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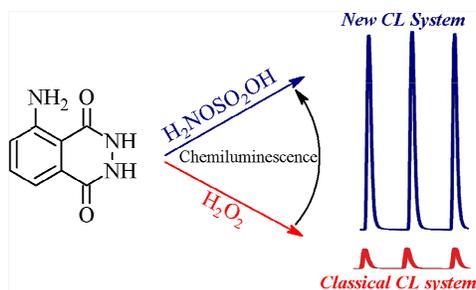
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ARTICLE TYPE

## Hydroxylamine-*O*-sulfonic acid as an efficient coreactant of luminol chemiluminescence for selective and sensitive detection

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Hydroxylamine-*O*-sulfonic acid, a versatile reagent for organic synthesis, has been explored as an effective coreactant for luminol chemiluminescence. Co<sup>2+</sup> can dramatically enhance chemiluminescence of this system. The sensitive detection of Co<sup>2+</sup>, luminol and HOSA, including highly selective Co<sup>2+</sup> detection, are achieved.

The chemiluminescence (CL) is a phenomenon in which chemically-generated molecules emit light in excited states.<sup>1</sup> It is a very powerful analytical technique because of its inherent features, such as better sensitivity, rapidity, wide dynamic range, controllable emission rate, relatively simple equipment, and low instrumentation costs because no external light source or optics are needed.<sup>2-4</sup> Among many CL luminophores, luminol has attracted tremendous scientific interest owing to its low cost, favorable properties, compatibility with large number of analytes and broad range of applications.<sup>5-17</sup> Luminol CL is one of the most popular and efficient CL system for metal ion determination,<sup>7,8</sup> immunoassays,<sup>9</sup> DNA probes and enzymatic biosensors,<sup>10-13</sup> microarrays,<sup>14</sup> cancer detection and therapy,<sup>13,14</sup> detection of free radicals,<sup>15</sup> forensic identification,<sup>16</sup> and pharmaceutical analysis.<sup>17</sup>

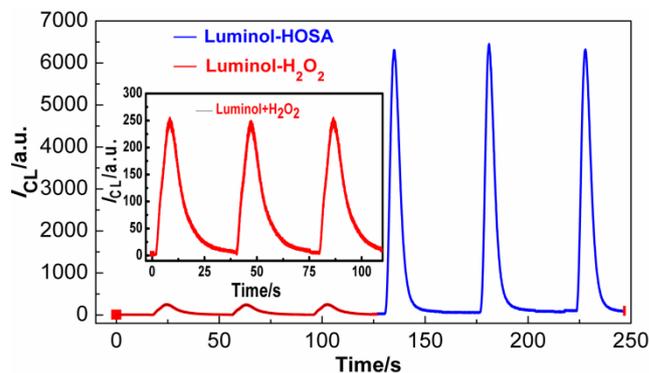
The luminol CL system needs some oxidants such as hydrogen peroxide, oxygen, potassium permanganate, ferricyanide, periodate, oxygen free radicals and hypochlorites<sup>5,18</sup> as well as some activators such as peroxidase, catalase, xanthine oxidase, cytochrome *c*, haemoglobin, deuterohemin, molecular ozone, halogens, persulphate anion and transition metal cations and their complexes to produce CL.<sup>7</sup> Since 1928,<sup>19</sup> the luminol-H<sub>2</sub>O<sub>2</sub> CL reaction has been most intensively studied and widely used,<sup>20</sup> which can be catalyzed by various substances including metal ions, metal complexes, peroxidases, and so on.<sup>21</sup> But H<sub>2</sub>O<sub>2</sub> is instable and can react with many metal ions and their complexes, resulting in poor selectivity.<sup>7</sup> There has been a continuing interest in the development of new coreactants for luminol CL, which can overcome these limitations and widen the application of the luminol CL.

