Title: "A green, rapid and efficient dual-sensors for highly selective and sensitive detection of cation (Hg$^{2+}$) and anion (S$^{2-}$) ions based on CMS/AgNPs composites"

Dear professors,

Many thanks for your greatly helpful comments and suggestions on the manuscript. They will increase the level of the article greatly. We have modified the manuscript accordingly, and detailed corrections are listed in the manuscript.

Reviewer #1:

I quite like the idea of this manuscript, which is to detect mercury cations and sulfide anions using carboxymethyl cellulose sodium/silver nanoparticles. The authors have identified a high priority problem, the dual detection of anions and cations in complex environments, and an innovative solution to solve that problem. Nonetheless, there are a number of issues that in my opinion need to be addressed prior to publication of this manuscript. These include:

1. In the abstract, the authors use the term ‘biosensor.’ This is not defined, nor is it clear why the authors need a ‘biosensor’ for their stated goal rather than simply a high-performance sensor. This should be qualified and explained further.

   Answer: Thanking for giving the significant comment to our research work. This is a constructive comment and we have revised the manuscript accordingly.

   The sentence “How to design and fabricate the high-performance biosensor for simultaneously and accurately detecting the Hg$^{2+}$ and S$^{2-}$ is critical.” has been revised into “How to design and fabricate the high-performance sensor for simultaneously and accurately detecting the Hg$^{2+}$ and S$^{2-}$ is critical.”
2. In all cases, the authors should be encouraged to use superscripts to denote the charges on the ions rather than including the charges at full size. The current full-size hampers readability of this manuscript.

Answer: Thanking for giving the precious comment to our research work. This is a constructive comment, and we have revised the manuscript accordingly.

3. In general, this manuscript would benefit from a thorough proofreading by a native English speaker. While it is certainly understandable in its current state, there are some syntax-based errors that should be corrected. In the introduction, the authors use a colloquial term “as well all known.” The term is “as we all know,” rather than “as we all known,” and neither term should be used in the formal research paper context.

Answer: Thanking for giving the precious comment to our research work. This is a meaningful suggestion, and we have modified it in the paper, meanwhile, we have carefully examined throughout the paper and revised accordingly.

4. A more concerning problem is the fact that the authors indicate that methods for Hg$^{2+}$ and S$^{2-}$ detection all require expensive instrumentation, and they ignore the fact that simpler methods for detection of these ions have already been reported. For example, the authors should familiarize themselves with papers such as:

Guo, Lulu; Song, Yonghai; Cai, Keying; Wang, Li "On-off" ratiometric fluorescent detection of Hg2+ based on N-doped carbon dots-rhodamine B@TAPT-DHTA-COF. Spectrochim. Acta A 2019, Ahead of Print; DOI: 10.1016/j.saa.2019.117703

Zhang, Yao; Zhang, Lan; Wang, Luyang; Wang, Guoqing; Komiyama, Makoto; Liang, Xingguo Colorimetric determination of mercury(II) ion based on DNA-assisted amalgamation: a comparison study on gold, silver and Ag@Au Nanoplates. Microchim. Acta 2019, 186, 1-8.

Shojaeifard, Zahra; Hemmateenejad, Bahram; Shamsipur, Mojtaba; Ahmadi, Raheleh Dual fluorometric and colorimetric sensor based on quenching effect of copper (II) sulfate on the copper

Engel, Laura; Tarantik, Karina R.; Pannek, Carolin; Woellenstein, Juergen Screen-printed sensors for colorimetric detection of hydrogen sulfide in ambient air. Sensors 2019, 19, 1182.

And many others like this. They should explain more clearly how their work fits into the context of work that has already been published in this area.

Answer: Thanking for giving the precious comment to our research work. This is a meaningful suggestion, and we have summarized these methods in the paper, as shown in Table S2.

