Rhodamine B Chemiluminescence Improved by Mimetic AuCu Alloy Nanoclusters and Ultrasensitive Measurement of H$_2$O$_2$, Glucose and Xanthine

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The less stability and robustness, high-cost preparation and maintenance of natural enzymes, especially horseradish peroxidase (HRP), challenge researchers to introduce effective alternatives for their wide applications. Herein, the peroxidase-like activity of AuCu bimetal nanoclusters (AuCu NCs) was investigated in the rhodamine B-H$_2$O$_2$ chemiluminescence (CL) system. AuCu NCs could effectively catalyzed the CL reaction, and a high intensive emission intensity was obtained. A comprehensive study was implemented to examine the effects of different stabilizing ligands and Au/Cu ratios on the catalytic activity of obtained NCs. Comparison experiments were also expanded to include Au and Cu nanoparticles with different sizes, too. The results verified the superior catalytic activity of penicillamine-stabilized AuCu bimetal NCs containing 50% cu atoms. Finally, the analytical application of the introduced CL system showed great sensitivity for H$_2$O$_2$ detection, with a detection limit of 0.13 nM. Moreover, the developed CL method was able to measure glucose and xanthine over wide concentration ranges of 0.1 - 400 and 0.1 - 200 μM, respectively. The method also indicated satisfactory reliability, confirmed by standard reference materials.

Keywords Bimetal nanoclusters, chemiluminescence, non-enzyme mimetic, analytical application

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Introduction

The recognition of hydrogen peroxide (H$_2$O$_2$) is recently a challenging topic for researchers in biological, medical and environmental areas. H$_2$O$_2$ plays a crucial role in the detection of some biomolecules, which is the reason why H$_2$O$_2$ is vastly of interest by analysts. In the presence of oxygen, some biological molecules (like glucose) are oxidized by their specific enzymes (glucose oxidase), resulting in the generation of H$_2$O$_2$. The concentration of produced H$_2$O$_2$ can be determined by different detection systems and directly linked to the concentration of biomolecules. This process has been applied to the design of many biosensors for the determination of various important analytes, for example glucose, xanthine, cholesterol, organophosphates, etc. It has also gained general acceptance and is applied in clinical centers. H$_2$O$_2$ detection techniques are mostly based on its oxidation ability to react with specific substrates and produce measureable materials. Horseradish peroxidase (HRP) is commonly used as catalyst in these reactions. For example, the H$_2$O$_2$ reaction with o-phenylene-diamine dihydrochloride (OPD) occurs in the presence of HRP, forming a yellow-orange product (2,3-diaminophenazine, DAP), which can be calorimetrically detected. Also, H$_2$O$_2$ can oxidize terephthalic acid (TA) to 2-hydroxyterephthalic acid (2-HTA), which emits a highly intensive fluorescence emission ($\lambda_{em} = 315$ nm and $\lambda_{em} = 425$ nm). HRP is necessary for this reaction, too. Introducing a highly efficient catalyst as an alternative for HRP is the main challenging issue, because of its expensive preparation and reservation, low stability and other deficiencies of natural enzymes. Nowadays, researchers witness the presence of nanomaterials with enzyme-like behaviors, indicating great advantages over other artificial enzymes, such as high stability, simple and low-cost synthesis, flexible catalytic properties, high surface area and facile preservation. Due to the individual properties of nanomaterials, they have been interesting materials in various fields, especially for catalysis applications. They have also attracted great attention as peroxidase-mimetic materials, applied to design promising sensing systems.

Chemiluminescence (CL) based methods have desirable analytical features, and are commonly preferable to design simple, low-cost and on-site analytical devices. CL detection methods are famous for their high sensitivity and wide dynamic linear ranges. Nowadays, researchers are interested in using nanomaterials with unique properties to improve the CL systems. Nanomaterials can play the role of emitters, catalysts or energy acceptors, improving the CL reactions and resulting in an enhanced emission. In recent times, various nanomaterials have been extensively utilized in CL-based detection systems to detect important analytes.

