

## 基于金纳米颗粒-半胱胺 SERS 基底的水中硝酸根检测

赖春红<sup>1\*</sup>, 赖林<sup>1</sup>, 张芝峻<sup>1</sup>, 张帅康<sup>1</sup>, 姜小明<sup>2</sup>, 刘家瑜<sup>1</sup><sup>1</sup>重庆邮电大学光电工程学院, 重庆 400065;<sup>2</sup>重庆邮电大学生物信息学院, 重庆 400065

**摘要** 硝酸根过量是导致水污染的主要原因之一。针对表面增强拉曼光谱技术直接检测水中硝酸根检出限低, 无法达到国家地下水环境质量标准的问题, 本课题组制备了一种半胱胺修饰金纳米粒子(AuNPs)的复合 SERS 基底, 利用带正电荷的半胱胺对周围带负电荷的金纳米颗粒进行功能化修饰, 增加 SERS 基底对硝酸根的亲和性, 提高其对硝酸根的检测灵敏度。实验结果表明: 使用去离子水浸泡自组装  $10^{-3}$  mol/L 半胱胺的盖玻片 3 h, 再修饰 20 mL 金溶胶, 这样制成的金纳米颗粒-半胱胺复合 SERS 基底的增强性能最佳, 对硝酸钾的增强因子为  $2.14 \times 10^5$ ; 8 片不同基底上硝酸钾 SERS 信号的相对标准偏差为 10.36%, 检出限为 0.01 mg/L, 达到国家地下水环境质量标准规定的 I 类水的检测标准, 对光谱法测量水中总氮含量具有重要意义。

**关键词** 光谱学; 金纳米颗粒-半胱胺; SERS 基底; 硝酸盐

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## 1 引言

硝酸根是离子营养物质, 它可通过农业耕作、污水泄漏、生活用水排放等方式流入水体中, 造成水污染, 严重影响人们的生产生活。目前, 硝酸根的检测方法主要有离子色谱法<sup>[1]</sup>和分光光度法<sup>[2]</sup>, 这两种方法虽然检测精度高但也存在一定的局限性。如: 离子色谱法需要使用专业仪器, 检测成本高昂; 分光光度法需要对样本进行预处理, 整个过程操作复杂、耗时长。

表面增强拉曼光谱(SERS)是分子“指纹”光谱。SERS 法具有灵敏度高、样本无需预处理、不受水干扰等优点, 已被广泛应用于医学<sup>[3]</sup>、化学<sup>[4]</sup>以及水环境检测领域<sup>[5]</sup>, 可以达到单分子检测水平<sup>[6]</sup>。目前, 水溶液中硫酸根、硝酸根、高氯酸根等阴离子<sup>[7-9]</sup>的检测备受青睐。2017 年, 李震<sup>[10]</sup>采用 SERS 法对水产养殖水体中的硝酸钠和亚硝酸钠进行检测, 结果显示, 它们的检出限分别是 16.47 mg/L 和 164.72 mg/L。该方法比较简单, 但对硝酸盐和亚硝酸盐的灵敏度较低, 无法达到国家标准。金、银、铜等金属与硝酸

根阴离子的亲和性较差, 功能化 SERS 基底可以增大基底与硝酸根的亲和性, 提高对硝酸根的检测灵敏度。2017 年, Tran 等<sup>[11]</sup>先采用正十八硫醇和十二硫醇的混合溶液功能化银纳米粒子(AgNPs), 然后利用电化学技术在氧化铟锡(ITO)上沉积功能化后的银纳米粒子, 完成 Ag/ITO 基底的制备, 最后利用碘基水杨酸对硝酸根进行重氮化处理; 采用该基底间接检测硝酸根的检出限是 0.14 mg/L。2021 年, Cai 等<sup>[12]</sup>采用自组装方法完成了玻璃-SiO<sub>2</sub>-银基底的制备, 并使用 p-ATP 和 Griess 试剂 B 混合溶液重氮化处理亚硝酸盐, 利用 SERS 光谱特征峰强度与亚硝酸盐浓度对数的相关性, 间接测得亚硝酸盐的检出限为 0.065 mg/L。以上方法虽然提高了硝酸根离子的检测灵敏度, 但需要对样本进行重氮化处理, 操作过程复杂, 且受数据分析处理的影响较大。