<table>
<thead>
<tr>
<th>Table S2</th>
<th>Comparison of the proposed Hg^{2+}/S^{2-} detection method with other reported methods</th>
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<tbody>
<tr>
<td>Methods</td>
<td>Probe</td>
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<tr>
<td>Colorimetric</td>
<td>β-CD AgNPs</td>
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<td></td>
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<tr>
<td>Colorimetric</td>
<td>SSA-AgNPs</td>
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<tr>
<td>Colorimetric</td>
<td>AUNSs</td>
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<tr>
<td>Electro chemistry</td>
<td>AgNPs</td>
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<tr>
<td>Colorimetric</td>
<td>AgNPs</td>
</tr>
<tr>
<td>Colorimetric</td>
<td>SA-AgNPs</td>
</tr>
<tr>
<td>Fluorescence</td>
<td>NCDs-RhB@COF</td>
</tr>
<tr>
<td>Electro chemistry</td>
<td>SPCE/Go/AuNPs</td>
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<tr>
<td>Colorimetric</td>
<td>Cip-AgNPs</td>
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<tr>
<td>Colorimetric</td>
<td>AgNPs</td>
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<tr>
<td>Colorimetric</td>
<td>CMS/AgNPs</td>
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</table>

2. Das, S., et al., Sensitive and robust colorimetric assay of Hg^{2+} and S^{2−} in aqueous solution directed by 5-sulfosalicylic acid-stabilized silver nanoparticles for wide range application in real samples. Journal of


5. The authors refer to an environmentally benign reducing agent, “Vc.” This agent needs to be more clearly delineated the first time it is used.

Answer: Thanking for giving the precious comment to our research work. This is a meaningful suggestion, and we have added some description in the paper.

“Vc is a water-soluble vitamin and non-toxic reducing agent. There are double O-H with C=C bonds in the structure of Vc, so it has strong reducing ability.”

6. The colloidal solution depends on electrostatic attraction between the carboxylic acid groups on the CMS and the Ag nanoparticles. If so, it seems that it is likely to have a strong pH dependence as well. Has this dependence been analyzed/ is the pH carefully controlled?
Answer: Thanking for giving the precious comment to our research work. This is a meaningful suggestion, and we have investigated the influence of the pH, the detail description can be found in the manuscript and supplementary materials.

Furthermore, the UV–vis spectral responses of CMS/AgNPs were recorded at different pH in Fig. S1. The pH of synthesized CMS/AgNPs sol is 8.39. The CMS/AgNPs shows no noticeable effects with the change in pH from 6.59 to 10.51. However, the CMS/AgNPs shows spectral changes at low pH (< 2.48) due to the COO\(^{-}\) in the CMS combined with H\(^{+}\) to form COOH, which reduced the stabilizing of AgNPs. These results confirm that CMS can stabilize AgNPs because of electrostatic adsorption between COO\(^{-}\) and [Ag (NH\(_3\))\(_2\)]\(^{+}\).

7. More details should be provided to answer this question. More information about the non-zero signals for cadmium (2+) and iron (2+) should be provided (see Figure 5B), especially around an explanation for this non-ideal selectivity and how such interfering ions are dealt with in complex environments. The authors are using a mixture of silver nanoparticles and carboxymethylcellulose, but the role of each in the observed performance is not clearly elucidated. The authors need to have control experiments that use just the carboxymethylcellulose and just the silver nanoparticles, and explain from there how the beneficial effect of both components of the sensor can occur. Similarly, the size of the silver nanoparticles appears to be somewhat arbitrarily chosen.