Metal nanoclusters (NCs) with their ultra-small sizes are very interesting nanomaterials for research. The individual and
size-tunable electronic and optical features, and also the quantum confinement effects of NCs, make them a preferred choice for analytical investigations. On the other hand, the very small size of NCs causes a strong dependency between their different properties and atom types in the cluster structure. This means that substituting just an atom in nanoclusters building may strongly change their properties. Recently, valuable studies in analytical areas have been done by bimetal NCs. Especially, some research groups have showed improved catalytic activity of Au-based bimetal NCs compared with pure Au NCs. Recently, a remarkably improved CL system has been reported using AuCu bimetal NCs. It was found that the weak CL emission of rhodamine B (RhoB)–H₂O₂ reaction intensified after the addition of AuCu NCs. The colorimetric experiments verified the great peroxidase mimetic activity of these NCs. A comprehensive comparison was carried out to investigate the effect of different capping agents, and also the effect of different ratios of Au/Cu in the structure of NCs. The results showed the superior peroxidase-like catalytic activity of penicillamine (PA)-capped AuCu NCs, containing 50% Cu atoms. There are some reports on modifying the catalytic activity of Au NCs by ligand engineering tactic. Moreover, the analytical application of presented nano-enzyme was examined for the detection of H₂O₂. Using the CL detection system, an exceptional sensitivity was obtained for H₂O₂. Afterwards, the presented method was used for measuring glucose and xanthine concentrations in real blood samples (Scheme 1), and the reliability of obtained results was validated by a standard reference material (SRM).

### Experimental

#### Reagents and chemicals

All utilized chemicals were obtained in analytical grade. Hydrogen peroxide (H₂O₂), glucose, o-phenanthroline (OPD), tetrachloroauric acid (HAuCl₄), albumin, sodium dihydrogen phosphate and sodium acetate were bought from Merck (Germany). Xanthine, xanthine oxidase (XOD) glucose oxidase (GOx), sodium dodecyl sulfate (SDS), terephthalic acid (TA), penicillamine (Pen) and glutathione (GSH) were purchased from Sigma. Deionized water (DI) was utilized for all experiments.

#### Apparatus

CL analyses were conducted using smart Sirius L luminometer (Berthold, Germany). The UV-visible absorption and fluorescence spectra were recorded by UV-1800 spectrophotometer and RF-5301 spectrofluorometer (Shimadzu, Japan), respectively. CL spectra were also obtained using the same spectrofluorometer, in a flow system. Characterization analyses were carried out to confirm the morphology and chemical structure of the synthesized nanoclusters, using a Mirax field-emission scanning electron microscope (FE-SEM, Tescan, Czech Republic), a JEM-2200FS transmission electron microscope (TEM, JEOL, Japan), D5000 X-ray diffraction (XRD, Siemens, Germany) spectrometer and a Tensor 27 Fourier-transform infrared (FT-IR, Bruker, Germany) spectrometer.

#### Synthesis of AuCu bimetal nanoclusters

Synthesis of AuCu NCs stabilized by PA was performed according a recently reported method. HAuCl₄ (150 μL, 5 mM) and PA (900 μL 30 mM) solutions were mixed together and vigorously stirred for 2 min. Next, Cu²⁺ solution (150 μL, 5 mM) prepared in nitric acid (0.1 M) was added to the mixture, and stirring was continued for another 2 min. The addition of copper precursor led to a color change from brown to white milky, verifying the generation of PA-AuCu NCs. The solution was shaken for 2 h (25°C, and dark media), and then, centrifuged (3000g) to remove any unreacted reagents. Afterwards, the precipitated NCs were re-dispersed in 1 mL DI and preserved in 4°C. This process led to the production of NCs with ~50% Cu atoms. NCs with different Au/Cu ratios (0, 25, 75, 100%) were synthesized using different amounts of Cu²⁺ solution. The synthesized NCs were specified by PA-Au NCs, PA-AuCu25 NCs, PA-AuCu50 NCs, PA-AuCu75 NCs, PA-Cu NCs, respectively, where the numbers in NCs names show the Cu percent applied for their synthesis. The synthesized NCs were satisfactorily stable at least for 8 weeks.