本研究团队制备了一种金纳米颗粒-半胱胺复合 SERS 基底, 利用带正电荷的半胱胺对金纳米颗粒进行功能化修饰, 通过优化半胱胺含量和金纳米颗粒密度获得最佳增强性能, 以提高直接检测水质

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通信作者: \*laich@cqupt.edu.cn

中硝酸根离子的检出限,达到国家地下水环境质量标准中 I 类水的检测标准。

## 2 实验部分

### 2.1 实验试剂

实验试剂包括二水合柠檬酸三钠(粉末,分析纯)、氯金酸(粉末,分析纯)、硝酸钾标准溶液(浓度为 1 mol/L,分析纯)、半胱胺(粉末,生物试剂)。

### 2.2 仪器设备

实验仪器包括拉曼光谱仪、He-Ne 激光器(633 nm, 10 mW)、扫描电镜 SEM (TESCAN MIRA3)和恒温磁力搅拌机。

### 2.3 金纳米颗粒-半胱胺复合 SERS 基底的制备

#### 2.3.1 金纳米粒子的制备

采用柠檬酸三钠还原氯金酸的方法制备金纳米粒子<sup>[13]</sup>,具体操作步骤如下:1)配制质量分数为 0.01%的氯金酸溶液,配制质量分数为 1%的二水合柠檬酸三钠溶液;2)将 100 mL 质量分数为

0.01%的氯金酸溶液置于恒温磁力搅拌机上加热至沸腾;3)快速加入 0.6 mL 质量分数为 1%的二水合柠檬酸三钠溶液,持续加热 20 min,待溶液变为紫红色时,停止加热,在室温下冷却后倒入广口瓶中,置于-4 °C 环境中保存。

#### 2.3.2 金纳米颗粒-半胱胺复合基底的制备

由于半胱胺带有正电荷基团,与金属具有较强的配位能力<sup>[14]</sup>,因此本文选用半胱胺功能化金纳米粒子。制备金纳米颗粒-半胱胺复合 SERS 活性基底的方法如图 1 所示。溶液配制:取 0.774 g 半胱胺粉末溶于去离子水中,定容至 100 mL,完成浓度为  $10^{-3}$  mol/L 的半胱胺溶液的制备。I:将盖玻片放置在浓度为  $10^{-3}$  mol/L 的半胱胺溶液中浸泡 4 h,浸泡结束后取出,在 60 °C 鼓风干燥箱中放置 20 min,进行干燥处理。II:将自组装半胱胺的盖玻片浸入去离子水中浸泡 3 h,取出后同样进行干燥处理。III:将烘干的盖玻片分别浸泡于 15、20、25 mL 的同一种金溶胶中,4 h 后取出,用同样的方式进行干燥。

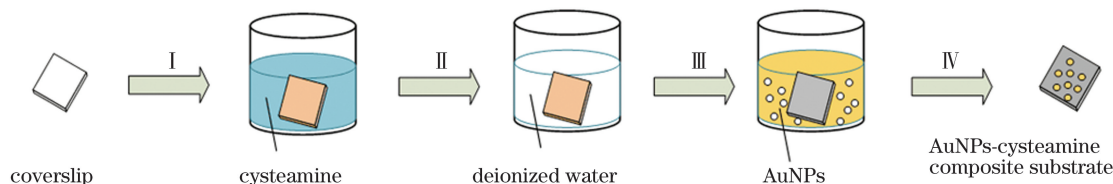


图 1 金纳米颗粒-半胱胺复合 SERS 基底的制备方案

Fig. 1 Specific preparation of AuNPs-cysteamine composite SERS substrate

### 2.4 金纳米颗粒-半胱胺复合 SERS 基底的实验测试

搭建拉曼实验测试平台,主要仪器和参数见 2.2 节。首先,将 1 mol/L 的硝酸钾标准溶液配制质量浓度分别为 0.001、0.01、0.1、1、10 mg/L 的硝酸钾溶液(以氮计);然后,将 10  $\mu$ L 质量浓度为 1 mg/L 的硝酸钾溶液滴加于金纳米颗粒-半胱胺复合 SERS 基底表面,并在 60 °C 烘干箱放置 20 min;最后,设置积分时间为 6 s,利用测试平台获取光谱数据。