Answer: Thanking for giving the precious comment to our research work. This is a meaningful suggestion, and we have added some description in the paper.
Fig. 7 (A) UV–vis absorption spectra and (B) ΔA of the proposed sensing system in the presence of 15 kinds various metal ions and mixed all ions. (The concentrations of all metal ions are 50 μM)

To evaluate the selectivity of CMS/AgNPs colloidal solution, besides Hg$^{2+}$, other 14 kinds of metal ions, including Cd$^{2+}$, Co$^{2+}$, Cu$^{2+}$, Fe$^{3+}$, Fe$^{2+}$, K$^+$, Al$^{3+}$, Ba$^{2+}$, Ca$^{2+}$, Mg$^{2+}$, Na$^+$, Ni$^{2+}$, Pb$^{2+}$, and Zn$^{2+}$ were selected. The absorption spectra of AgNPs with various metal ions were given in Fig. 7A. Comparing these plots, only the Hg$^{2+}$ one had a response for AgNPs, and upon the addition of Hg$^{2+}$, the absorption peak decreased and exhibited an obvious blue shift. While for other 14 metal ions, the absorption spectra were almost unchanged even at the maximum concentration (50 μM). As shown in Fig. 7B, the change of ΔA was 0.36 in the presence of Hg$^{2+}$, but there was no obvious change observed in the presence of other metal ions. The color of the sensing system did not change after the addition of other metal ions (50 μM) except for Hg$^{2+}$, which made the system from yellow to colorless (inset, Fig. 7B). In addition, in the presence of all anions, the relative absorbance values were analogous to that of Hg$^{2+}$ value. These results clearly indicated that other related anions did not interfere with the spectral and colorimetric detection of Hg$^{2+}$. The experimental results verify that our proposal shows unique selectivity for Hg$^{2+}$. 
To evaluate the selectivity of this sensing system, 15 kinds various negative ions such as NO$_3^-$, NO$_2^-$, CO$_3^{2-}$, HCO$_3^-$, HPO$_4^{2-}$, H$_2$PO$_4^-$, PO$_4^{3-}$, F$, $Cl$, Br$, $I$, SO$_3^{2-}$, S$_2$O$_8^{2-}$, and SO$_4^{2-}$ ions (50 μM) were added to CMS/AgNPs colloidal solution respectively. Fig. 10B gave the absorption spectra changes of AgNPs after addition of negative ions. Followed by the addition of S$_2^-$, the absorption peak of AgNPs decreased significantly, the ΔA was 0.65 in Fig. 10C with the color changes from yellow to brown (Fig. 10A), while there were no obvious changes for other negative ions. In addition, the relative absorbance values were analogous to that of S$_2^-$ value in the presence of all anions. These results clearly indicated that other related anions did not interfere with the spectral and colorimetric detection of S$_2^-$. These results demonstrate that our proposal shows distinguishing selectivity toward S$_2^-$. 

In this paper, carboxymethyl cellulose sodium/silver nanoparticles (CMS/AgNPs) colloidal solution show highly detection for Hg$^{2+}$ and S$^{2-}$ due to the synergy effect of the two compounds, while the CMS was utilized as both the stabilizer and the hydrophilic substrate for AgNPs. Just the CMS without AgNPs did not show any signals for the detection of metal ions, and the AgNPs without CMS are easy to aggregate into large particles due to the high surface-energy, thus affecting the detective results.
We have conducted a series of parallel experiments, the same experiment condition with the certain reductant can achieve a uniform size of the silver nanoparticles for 20 nm.

8. More information about how such a size was selected and how the choice of particle size influences detection performance should be provided as well.

Answer: Thanking for giving the precious comment to our research work. This is a meaningful suggestion, and we have added some description in the paper.

![Fig. S2 TEM images of different reducing agent: (A) NaBH₄, (B) Sodium Citrate and (C) Glucose](image)

**Table S1** Comparison of the proposed Hg²⁺ detection method with different reducing agent

<table>
<thead>
<tr>
<th>Reducing agent</th>
<th>Size of AgNPs/(nm)</th>
<th>Linear range/(µM)</th>
<th>LOD/(µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaBH₄</td>
<td>~10</td>
<td>20-120</td>
<td>0.32</td>
</tr>
<tr>
<td>Glucose</td>
<td>~40</td>
<td>0-60</td>
<td>0.12</td>
</tr>
<tr>
<td>Sodium Citrate</td>
<td>~30</td>
<td>0-50</td>
<td>0.013</td>
</tr>
<tr>
<td>Vc</td>
<td>~20</td>
<td>0-50</td>
<td>0.018</td>
</tr>
</tbody>
</table>