On the other hand, different stabilizing agents (including BSA, GSH) were applied for synthesizing NCs, to compare their properties. Besides, some citrate-capped Au and Cu nanoparticles (NPs) in different sizes were also synthesized for comparison experiments. The synthesis processes were according to reported references.

#### Colorimetric studies

The OPD-H₂O₂ reaction was applied for a colorimetric study.
of the NCs mimetic activity. Briefly, PA-AuCu NCs or a pure Au/Cu NCs solution (250 μL) and a H₂O₂ solution (0.2 mM, 250 μL) were respectively added to a 2-mL reaction solution (0.05 M acetate buffer, pH 4, containing 2.5 mM OPD). Then, the volume of the mixed solution was increased to 5 mL, by DI (2.5 mL), and gently (10 min) shaken to complete the reaction. Finally, the absorbance of solution was recorded at λ = 417 nm.

Chemiluminescence spectra

The CL-wavelength spectra were recorded using a flow-based system including two polytetrafluoroethylene channels (inner diameter = 1 mm) and a glass flow-cell (volume = 200 μL) in front of the emission monochromator (slit = 20 nm) of spectrofluorometer. The mixed solution of RhoB (0.5 mM), SDS (0.5 mM) and NaOH (0.01 M) with or without AuCu NCs via the first channel, and H₂O₂ solution (1 μM) via the second channel were delivered (10 mL min⁻¹) into the flow-cell. The radiation source was turned off, and the emission monochromator was scanned in the range of 400 – 700 nm.

Chemiluminescence experiments

Glucose enzymatic oxidation. A constant volume (50 μL) of a standard glucose solution in different concentrations (or prepared serum sample) was mixed with a 250-μL acetate buffer solution (0.05 M, pH 4.4) containing 60 U mL⁻¹ GOx. To complete the reaction of glucose and dissolved O₂, the solution was incubated for 10 min (37°C). Then, the produced H₂O₂ was measured by the CL detection system, after some dilution steps using DI.

H₂O₂ determination. The detection of H₂O₂ was done using a flow-based CL system and a RhoB-H₂O₂ reaction catalyzed by PA-AuCu50 NCs (Fig. S1, Supporting Information). The flow system contained four channels (polytetrafluoroethylene tubes with an inner diameter of 1 mm) and a CL flow-cell (100 μL) in front of the detector (photomultiplier, PMT) of luminometer. A RhoB (0.3 mM), SDS (0.2 mM) and NaOH (0.01 M) solution (channel a) was mixed with a PA-AuCu50 NCs catalyst solution (channel b), and was then directly transferred to a CL cell. DI as carrier (in channel c) was transported via a six-port valve having a 100-μL loop. And a H₂O₂ solution (channel d) was collected in the loop (valve is in loading mode). By turning the valve to the injection mode, the H₂O₂ solution stored in the loop was carried by the carrier stream toward the cell. The generated CL emission from the RhoB-H₂O₂ reaction was recorded during the time, and the maximum intensity was used as analytical signal.

Blood samples

Human blood samples were taken from healthy volunteers and centrifuged (8000 rpm) for 25 min). Then, the serum samples were diluted (1:50000) by a 5 mM phosphate buffer solution (pH 7), and a proper volumes of them were used for analysis aims.

