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Organo-modified layered double hydroxide-catalyzed Fenton-like ultra-weak chemiluminescence for specific sensing of vitamin B₁₂ in egg yolks

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ABSTRACT

In general, the chemiluminescence (CL) sensing of vitamin B₁₂ is achieved by determining Co(II) liberated from acidified vitamin B₁₂ by a luminol system. However, the luminol system for sensing vitamin B₁₂ has poor selectivity due to serious interference from other metal ions. In this study, as a novel CL amplifier of the Co(II)+H₂O₂+OH⁻ ultra-weak CL reaction (Fenton-like system), dodecylbenzene sulfonate (DBS)-layered double hydroxides (LDHs) have been applied to the specific determination of vitamin B₁₂ by liberating Co(II). The CL intensity increased with increasing the concentration of vitamin B₁₂ in a wide range from 1.0 ng mL⁻¹ to 5 μg mL⁻¹ with a detection limit of 0.57 ng mL⁻¹ (S/N=3). The proposed method has been successfully applied to determine vitamin B₁₂ in egg yolk with simple procedures, shorter time and higher selectivity. Recoveries from spiked real samples were 96–103%. The results of the proposed method for sensing vitamin B₁₂ in real samples were agreed with those obtained by the standard inductively coupled plasma mass spectrometry (ICP-MS) method. To the best of our knowledge, this is the first report on the CL sensing of vitamin B₁₂ with high selectivity in the absence of luminol.

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1. Introduction

Vitamin B₁₂ is an essential nutrient linked to human growth and cell development [1]. The daily requirement of vitamin B₁₂ is relatively low in comparison to other vitamins, and thus exposure to excessive amounts of vitamin B₁₂ can induce asthma and folic acid deficiency [2]. On the other hand, its deficiency is associated with a variety of disorders and human diseases, such as weakness, fatigue, renal dysfunction, diabetes, pernicious anemia and nerve degeneration [3]. It is well known that vitamin B₁₂ must be obtained through diet because it cannot be synthesized in the human body [4]. Acting as a nutrient-dense food, eggs contain a substantial amount of various essential vitamins, and thus they are considered as an important source of vitamin B₁₂, in which vitamin B₁₂ is mainly found in egg yolks [5]. As a result, the accurate measurement of vitamin B₁₂ in egg yolks is desirable for research purposes and routine clinical use.

The classical analytical techniques for quantifying vitamin B₁₂ currently include microbiological [6], spectrophotometric [7],

fluorescent [8], inductively coupled plasma mass spectrometry (ICP-MS) [9], and electrochemical methods [10]. Nowadays, a chemiluminescence (CL) technique for the determination of vitamin B₁₂ is becoming a promising technique with reliability, fast response, cheap instrument, simple operation and high sensitivity [11–15]. Generally, the quantification of vitamin B₁₂ could be achieved by determining Co(II) liberated from acidified vitamin B₁₂ using the luminol CL system. However, these CL methods suffered from the low selectivity due to serious interference from other metal ions. Therefore, it is highly desired to employ the other CL systems in the absence of luminol for improving the CL selectivity towards vitamin B₁₂. To the best of our knowledge, the specific and precise CL measurements of vitamin B₁₂ are not available except for the luminol system at present. As a result, it is still an active field as well as a great challenge to attain this goal.

Layered double hydroxides (LDHs) are an important class of host-guest layered nanomaterials consisting of positively charged metal hydroxide sheets with charge-balancing intercalated anions and water molecules [16,17]. LDHs exhibit a well-defined layered structure with relatively large surface area, high porosity, high layer charge density and interlayer anion mobility [18]. Therefore, LDHs have been extensively employed as catalysts, ion exchangers, and adsorbents [19–22]. Recently, we have found that the galleries

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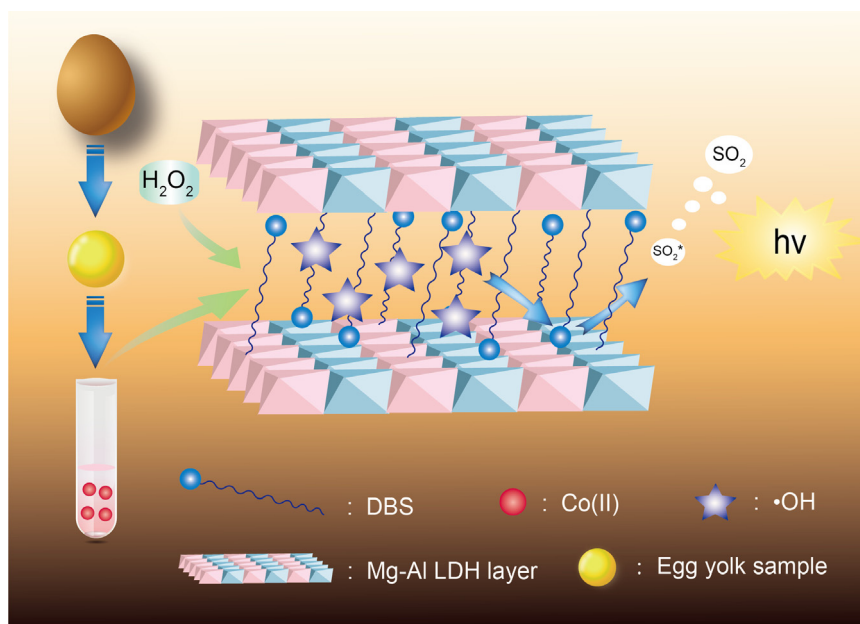


Fig. 1. Possible mechanism for the DBS-LDH-Co(II)+ H_2O_2 + OH^- system.

of the LDH could lead to the concentration of the CL reactants/intermediates on the surface or in the interlayer of the LDHs, facilitating the occurrence of the CL reactions [23,24]. It has been reported that the LDHs can enhance the CL signals of some ultra-weak CL systems, such as the IO_4^- - H_2O_2 system, and peroxytrifluoromethane system [25–27]. Subsequently, the LDH-amplified ultra-weak CL emissions were successfully used for a wide variety of analytes in environmental and food samples [28–30].