## 3 结果与分析

### 3.1 半胱胺及复合 SERS 基底的表征

为研究半胱胺与金纳米颗粒的结合情况,对半胱胺和金纳米颗粒-半胱胺复合 SERS 基底的形貌进行扫描电镜(SEM)表征,表征结果如图 2 所示。图 2(a)为未浸泡去离子水的半胱胺的 SEM 图,可知半胱胺堆叠较明显,结构不太清晰。图 2(b)为浸

泡去离子水后的半胱胺的 SEM 图,可以清楚地看出半胱胺呈类似珊瑚的网状结构,且网状分布得较均匀。图 2(c)是未浸泡去离子水的金纳米颗粒-半胱胺复合 SERS 基底的 SEM 图,可见,半胱胺与金纳米颗粒结合得较为稀疏。图 2(d)是浸泡去离子水后的金纳米颗粒-半胱胺复合 SERS 基底的 SEM 图,可见,金纳米颗粒与半胱胺结合得比较紧密且均匀。未浸泡去离子水的半胱胺层层堆叠且分布不均,修饰结合金纳米颗粒时仅表面部分吸附金颗粒,因此半胱胺与金颗粒的结合较为稀疏。经去离子水浸泡后的半胱胺呈现出均匀的类珊瑚网状结构,可以充分修饰金纳米颗粒,获得均匀、紧密结合的金纳米颗粒-半胱胺复合 SERS 基底。

### 3.2 不同密度金纳米颗粒与半胱胺结合的 SERS 基底

使用同一批半胱胺分别修饰 15、20、25 mL 金溶胶制备金纳米颗粒-半胱胺复合 SERS 基底,基底的 SEM 表征如图 3(a)~(c)所示。可以看出:

15 mL 金溶胶与半胱胺的结合不均匀,且较为稀疏;20 mL 金溶胶与半胱胺的结合较为均匀,有利

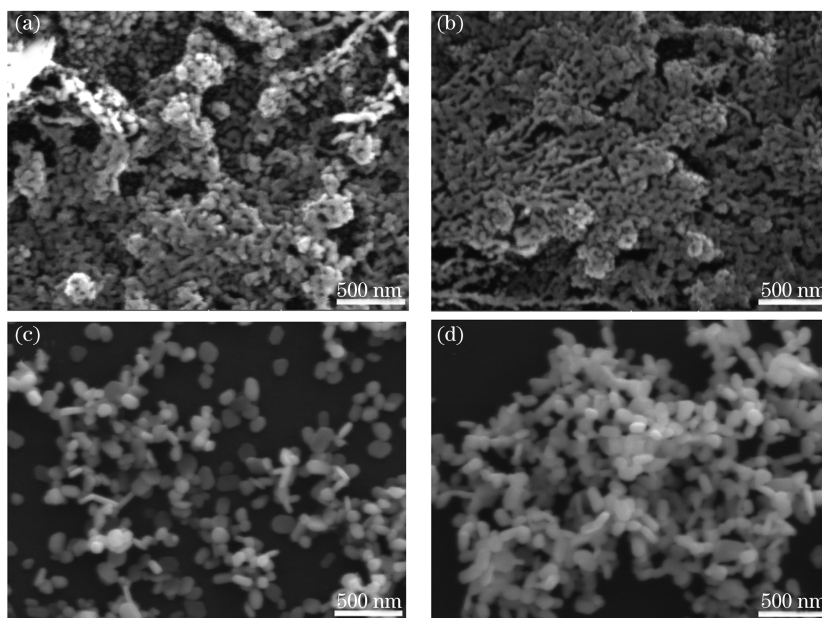


图 2 半胱胺和金纳米颗粒-半胱胺复合 SERS 基底形貌的 SEM 表征。(a) 未浸泡去离子水的半胱胺;(b) 浸泡去离子水后的半胱胺;(c) 未浸泡去离子水的金纳米颗粒-半胱胺复合 SERS 基底;(d) 浸泡去离子水后的金纳米颗粒-半胱胺复合 SERS 基底

Fig. 2 SEM characterization of cysteamine and AuNPs-cysteamine composite SERS substrate. (a) Cysteamine without soaking in deionized water; (b) cysteamine after soaking in deionized water; (c) AuNPs-cysteamine composite SERS substrate without soaking in deionized water; (d) AuNPs-cysteamine composite SERS substrate after soaking in deionized water