Results and Discussion

Penicillamine-stabilized AuCu bimetal nanoclusters

The starting point for the preparation of AuCu bimetal NCs is an interaction between PA ligands and Au³⁺ cations, forming colorless PA-Au(I) complexes. After the addition of Cu²⁺, PA-Au(I)Cu(I) bimetal complexes are produced and, subsequently excess PA reduces these complexes to AuCu NCs. Moreover, PA molecules attach on the surface of formed NCs and play as stabilizing agents. This effect prevents further growing or aggregation of NCs. In this section, the characterization
analyses of obtained NCs are presented. SEM and TEM images of PA-stabilized AuCu NCs are indicated in Figs. 1A and 1B, respectively. Ultra-small particles with relatively uniform morphology and an average size of ~1.8 nm (using TEM image) were obtained by the presented synthesis process. Besides, the energy-dispersive X-ray (EDAX) spectrum (Fig. 1C) showed the existence of Au and Cu atoms in the synthesized NCs, with an Au/Cu ratio of 56.2:43.8. This obtained value is very close to the claimed value of 50:50 in the synthesis section. The ratios of Au/Cu for different prepared NCS are given in Table S1 (Supporting Information), and compared with the claimed values. On the other hand, investigation of XRD pattern of PA-AuCu50 NCS illustrated a broad diffraction spectrum (Fig. 1D), which proves an amorphous structure for prepared NCS. Moreover, FT-IR spectrum of synthesized PA-AuCu NCs was recorded to study their surface properties (Fig. 1E). The results verified the presence of COOH (1621 cm⁻¹), N-H (3550 cm⁻¹), C-H (2980 cm⁻¹) and C-S (658 cm⁻¹) bonds on the surface of NCs. Also, compared with the PA FT-IR pattern, the absorption peak at about 2500 cm⁻¹ was not observed for stretching vibration mode of S-H bonds in the spectrum of NCs. This means that the connection of PA ligands on the NCs surface occur via Cu-S or Au-S bonding.23,27

The fluorescence spectra (Fig. 1F) showed an increase in the emission intensity by inserting Cu atoms to the structure of Au NCs. The maximum intensity was obtained for AuCu25 NCs. Also, by increasing the Cu content in NCs structure, a considerable shift to larger wavelengths was occurred in the emission spectra of bimetal NCs. This observation is related to the modification of metallophilic bonding in NCs structure after introducing Cu atoms. Consequently, the oxidation features and also the energy levels of obtained bimetal NCs would be different from pure NCS. The quantum yield (QY) of Au, AuCu25, AuCu50, AuCu75 and Cu NCs were 1.2, 5.6, 3.9, 2.4 and 1.1, respectively, calculated using tyrosine (QY = 14%) as reference material. Finally, the prepared PA-AuCu NCs were stable at least for 2 months. After preservation at 4°C for 1 and 2 months. The emission of NCs decreased just 2.9 and 6.0%, indicating their good stability.

Investigation of catalytic activity of AuCu bimetal NCs by colorimetric experiments

In order to examine the catalytic activity of synthesized NCS by visual and colorimetric methods, OPD was used as a common peroxidase substrates (Fig. 2). In the presence of an effective peroxidase, H2O2 can oxidize OPD to produce a yellow colored product (DAP). This reaction is commonly used as a simple and rapid colorimetric tool to study the performance of mimetic materials. Materials with greater catalytic activity generate a higher absorption intensity at λ = 417 nm. As can be seen in Fig. 2, bimetal NCs caused higher absorption intensities than pure Au and Cu NCs, and so, their mimetic activity is noticeably better. AuCu bimetal NCs showed a greater catalytic performance than HRP, too. All of the experiments were performed under the optimized condition (including the reagents concentration, pH and type of buffer, and bimetal NCs). The process is briefly mentioned in section above. On the other hand, the high efficiency of AuCu NCs can be clearly observed by photographic images of H2O2-OPD solutions, in the presence of different catalysts (inset of Fig. 2). No sensible color was generated for OPD solution without the H2O2 or catalyst. To perform a comprehensive study on the effects of a stabilizing agent and the copper content in the NCs structures, different stabilizing ligands (BSA and GSH) were used for the synthesis of NCs with different Au/Cu ratios. The prepared NCs were applied as catalyst for OPD-H2O2 reaction and the obtained absorption intensities were summarized in Fig. S2 (Supporting Information). The results illustrated the higher catalytic efficiency of PA-AuCu50 NCS than other synthesized NCS.