From **Fig. S2**, we could see that the stronger the reducing ability of the reducing agent, the smaller the average particle size of the synthesized AgNPs. **Table S1** showed that the smaller the
particle size of AgNPs, the lower the LOD of Hg$^{2+}$. The reason is that the particles are small, and the stability is not good, so low concentration of Hg$^{2+}$ will cause aggregation of AgNPs.

9. Finally, there are different ways of connecting silver nanoparticles to carboxymethylcellulose. The authors are using non-covalent electrostatic interactions. It would benefit this paper to investigate other mechanisms of attachment and how the detection performance changes based on the differences in the attachment mechanisms provided.

Answer: Thanking for giving the precious comment to our research work. This is a constructive comment, which has pointed the direction for the further investigation.

10. Overall, while the paper is interesting and the idea is good, there are some minor issues that need to be addressed. Moreover, there are some more major issues around the lack of control experiments that also reduce my overall enthusiasm for this manuscript as currently written.

Answer: Thanking for giving the precious comment to our research work. We have conducted a series of control experiments, such as the proportion of reactants, the choice of reductant or some related measurements, the detail analysis is shown in the manuscript.
Fig. 2 UV–vis spectra of CMS/AgNPs: (A) Different volumes of Tollens’ reagent (0.1M), (B) Different proportion of Vc (C) Different reducing agents and (D) reduction stability of Vc

Fig. 2 is the optimal screening of the experiment. The absorbance of AgNPs increases with the increase of the amount of silver ions in Fig. 2A. The absorbance of AgNPs decreases and red shift occurs in 200 μL Tollens’ reagent, indicating that the nano-silver particles become larger at a higher concentration, so the best amount is 100 μL Tollens’ reagent. The absorbance of AgNPs increases with the increase of the amount of Vc in Fig. 2B. The absorbance of AgNPs decreases and red shift occurs in 200 μL Tollens’ reagent, indicating that the nano-silver particles become larger at a higher concentration, so the best amount is 100 μL Tollens’ reagent. Fig. 2C is UV–vis spectra of CMS/AgNPs by synthesized with different reducing agents, indicating that Vc is weaker in reducing ability than NaBH₄, but stronger than glucose and sodium citrate. The latter two require a catalyst for the reaction to synthesize AgNPs. The synthesized AgNPs with NaBH₄ was weaker in stability than Vc, so Vc was the best and green reducing agents. The stability of the synthesized AgNPs is optimal in Fig. 2D. We can see from Fig. 2 that the best experimental conditions are CMS aqueous solution (0.1% [w/v], 100 mL) and 1:1 molar ratio of Tollens’ reagent (0.1 M, 100 μL) to Vc (0.1 M, 100 μL). Further, the experiments show that Vc is weaker in reducing ability than B, but stronger than glucose and sodium citrate. The latter two require a catalyst for the reaction.

Fig. 3C shows the FT-IR spectra of CMS and CMS/AgNPs. For CMS/AgNPs, the band centered at around 3456 cm⁻¹ can be attributed to the stretching vibration of hydroxyl group; the band at about 2885 cm⁻¹ is assigned to the C–H group; the band at around 1605 cm⁻¹ is attributed to the stretching vibration of carboxyl group; the band at around 1396 cm⁻¹ is corresponded to the C–H bending mode; the absorption band at 1079 cm⁻¹ is ascribed to C–O–C stretching mode from the glucosidic units; the peak at 612cm⁻¹ was related to the deformation vibration of hydrogen bond. Fig. 3C shows the FT-IR spectra of CMS which is similar to the CMS/AgNPs, indicating that there is no chemical reaction between the formed AgNPs and CMS.