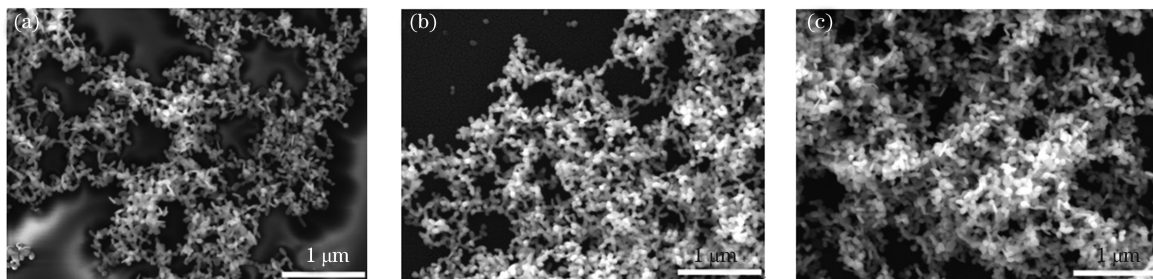


图 3 不同密度金纳米颗粒与半胱胺结合后的 SEM 图。(a) 15 mL 金溶胶;(b) 20 mL 金溶胶;(c) 25 mL 金溶胶  
Fig. 3 SEM images of AuNPs with different densities after binding to cysteamine. (a) 15 mL gold sol; (b) 20 mL gold sol; (c) 25 mL gold sol

于更多“热点”产生;25 mL 金溶胶与半胱胺结合紧密,但产生了堆叠。

### 3.3 SERS 基底的增强性能

#### 3.3.1 半胱胺含量及金纳米颗粒含量对 SERS 基底增强性能的影响

半胱胺的拉曼特征峰在  $646, 721, 937 \text{ cm}^{-1}$  处,而硝酸根离子的特征峰在  $1045 \text{ cm}^{-1}$  处,因此基底本身的拉曼信号不会对硝酸钾的检测产生影响。在本实验中,使用金纳米颗粒-半胱胺复合 SERS 基底检测硝酸根的拉曼特征峰在  $1037 \text{ cm}^{-1}$  附近,谱峰略微蓝移。这可能是在基底组装过程中,纳米颗粒的形状、密度和间隙发生变化引起的<sup>[15]</sup>。将质量浓度为  $1 \text{ mg/L}$  的硝酸钾溶液按照 2.4 节中所述操作

步骤对基底进行检测,图 4(a)是不同含量半胱胺对基底增强效果的影响,可见,浸泡去离子水的金纳米颗粒-半胱胺复合 SERS 基底对硝酸钾的检测强度为 130,而未浸泡去离子水的金纳米颗粒-半胱胺复合 SERS 基底对硝酸钾的检测强度为 66。未浸泡去离子水的金纳米颗粒-半胱胺复合 SERS 基底增强较弱的原因是半胱胺堆叠,仅表面吸附金纳米颗粒,产生的“热点”较少,因此拉曼信号增强较弱;而经去离子水浸泡的复合 SERS 基底,半胱胺与金纳米颗粒充分均匀结合,产生了更多的“热点”,因此拉曼信号增强较大。这一结果表明,使用去离子水浸泡后的金纳米颗粒-半胱胺复合 SERS 基底的增强性能更好。图 4(b)是不同密度金纳米颗粒-半胱胺

复合 SERS 基底对 1 mg/L 硝酸根的拉曼测试结果,可以看出,15、20、25 mL 金溶胶与半胱胺结合的复合 SERS 基底的拉曼信号强度分别为 51、88、48,其中 20 mL 金溶胶制备的基底的增强性能最

好。这主要是因为:金颗粒含量太少时,其分布太过稀疏,增强性能较弱;金颗粒含量太多时,会造成金颗粒聚集,也会降低增强性能;当金颗粒含量适中时,其与半胱胺均匀结合,增强性能最好。