Chemiluminescence investigations on the catalytic activity of AuCu bimetal NCs

The applied CL system for studying the catalytic behavior of bimetal NCs was based on the RhoB-H2O2 reaction. Under an alkaline condition, this reaction leads to very weak CL emission. The produced emission has been ascribed to the partial dissociation of H2O2 molecules to reactive •OH radicals, which can oxidize RhoB.40 The •OH radicals could degrade the RhoB molecules, confirmed a by decrease in the absorption intensity of RhoB in the presence of H2O2. The chief intermediate reported for this system is oxidized RhoB radicals in an excited state (RhoB*ox), which then transfer energy to RhoB molecules. Finally, the CL emission is generated by excited RhoB (RhoB*) at about 575 nm.41 Herein, it was found that the bimetal NCs cause a sensible improvement in the CL emission. The obtained CL peaks in flow system for alkaline RhoB-H2O2 solution in the presence of PA-stabilized Au, Cu and AuCu NCs are indicated in Fig. 3A. A great CL emission was obtained for solution which contained PA-AuCu NCs. Also, the effects of various stabilizing agent and different Au/Cu ratios are shown in Fig. S3 (Supporting Information). The results imply the more efficient activity of PA-AuCu50 NCS than that of other examined NCs. Also, the comparison experiments were expanded to Au and Cu nanoparticles with different sizes (Fig. S3), confirming the better results for PA-AuCu50 NCs.

Some experimentations were designed to investigate the mechanism of produced CL emission. First, the RhoB-H2O2 CL system was implemented in the batch condition, in the absence or presence of NCs and the CL intensity-time profiles were recorded (Fig. 3B). Without any catalyst, a very weak emission was observed; however, it was remarkably increased when the NCs were added. In the presence of pure Au and Cu NCs, the CL intensity was enhanced by about 15 and 40 times, respectively. These data indicate the good catalytic activity of Au and Cu NCs in the applied CL reaction. More wonderfully, the bimetal AuCu NCs improved the CL emission by about 130 fold. This result demonstrates the superior action of bimetal NCs. On the other hand, the maximum intensity of CL emission was...
obtained in shorter times (7 s) in the presence of AuCu NCs, than that of CL system without NCs (82 s) (Fig. 3B). This observation is another document that strongly verifies the high catalytic activity of NCs. Also, the maximum intensities of CL emission for CL systems in the presence of Au and Cu NCs were obtained in 18 and 23 s, respectively. The CL experiments in the presence of precursors, used for the synthesis of NCs (such as, PA, Au3+ and Cu2+) indicated a negligible increase in the CL intensity. Also, CL experiments were examined in the simultaneous presence of pure Au and Cu NCs, which increased the CL intensity by about 45 times.

Afterwards, some experiments were performed to record the CL-wavelength spectra, using a flow-based system (section above). The spectra are indicated in Fig. 3C, comprising a clear peak at about 571 nm. The observed spectra were similar to the fluorescence spectrum of RhoB, and the NCs did not change the peak wavelength. Consequently, the CL emission is produced by the RhoB* species. Finally, the catalytic action of the applied NCs on the RhoB–H2O2 reaction was further studied by their effect on the H2O2 reaction with TA. TA is a fluorometric-specific tool used to follow the concentration of •OH radicals. In the presence of an efficient catalyst, H2O2–TA reaction is occurred to produce highly fluorescent 2-hydroxyterephthalic acid (2-HTA, λem = 420 nm, λex = 315 nm). AuCu NCs could remarkably improve the generated fluorescence intensity of the H2O2–TA reaction. Thus, the catalytic action of NCs could be ascribed to their facilitating effect on the dissociation of H2O2 to hydroxyl radicals, which can rapidly oxidize the RhoB molecules and produce CL emission.