Hydroxylamine-*O*-sulfonic acid (HOSA) is a versatile and stable synthetic reagent in preparative organic chemistry.<sup>22-24</sup> The electrophilic properties of this reagent are observed under basic conditions, while the nucleophilic character is evident under neutral or acidic conditions.<sup>23</sup> It decomposes in alkaline conditions to generate a diimide species, nitrogen and ammonia.<sup>25</sup> It has been extensively used in the synthesis of dye, pharmaceutical and pesticide intermediates.<sup>26</sup> Other important areas of application of HOSA are amination and reductive

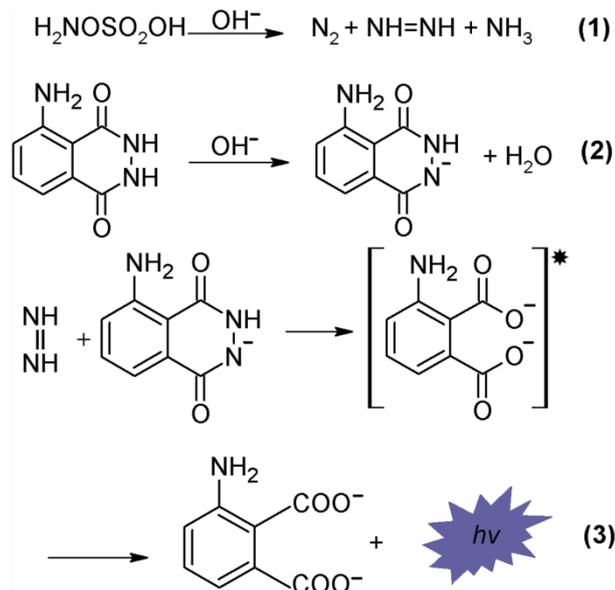
deamination reactions, nitrile and oxime formation, and the preparation of amides and diazo compounds. HOSA can also be used for the synthesis of large variety of heterocycles and related derivatives.<sup>24</sup> The heterocyclic hydroxylamine-*O*-sulfonates have significant potential for use in synthesis of anticancer, antiviral, and antimicrobial agents.<sup>27</sup>

In this study, HOSA is exploited as an effective and stable coreactant of luminol CL. It can react with luminol to produce strong CL signals in the absence of any other activators. The CL intensities can be significantly enhanced by Co<sup>2+</sup>. The luminol-HOSA system was used to detect Co<sup>2+</sup>, luminol and HOSA with excellent sensitivity. Noteworthy, the method exhibits excellent selectivity for the detection of Co<sup>2+</sup>.

Fig. 1 shows the CL intensity-time curves of the luminol-HOSA system and the luminol-H<sub>2</sub>O<sub>2</sub> system. By comparison, the CL peak intensity of the luminol-HOSA system is about 25 times higher than that of the luminol-H<sub>2</sub>O<sub>2</sub> system. It indicates that HOSA is an effective coreactant for luminol CL. As shown in Fig. S1 (ESI<sup>†</sup>), the maximum CL emission wavelength is about 450 nm, consistent with the typical spectrum of luminol.<sup>28</sup> It has already been reported that HOSA can decompose and generate diimide species in alkaline solutions (eq. (1) in Scheme 1).<sup>24, 29</sup> These diimide species can react with luminol efficiently to produce strong CL as shown in eq. (3) of Scheme 1.

