSA-AuNCs and N, S-CQDs were mixed at a volume ratio of 1:2 and stored at 4 °C for the coming use. 5 mL N,

S-CQDs@BSA-AuNCs as a working solution was mixed with Ce^{4+} solution at different concentrations. Deionized water was added into the mixture for a fixed volume to 10 mL, and stabilized for a period of time. Afterwards, the fluorescence test was performed at the excitation wavelength of 345 nm.

5 mL N, S-CQDs@BSA-AuNCs solution was firstly mixed with 100 μL 1 mmol/L Ce^{4+} , and then mixed with AA solution at different concentrations at a constant volume of 10 mL under stirring for 2 min. The fluorescence test was performed at the excitation wavelength of 345 nm after stability for a period of time.

Results and discussion In this work, nitrogen and sulfur doped carbon quantum dots (N, S-CQDs) and gold nanoclusters (BSA-AuNCs) are combined to form a N, S-CQDs@BSA-AuNCs fluorescence sensor for the continuous determination of Ce^{4+} ion and AA. BSA-auncs were synthesized with bovine serum protein (BSA) as a stabilizer. The BSA-AuNCs have superior fluorescence properties, good biocompatibility, high quantum yield, excellent colloidal stability and ultra-small size. It is indicated that BSA-AuNCs selectively recognizes Ce^{4+} ions based on a fluorescence enhancement mechanism, and an "open" NIR fluorescence sensor platform for detecting Ce^{4+} is established. The Ce^{4+} mediated BSA-AuNCs "off" NIR fluorescence sensing strategy is developed to detect AA *via* adding AA to the NIR fluorescence sensor platform of N, S-CQDs@BSA-AuNCs system.

Conclusions The N, S-CQDs@BSA-AuNCs ratio fluorescence sensor was constructed by N, S-CQDs and BSA-AuNCs for the continuous determination of Ce^{4+} and AA. When Ce^{4+} was added to the system, BSA-AuNCs were oxidized by Ce^{4+} and aggregated into coarser particles with an enhanced fluorescence. Therefore, an "open" NIR fluorescence sensing platform for Ce^{4+} detection was constructed. The fluorescence intensity ratio of $I_{\text{F}_{429}}/I_{\text{F}_{692}}$ showed a linear detection relationship with the concentration of Ce^{4+} , and the detection limit was 0.12 $\mu\text{mol/L}$. Subsequently, a Ce^{4+} mediated NIR fluorescence sensing strategy based on N, S-CQDs@BSA-AuNCs "off" was developed to detect AA, with a detection limit of 0.19 $\mu\text{mol/L}$ *via* adding the NIR fluorescence intensity of AA quenching N and S-CQDs@BSA-AuNCs/ Ce^{4+} system. The ratio fluorescence sensor was applied to the detection of Ce^{4+} concentration in actual tap water samples, AA concentration in commercial peach juice and vitamin C tablets.

Keywords ascorbic acid; cerium ions; ratiometric fluorescence sensor; carbon quantum dots