In order to further evaluate the reactive oxygen species, the effects of t-butyl alcohol (as a common •OH radical scavenger) and benzoquinone (as the O2•− radical quencher) were investigated on the CL emission of RhoB–H2O2–AuCu NCs system. In the presence of 0.2 mM t-butyl alcohol, a remarkable quenching in the CL emission was observed, and the CL intensity was decreased by 83%. However, a little diminishing effect on the CL intensity occurred in the presence of benzoquinone, with the same concentration. After addition of 0.2 mM benzoquinone, the CL intensity was reduced by only 17%. Even, the higher concentrations of benzoquinone (0.6 mM) decreased the CL intensity by only 25%. Consequently, O2•− and •OH radicals create during the CL reaction and participate in the oxidation of RhoB, but, the •OH radicals have the main role.

Analytical applications

The useful application of presented non-mimics was examined for the detection of H2O2, glucose and xanthine. The CL intensity of RhoB–H2O2–AuCu NCs was proportionally enhanced by increasing the concentrations of H2O2. It can thus be exploited to design a simple and rapid method to measure the H2O2 level. Also, determinations of glucose and xanthine were performable by the developed CL system, after their enzymatic oxidation and H2O2 production. In this step, to achieve the optimum sensitivity, influences of some practical parameters were investigated on the obtained CL emission (Fig. S4, Supporting Information). The best sensitivity (Ic/I0 show the chemiluminescence intensities in the presence and absence of hydrogen peroxide) were obtained by 300 μL PA-AuCu50 NCs and 0.3 mM RhoB, in 0.01 M NaOH condition. CL intensity was linearly increased by increasing the amounts of NCs, up to 300 μL. The higher amounts led to a decrease in the CL emission intensity (Fig. S4D), perhaps because of their inner filter effect. Also, it was observed that the addition of SDS surfactant in the RhoB–H2O2–AuCu NCs CL system led to a noticeable increase in CL emission intensity. It can be related...
to the construction of some micro-environments by a surfactant, which supports and more stabilize the CL intermediate species. The best CL signal was obtained with 0.2 mM SDS concentration.

Analytical figures of merit

Figure 4A shows the CL intensities of RhoB–H2O2–AuCu NCs against some different concentration of H2O2. The linear section of calibration graph is in the range of 0.2 – 2000 nM H2O2 concentrations (inset of Fig. 4A). The corresponding CL peaks are also indicated in Fig. 4B. As can be seen from the results, the method provided a high sensitivity for the detection of H2O2. A great detection limit of 0.13 nM (3σb/slope, σb presents the standard deviation for signals of 6 blank solutions) was obtained. The obtained CL–time profiles for low H2O2 concentrations are indicated in Fig. S5 (Supporting Information), which confirm a measurable CL intensity (distinguishable from blank profile) for H2O2 concentration as low as 0.2 nM. Compared with colorimetric OPD-H2O2–AuCu NCs and fluorometric TA–H2O2–AuCu NCs systems, the developed CL system led to a better sensitivity (Table S2, Supporting Information). Besides, the CL method has other worthy advantages, including a wide dynamic range, a simple apparatus and a rapid detection process. On the other hand, the catalytic activity of AuCu NCs on the more common CL reaction, luminol–H2O2, was examined and applied for the detection of H2O2. The results are indicated in Table S2. Consequently, the usefulness of RhoB was confirmed because: 1) the luminol solution is unstable; 2) luminol has been widely used, but there are few studies about the rhodamine B CL systems (and this study can be useful for expanding the area of useful CL systems); 3) rhodamine B is cheaper than luminol; 4) there is no report on the effect of nanoclusters on the rhodamine B system; and 5) more important, the detection limit of rhodamine CL system for the determination of H2O2 is

Fig. 4  (A) Calibration graph for the determination of H2O2 using the developed CL system; the inset shows the linear section of calibration graph; (B) the CL profiles in the presence of different concentrations of H2O2; (C) and (D) linear calibration graphs for the determination of glucose (C) and xanthine (D).