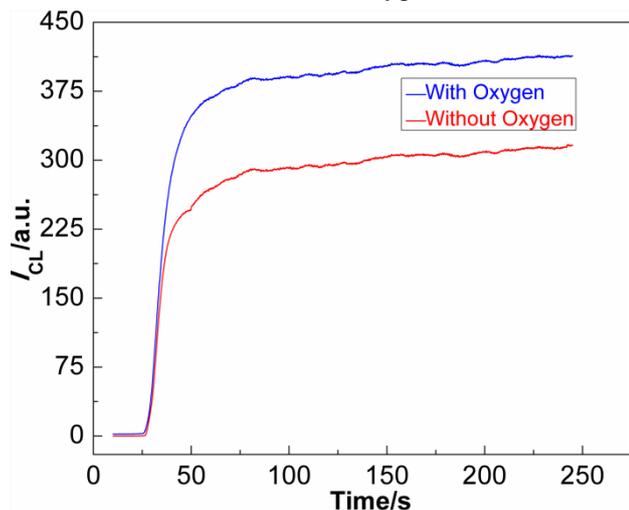


**Fig. 1** The CL intensity-time curves for the luminol-H<sub>2</sub>O<sub>2</sub> (red line) and luminol-HOSA systems (blue line). Inset: enlarged CL intensity-time curve for the luminol-H<sub>2</sub>O<sub>2</sub> system. [luminol]: 10 μM; [HOSA]: 1 mM; [H<sub>2</sub>O<sub>2</sub>]: 1 mM; photomultiplier tube voltage: 850 V



**Scheme 1** Reaction mechanism for the luminol-HOSA CL system

Since oxygen can react with luminol to generate CL under some conditions,<sup>30</sup> the effect of oxygen on the luminol-HOSA CL system has been investigated. The testing solutions, the luminol solution and the HOSA solution, were purged with nitrogen for 30 min. As shown in Fig. 2, the CL intensity decreases only about 19% for luminol-HOSA CL system when the dissolved oxygen was removed from the solutions by the purge of nitrogen. It shows that oxygen is not necessary for the generation of CL of the luminol-HOSA CL system. These results also indicate that the dissolved O<sub>2</sub> can enhance CL intensity to some degree.<sup>31</sup> It has been reported that the yield of diimide may be less if oxygen is excluded.<sup>32</sup> The generation of less diimide species may lead to the decrease in CL in the absence of oxygen.



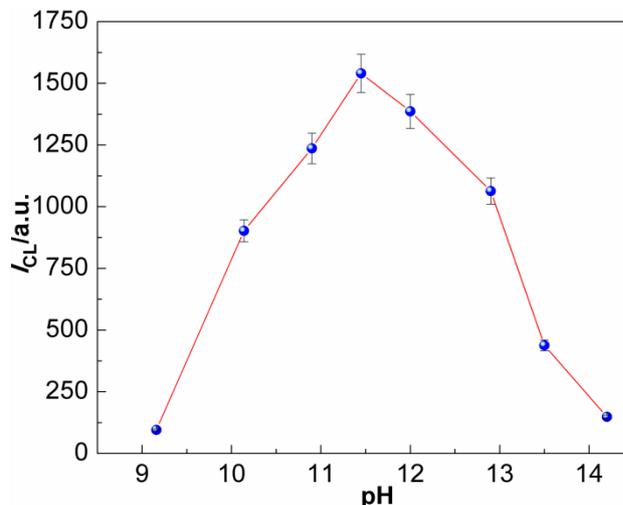
**Fig. 2** The CL intensity-time curves in the absence (red line) and presence (blue line) of oxygen. [luminol]: 10  $\mu\text{M}$ ; [HOSA]: 2 mM; photomultiplier tube voltage: 700 V.

According to the CL-generating mechanism for luminol oxidation, basic conditions are favorable for the CL.<sup>33</sup> Thus, the effect of the pH on the CL intensity was investigated under basic pH conditions. As shown in Fig. 3, the CL intensities increase with increasing pH from 9.14 to 11.45, and then decrease as the pH increases further. The increase in CL intensity with increasing

pH from 9.14 to 11.45 is attributed to the faster generation of effective diimide species from HOSA at higher pH and the deprotonation of luminol. When the pH becomes higher than 12, the decomposition of HOSA may generate excessive nitrogen, ammonia and sulfate ions but less reactive diimide species.<sup>24</sup> The generation of less reactive diimide species results in the decrease in CL intensity.