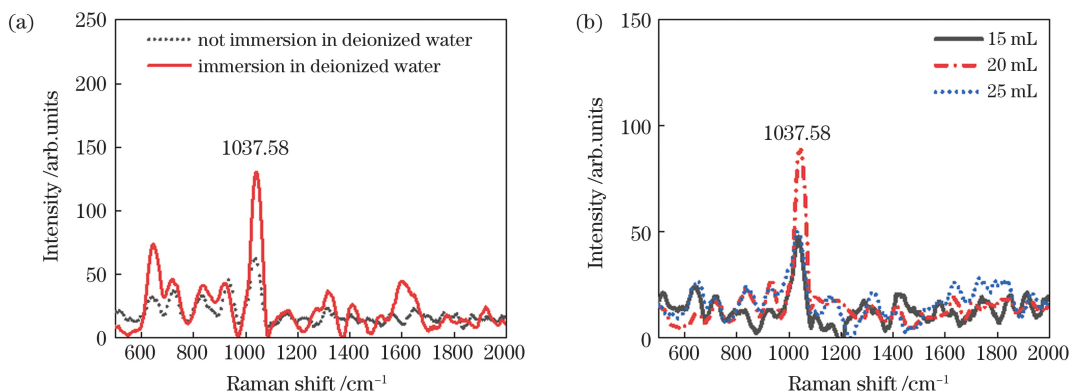


图 4 半胱胺及金纳米颗粒含量对 SERS 基底增强性能的影响。(a)不同含量半胱胺结合金纳米颗粒的 SERS 基底的性能对比;(b)不同密度金纳米颗粒结合半胱胺的 SERS 基底的性能对比

Fig. 4 Effect of cysteamine and gold nanoparticle contents on enhancement performance of SERS substrate. (a) Performance comparison of SERS substrate with different contents of cysteamine combined with AuNPs; (b) performance comparison of SERS substrate with different densities of AuNPs combined with cysteamine

### 3.3.2 金纳米颗粒-半胱胺复合 SERS 基底对硝酸钾的检出限测试

对质量浓度分别为 0.001、0.01、0.1、1、10 mg/L 的标准硝酸钾溶液进行基底增强性能测试,图 5 是检测结果。从图 5 中可以看出:随着硝酸钾溶液浓度的降低,拉曼信号强度逐渐减小;当硝酸钾溶液的质量浓度为 0.01 mg/L 时,能清晰地看到 1037 cm<sup>-1</sup> 处存在硝酸钾的拉曼特征峰,信号强度为 60;当硝酸钾溶液的质量浓度为 0.001 mg/L 时,未检测到硝酸钾的拉曼信号。因此,金纳米颗粒-半胱胺复合 SERS 基底对标准硝酸钾溶液的检出限是 0.01 mg/L,满

足国家地下水环境质量标准规定的 I 类水中总氮含量(以氮计)小于等于 0.2 mg/L 的要求<sup>[16]</sup>。

### 3.3.3 金纳米颗粒-半胱胺复合 SERS 基底增强因子及重现性评估

为了获得金纳米颗粒-半胱胺复合 SERS 基底更精确的增强性能,对基底增强因子( $E_F$ )进行评估。

$E_F$  的计算公式<sup>[17]</sup>为  $E_F = \frac{I_{\text{SERS}}/N_{\text{SERS}}}{I_{\text{bulk}}/N_{\text{bulk}}}$ ,其中,

$I_{\text{SERS}}$  为吸附在金纳米颗粒-半胱胺复合 SERS 基底上的硝酸根的拉曼信号的强度, $N_{\text{SERS}}$  为基底表面激光所照射的探针分子数, $I_{\text{bulk}}$  为无增强时硝酸根的拉曼信号的强度, $N_{\text{bulk}}$  为无增强时激光能照射到的有效分子数。为方便计算,忽略激光光斑、穿透深度及探针分子的吸附密度对基底的影响, $N_{\text{bulk}}$  与  $N_{\text{SERS}}$  之比近似于无增强基底与增强基底上硝酸钾的浓度之比<sup>[18]</sup>。图 6 为硝酸钾在金纳米颗粒-半胱胺复合 SERS 基底与无增强基底上的拉曼光谱图,质量浓度为 0.01 mg/L 的硝酸钾在金纳米颗粒-半胱胺复合基底上的拉曼信号的强度为 60,而质量浓度为 1000 mg/L 的硝酸钾在无增强基底上的拉曼信号的强度为 28。根据增强因子计算公式可得到拉曼增强因子约为  $2.14 \times 10^5$ 。基底可重现性是影响基底实际应用的重要参数之一,为评估基底的