Fig. 5 (A) CL intensities of the RhoB-H2O2 system applied for the detection of different compounds (10 mM) in the presence or absence of (A) xanthine (100 μM) and (B) glucose (200 μM) in optimum condition.
somewhat better than that of luminol CL system. Also, linear calibration graphs for the determination of glucose and xanthine by developed CL method were obtained in the concentration ranges of 0.1 - 400 and 0.1 - 200 μM, with detection limits of 30 and 38 nM, respectively (Figs. 4C and 4D).

The method indicated a good precision for the detection of glucose and xanthine. The relative standard deviation (RSD) values of 2.57 and 3.39% were obtained for the measurement of 6 similar solutions, containing 20 μM glucose and xanthine, respectively.

Selectivity
The specificity of the presented CL method for glucose and xanthine detection was studied using some glucose (200 μM)/xanthine (100 μM) solutions, containing certain concentrations (10 mM) of different compounds. A 5% change in the final signal was considered as the tolerance limit for the interfering influence of each examined compound of tested compounds (Fig. 5). It is clear that the tested compounds have no interfering effects on the CL signal. These results confirmed the good selectivity and reliable applicability of presented method for the measurement of biomolecules in real samples. This high selectivity is of specific enzymatic reactions.

The influences of some biological components were evaluated on the CL signals for H2O2, too (Fig. S6, Supporting Information). It was observed that some of the tested compounds (especially, glutathione, hemin, urea and uric acid) had an interfering effect on the determination of H2O2, and the CL signal was considered as the tolerance limit for the interfering charge on the Web at http://www.jsac.or.jp/analsci/.

Analysis of real samples
The presented CL method was applied for measurements of glucose and xanthine in human serum samples (Table 1). The validity of the obtained results was also verified by adding certain quantities of the glucose/xanthine standard solution into the samples. Spiked samples were then prepared and measured according to the presented CL method. The recoveries are shown in Table 1, verifying the proper reliability of detection process. More validation was obtained by applying the CL method to analyze the standard reference material (SRM 965b) for glucose. The result (4.126 ± 0.113 mM) was in good agreement with the certified value (4.194 ± 0.059 mM), reported by National Institute of Standards and Technology (NIST).

Table 1 Application of the developed chemiluminescence system for the determination of xanthine and glucose in real samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Glucose</th>
<th>Xanthine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Added/mM</td>
<td>Found a</td>
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<tr>
<td>Healthy person</td>
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<td>3.85 ± 0.12</td>
</tr>
<tr>
<td>(Woman, age 41)</td>
<td>2.0</td>
<td>5.82 ± 0.08</td>
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<tr>
<td>Diabetic patient</td>
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<td>7.94 ± 0.09</td>
</tr>
<tr>
<td>(Woman, age 58)</td>
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<td>3.21 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>42.57 ± 0.37</td>
</tr>
</tbody>
</table>

a. Mean of three determinations (mM) ± standard deviation.

Conclusions
In summary, the peroxidase-like activity of AuCu bimetal NCs with various stabilizing agents and different Au/Cu ratios were examined in the CL reaction of RhoB-H2O2. AuCu50 NCs stabilized by penicillamine caused the best CL emission. Colorimetric experiments also verified the high catalytic activity of PA-AuCu50 NCs. This catalytic action of NCs was related to their facilitating effect on the decomposing of H2O2 to •OH radicals, which can rapidly oxidize RhoB molecules. Moreover, the NCs assisted CL system was examined for the detection of H2O2, resulting in a great sensitivity. Besides, the method was used for the quantification of glucose and xanthine, after their oxidation by specific enzymes and the production of H2O2. The developed CL method provides good detection limits of 0.13, 30 and 38 nM for H2O2, glucose and xanthine, respectively. Finally, the reliability of method was verified by the analysis of real sera and the SRM sample.

Acknowledgements
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Supporting Information
The schematic image of CL flow-injection setup, EDAX results, optimization graphs and comparison results of the catalytic activity of different applied nano-structures are located in Supporting Information. This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/

References