Recently, we reported that organo-modified LDHs (*i.e.*, dodecylbenzene sulfonate DBS-modified LDHs) could significantly enhance an ultra-weak CL from Fenton-like reaction ($Co(II)+H_2O_2+OH^-$) [31]. The DBS-modified LDH-induced CL enhancement of Fenton-like reaction was ascribed to the hydrophobic microenvironment of the intercalated DBS in LDHs, facilitating the formation of reaction intermediates. In this study, we tried to detect $Co(II)$ liberated from acidified vitamin B_{12} using the DBS-modified LDH-catalyzed Fenton-like CL system (Fig. 1). It was found that the CL intensity was proportional to the log concentration of vitamin B_{12} in the range from 1.0 ng mL^{-1} to $5\text{ }\mu\text{g mL}^{-1}$, and the limit of detection ($S/N=3$) was found to be as low as 0.57 ng mL^{-1} . Therefore, a rapid, selective, sensitive and simple CL method to assay vitamin B_{12} was successfully developed. Validation of the proposed method was checked by determining vitamin B_{12} in egg yolks. To the best of our knowledge, here is the first example for CL sensing of vitamin B_{12} with high selectivity in the absence of luminol.

2. Experimental

2.1. Reagents

Analytical grade chemicals including NaOH, $Mg(NO_3)_2 \cdot 6H_2O$, $Al(NO_3)_3 \cdot 9H_2O$, HCl, HNO_3 , H_2O_2 , $FeSO_4 \cdot 7H_2O$, $FeCl_3$, $CuCl_2 \cdot 2H_2O$, $MnSO_4 \cdot H_2O$, $Ni(NO_3)_2 \cdot 6H_2O$, $Pb(NO_3)_2$, $CrCl_3 \cdot 6H_2O$, and $ZnCl_2$ were purchased from Beijing Chemical Reagent Company (Beijing, China). Uranine was purchased from Acros. Sodium dodecyl benzene sulfonate (SDBS) was purchased from Tokyo Chemical Industry Co. Ltd. Pure crystalline vitamin B_{12} was purchased from Beijing HWRK Chem Co. Ltd. Working solutions of H_2O_2 were prepared daily from 30% (*v/v*) H_2O_2 . A stock solution of vitamin B_{12}

(1.0 mg mL^{-1}) was prepared by dissolving 0.100 g of crystalline vitamin B_{12} in 100 mL deionized water. All reagents were of analytical grade and used without further purification. All solutions were prepared with deionized water (Milli Q, Millipore, Barnstead, CA, USA).

2.2. Apparatus

The powder X-ray diffraction (XRD) measurement was performed on a Bruker (Germany) D8 ADVANCE X-ray diffractometer equipped with graphite-monochromatized $Cu/K\alpha$ radiation ($\lambda=1.5406\text{ \AA}$). The 2θ angle of the diffractometer was stepped from 2° to 70° at a scan rate of $0.02^\circ/s$. The particle sizes and external morphology of the samples were observed on a transmission electron microscope (TEM, Tecnai G220, FEI Company). Scanning electron microscopy (SEM) was measured on a Hitachi (Japan) S-4700 field-emission scanning electron microscope. The fluorescence spectra were obtained using a F-7000 fluorescence spectrophotometer (Hitachi, Tokyo, Japan) at a scanning rate of 1500 nm/min . The excitation slit and the emission slit were maintained at 5.0 nm and 2.5 nm , respectively. The CL detection was conducted on an Ultra-Weak Luminescence Analyzer, which was purchased from institute of Biophysics, Chinese Academy of Science, Beijing, China (Biophysics Chemiluminescence, BPCL). An Agilent 7700 ICP-MS system (Agilent Technologies, Santa Clara, CA) was used to detect the $Co(II)$ in egg yolks.

2.3. Synthesis of Mg-Al- NO_3 LDHs

The Mg-Al- NO_3 LDHs with different Mg/Al molar ratios were obtained by a co-precipitation method. The precipitation process was taken under low supersaturation conditions at constant pH. For Mg/Al molar ratio of 3, the salt solution (80 mL) containing $Mg(NO_3)_2 \cdot 6H_2O$ (0.06 mol) and $Al(NO_3)_3 \cdot 9H_2O$ (0.02 mol) was added dropwise to a 250 mL four-necked flask under vigorous stirring at room temperature, the pH value was adjusted to 10 with 2.0 M NaOH. The resulting white precipitate was continually stirred for 24 h at 65°C . The whole reaction process was purged with N_2 throughout the experiment to avoid carbon dioxide uptake. The products were centrifugated, washed with degassed and deionized

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