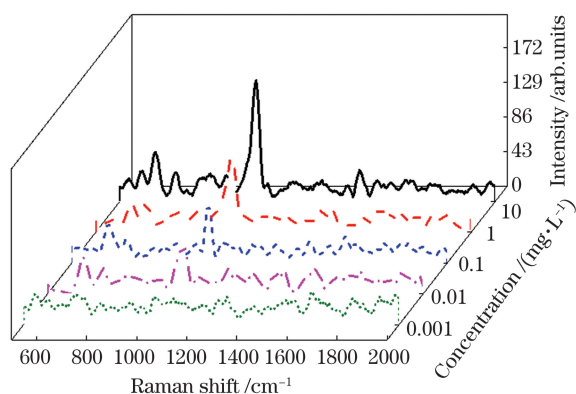


图 5 金纳米颗粒-半胱胺复合 SERS 基底上不同浓度硝酸钾的拉曼光谱

Fig. 5 Raman spectra of different concentrations of potassium nitrate on AuNPs-cysteamine composite SERS substrate

可重现性,本课题组在 8 片不同的基底上使用 1 mg/L 硝酸钾进行了测试,测试结果如图 7 所示。计算可得相对标准偏差(RSD)为 10.36%,表明该基底具

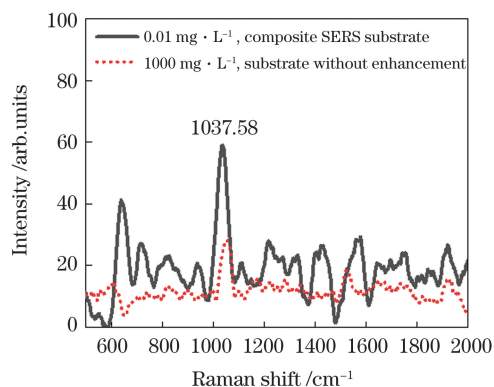


图 6 硝酸钾在金纳米颗粒-半胱胺复合 SERS 基底与无增强 SERS 基底上的拉曼光谱

Fig. 6 Raman spectra of potassium nitrate in AuNPs-cysteamine composite SERS substrates and SERS substrate without enhancement

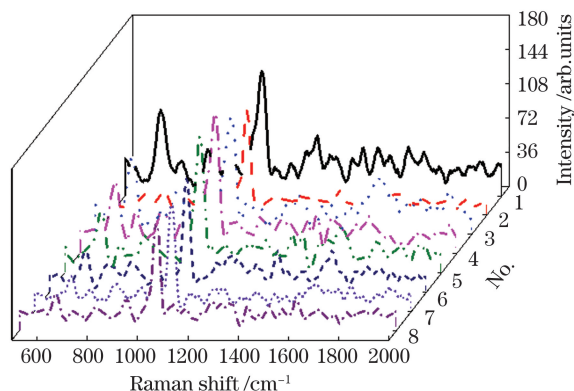


图 7 硝酸钾在 8 片不同金纳米颗粒-半胱胺复合 SERS 基底上的拉曼光谱

Fig. 7 Raman spectra of potassium nitrate on 8 different AuNPs-cysteamine composite SERS substrates

有良好的可重现性。

## 4 结 论

本课题组制备了一种用于检测水质中硝酸根的金纳米颗粒-半胱胺复合 SERS 基底。利用化学还原法制备金纳米粒子,通过带正电荷的半胱胺功能化周围带负电荷的金纳米粒子,增加金纳米颗粒与硝酸根阴离子的亲和性,提高了信号的检测灵敏度。实验结果表明:用去离子水浸泡自组装  $10^{-3}$  mol/L 半胱胺的盖玻片 3 h,再将其置于 20 mL 金溶胶中进行修饰,如此制备而成的金纳米颗粒-半胱胺复合 SERS 基底的增强性能最好,增强因子为  $2.14 \times 10^5$ ; 8 片不同基底上硝酸钾 SERS 信号的相对标准偏差为 10.36%,该基底对标准硝酸根溶液的检出限为 0.01 mg/L,达到了国家地下水环境质量标准规定的 I 类水的检测标准。金纳米颗粒-半胱胺复

合 SERS 基底的研制为拉曼光谱法检测水质中总氮含量奠定了基础,具有较广阔的应用前景。

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## Nitrate Detection in Water Based on AuNPs-Cysteamine SERS Substrate

Lai Chunhong<sup>1</sup>, Lai lin<sup>1</sup>, Zhang Zhijun<sup>1</sup>, Zhang Shuaikang<sup>1</sup>, Jiang Xiaoming<sup>2</sup>, Liu Jiayu<sup>1</sup>

<sup>1</sup> School of Optoelectronics Engineering, Chongqing University of Posts and Telecommunications, Chongqing 400065, China;