This system was used for the detection of HOSA. The CL intensity has a good linear relationship over HOSA concentrations from 1 to 2000  $\mu\text{M}$  with a correlation coefficient ( $r$ ) of 0.9978 (Fig. S2, ESI<sup>†</sup>). The linear equation is  $I = 11.26 + 0.94c$  (where  $c$  is the concentration in  $\mu\text{M}$ ). The limit of detection (LOD) for HOSA is calculated to be 0.97  $\mu\text{M}$ . The method exhibits good reproducibility with a relative standard deviation (RSD) of 4.73% for nine consecutive determinations of 100  $\mu\text{M}$  HOSA. To the best of our knowledge, it is the first report about the detection of HOSA.

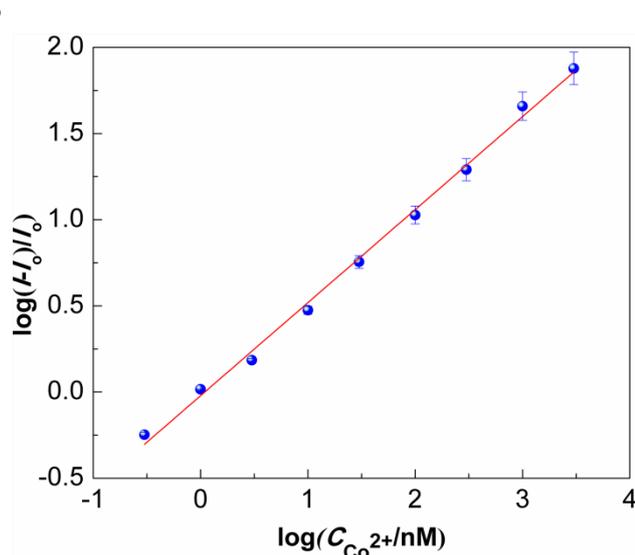
The luminol-HOSA system can detect luminol with excellent sensitivity. Moreover, the logarithm of CL intensity ( $\log I$ ) has good linear relationship with the logarithm of concentrations of luminol ( $\log c$ ) from 0.1 to 3000 nM (Fig. S3, ESI<sup>†</sup>). The linear equation is  $\log I = 1.98 + 0.95 \log c$  (where  $c$  is the concentration in nM) ( $r = 0.9942$ ). The LOD is 0.03 nM, which is comparable to the best LOD of other reported luminol detection methods.<sup>7, 34</sup> The RSD for nine consecutive measurements of 500 nM luminol is 3.89%.



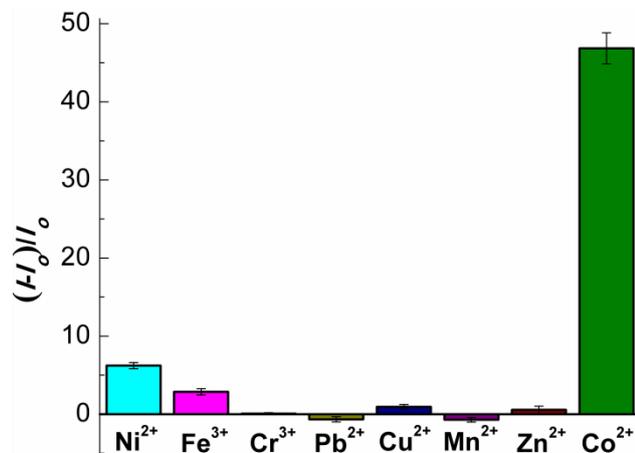
**Fig. 3** Effect of pH on the CL intensities. [luminol]: 10  $\mu\text{M}$ ; [HOSA]: 1 mM; pH: 9.2, 10.1, 10.9, 11.5, 12.0, 12.9, 13.5, 14.2; photomultiplier tube voltage: 800 V.