<sup>2</sup> School of Bioinformatics, Chongqing University of Posts and Telecommunications, Chongqing 400065, China

### Abstract

**Objective** Nitrate is an ionic nutrient that can flow into rivers, lakes, and seas through agricultural fertilizers, organic nitrogen-containing soils, industrial and domestic wastewater, as well as other sources, leading to the eutrophication of water bodies. Therefore, the detection of nitrate content is an important indicator of water quality. The main detection methods for nitrate include ion chromatography and spectrophotometry. Ion chromatography requires professional instruments, resulting in a high cost, whereas spectrophotometry requires pretreatment of the sample, resulting in a complicated and time-consuming process. Surface-enhanced Raman spectroscopy (SERS) has high sensitivity, does not require pretreatment of the sample, and does not experience interference from water. Thus, it is applicable to detect substances in aqueous solution. Presently, nitrate detection in water using SERS has low detection limits, which cannot meet the national environmental quality standards for groundwater. In this study, a composite SERS substrate is reported with positively charged cysteamine modified around negatively charged gold nanoparticles. The reported substrate can directly detect nitrate while reaching the detection standards of Class-I water.

**Methods** In this paper, Au-cysteamine composite SERS substrates were prepared using a combination of chemical reduction and self-assembly methods. First, gold nanoparticles were prepared through the reduction of chloroauric acid using trisodium citrate. Second, cysteamine, having strong coordination with metals and charged groups, was used to functionally modify the surrounding negatively charged gold nanoparticles to increase the affinity of gold nanoparticles to nitrate anions and improve the signal-detection sensitivity. Finally, the morphology of the cysteamine and AuNPs-cysteamine composite SERS substrates were characterized by scanning electron microscopy (SEM), and the effects of cysteamine stacking density and gold nanoparticle density on the performance of the substrates were investigated to improve the signal sensitivity to nitrate anions.

**Results and Discussions** The AuNPs-cysteamine composite SERS substrate prepared in this paper has high sensitivity to nitrate anions in water and can meet the detection standard for Class-I water in the national groundwater environmental quality standard. SEM showed a coral-like network formed by the cysteamine soaked in deionized water, which had a uniform distribution and was tightly and uniformly bound to the gold nanoparticles (Fig. 2). The self-assembled coverslip with cysteamine was immersed in 20 mL of gold sol, and the bound cysteamine and gold nanoparticles were more uniformly distributed, which produced more hotspots (Fig. 3). The AuNPs-cysteamine composite SERS substrate performed better in experiments when soaked in deionized water than otherwise, owing to the uniform distribution of cysteamine. The AuNPs-cysteamine composite SERS substrate prepared by the same batch of cysteamine modified with 20 mL of gold sol had moderate detection performance owing to the content of gold nanoparticles, whereas the best performance was obtained after binding with cysteamine (Fig. 4). The detection limit of the AuNPs-cysteamine composite SERS substrate prepared by self-assembly using cysteamine soaked in deionized water and 20 mL of gold sol was 0.01 mg/L (Fig. 5) with an enhancement factor of  $2.14 \times 10^5$  (Fig. 6), and the relative standard deviation (RSD) of the potassium nitrate signal on eight different AuNPs-cysteamine composite SERS substrates was 10.36% (Fig. 7), which meets the detection standard of Class-I water.

**Conclusions** In this paper, an AuNPs-cysteamine composite SERS substrate for the detection of nitrate in water is prepared. To increase the affinity of gold nanoparticles toward nitrate anions, gold nanoparticles are prepared by chemical reduction, and the signal-detection sensitivity is improved by functionalizing the surrounding negatively charged gold nanoparticles with positively charged cysteamine. The experimental results show that the best enhancement performance is obtained when AuNPs-cysteamine composite SERS substrate is prepared by soaking coverslips with  $10^{-3}$  mol/L cysteamine for 3 h using deionized water and then modifying 20 mL of gold sol, with an enhancement factor of  $2.14 \times 10^5$ . The RSD of potassium nitrate SERS signal on eight different AuNPs-cysteamine composite SERS substrates was 10.36%; moreover, the detection limit for standard nitrate solution is 0.01 mg/L. Therefore, the development of AuNPs-cysteamine composite SERS substrate has laid the foundation for the detection of nitrate nitrogen in water by Raman spectroscopy and has a great prospect of application.

**Key words** spectroscopy; AuNPs-cysteamine; SERS substrate; nitrate