The luminol/H<sub>2</sub>O<sub>2</sub> CL reaction can be triggered through a wide range of transition metal cations as catalyst with mitigated performances. Co<sup>2+</sup> is one of the most popular transition metal cations that can promote and enhance luminol/H<sub>2</sub>O<sub>2</sub> CL intensities. So the effect of Co<sup>2+</sup> on the CL intensity of the luminol-HOSA system was investigated. The results show that Co<sup>2+</sup> can significantly increase the CL intensity of the luminol-HOSA system, which is then utilized to detect Co<sup>2+</sup>. Fig. 4 shows the linear calibration curve for Co<sup>2+</sup>. The CL enhancement efficiency of  $\log(I-I_0)/I_0$  has a linear relationship over log concentrations ( $\log c$ ) of Co<sup>2+</sup> from 0.3 to 3000 nM with a linear equation of  $\log(I-I_0)/I_0 = -0.02 + 0.54 \log c$  (where  $c$  is the concentration in nM) ( $r = 0.9960$ ). The LOD is 0.13 nM. The RSD ( $n = 9$ ) for the detection of 100 nM Co<sup>2+</sup> is 4.35%. In comparison with other reported CL Co<sup>2+</sup> detection methods, our method is very sensitive and simple (Table.S1 ESI<sup>†</sup>).<sup>35-42</sup> The

detection of  $\text{Co}^{2+}$  in the absence of HOSA was also investigated. The CL intensities in the absence of HOSA are extremely weak. The linear range is 10-3000 nM and the LOD is 3.0 nM in the absence of HOSA. By comparison, the use of HOSA can significantly improve sensitivity. Moreover, CL intensity in the presence of HOSA is much stronger than CL intensity in the absence of HOSA. The much stronger CL intensity in the presence of HOSA will enable the use of low cost and portable detectors.



**Fig. 4** Linear calibration curve for  $\text{Co}^{2+}$ .  $I$  represents the CL intensity of the system after the addition of  $\text{Co}^{2+}$ ,  $I_0$  represents the CL intensity of the control sample and  $(I-I_0)/I_0$  represents the CL enhancement efficiency after the addition of  $\text{Co}^{2+}$ . [HOSA]: 2 mM; [luminol]: 10  $\mu\text{M}$ ; [ $\text{Co}^{2+}$ ]: 0.3, 1, 3, 10, 30, 100, 300, 1000, and 3000 nM; photomultiplier tube voltage: 600 V.



**Fig. 5** Selectivity for the detection of  $\text{Co}^{2+}$ .  $I$  represents the CL intensity of the system after the addition of metal ions,  $I_0$  represents the CL intensity of the control sample and  $(I-I_0)/I_0$  represents the CL enhancement efficiency after the addition of metal ions. The concentrations of all the metal ions are 10  $\mu\text{M}$ . [HOSA]: 2 mM; [luminol]: 10  $\mu\text{M}$ ; photomultiplier tube voltage: 600 V.

The detection of metal ions by conventional luminol CL methods experiences easy interference from other metal ions. It is thus desirable to develop a selective method for metals ion detection. To assess the selectivity of the new CL system, the effects of typical common metal ions were investigated. As

shown in Fig. 5, the CL intensity increases dramatically in the presence of  $\text{Co}^{2+}$ . In contrast, the CL intensity changes negligibly in the presence of other metal ions. The results suggest that the  $\text{Co}^{2+}$  detection method based on the luminol-HOSA system has excellent selectivity. The remarkable selectivity makes the luminol-HOSA system distinctive from other luminol systems that generally do not show good selectivity.<sup>43</sup>

In conclusion, this work has exploited a new and efficient coreactant HOSA for luminol CL reaction. The CL intensity of the luminol-HOSA system can be significantly enhanced by  $\text{Co}^{2+}$ . The newly developed luminol-HOSA CL system enables the highly sensitive detection of luminol, HOSA, and  $\text{Co}^{2+}$ . Moreover, the luminol-HOSA CL system exhibits excellent selectivity for the detection of  $\text{Co}^{2+}$  against other transition metal ions. The remarkable selectivity against other transition metal ions makes the luminol-HOSA CL system attractive for a broad range of applications. It is expected that HOSA is a versatile coreactant for other CL systems and may find broad applications in CL chemical/biological sensing.

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## Notes and references

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- † Electronic Supplementary Information (ESI) available: Experimental section, supplementary figures and table. See DOI: 10.1039/b000000x/
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