

# Chemiluminescent flow-through sensor for 1,10-phenanthroline based on the combination of molecular imprinting and chemiluminescence

Jin-Ming Lin\* and Masaaki Yamada

Department of Applied Chemistry, Graduate School of Engineering, Tokyo Metropolitan University, 1-1 Minami-Ohsawa, Hachioji, Tokyo 192-0397, Japan.

E-mail: lin@ecomp.metro-u.ac.jp

Received 30th November 2000, Accepted 9th February 2001

First published as an Advance Article on the web 9th March 2001

A functional polymer for the catalysis of the chemiluminescent reaction and molecular recognition ability of 1,10-phenanthroline was prepared based on the molecular imprinting method. The structural and catalytic roles of transition metal ion interactions were applied in the material design. A ternary complex, 4-vinylpyridine–Cu(II)–1,10-phenanthroline (2:1:1), was synthesized and used as a functional monomer. The ligand 1,10-phenanthroline in the ternary complex was the template used to form the molecularly imprinted polymer. Another monomer, styrene, and the cross-linking reagent divinylbenzene were copolymerized with the ternary complex. The polymer containing the ternary complex is an efficient catalyst for the decomposition of hydrogen peroxide. During the hydrogen peroxide decomposition, superoxide radical ion is formed, which reacts with 1,10-phenanthroline and gives a chemiluminescent emission. The 1,10-phenanthroline was destroyed during the chemiluminescent reaction, leaving a cavity and copper binding sites for another 1,10-phenanthroline molecule. The prepared polymer particles were packed into a glass tube and developed as a molecular recognition chemiluminescent flow-through sensor for 1,10-phenanthroline. The sensitivity and selectivity of the sensor were tested.

Molecular imprinting is a useful technique for the preparation of functional materials with molecular recognition properties. Research on and the applications of the molecular imprinting method have accelerated since a drug assay using antibody mimics made by this method was reported by Mosbach's group in 1993,<sup>1</sup> and many research reports in this field have appeared in recent years.<sup>2–5</sup> A great number of molecularly imprinted polymers have been synthesized and their molecular recognition properties have been utilized in a variety of separation and sensing techniques. In the last 3 years there have been several detailed reviews on the applications of molecularly imprinted polymers in the analytical chemistry.<sup>6–9</sup>

In principle, molecularly imprinted polymers are synthesized by cross-linking complexes of template molecules and functional monomers. After removing the template molecule from the polymers, binding sites are formed by functional monomer-derived residues complementary for the template molecule. The molecularly imprinted polymer acts as an artificial receptor/antibody exhibiting tailor-made selectivity for the template molecule. Usually, the template molecule is also the analyte. In most cases, the analytes selectively adsorbed in the cavities of the molecularly imprinted polymer were eluted with an organic solvent and detected with a UV or fluorescence detector. Therefore, if the affinity is strong, a long time and a large volume of organic solvent to wash the polymer are needed.

It is well known that chemiluminescence (CL) has two main characteristics: CL is emitted during oxidation–reduction reaction and one or more reactants are destroyed after the CL reaction. The sensitivity and the simple instrumentation of CL have led to this method being widely used in many fields.<sup>10–12</sup> However, the high sensitivity of the CL method faced the challenge of selectivity, as most CL systems could not be used to determine the analytes directly. They often are used for post-column detection, e.g., in high-performance liquid chromatography<sup>13,14</sup> and capillary electrophoresis.<sup>15,16</sup> The CL systems used as post-column detectors have also met their problems,

e.g., liquid chromatography needs an eluent, which is often a mixture of an organic solvent and salts. This eluent not only affects the separation but also influences the CL emission.

Great efforts have been made to improve the selectivity of CL. Two effective methods could be considered. One is to set up the CL systems without special CL reagents, such as luminol and lucigenin. Even though these special CL reagents have high CL quantum yields, their CL emissions vary with the metal ions, buffer conditions and other additives. A few relatively highly selective CL systems, such as the  $\text{KIO}_4\text{--CO}_3^{2-}$  system for  $\text{H}_2\text{O}_2$ ,<sup>17,18</sup> the  $\text{BrO}^- \text{--OH}^-$  system for ammonium ion<sup>19</sup> and the  $\text{MnO}_4^- \text{--H}^+$  system for ascorbic acid<sup>20</sup> determinations, have been proposed. Another approach is to prepare a material with molecular recognition and CL characteristics. An initial attempt to combine the high selectivity of molecular imprinting with the high sensitivity of the CL method was successful in our previous work.<sup>21</sup> To our knowledge, there is no other report on the application of molecular imprinting in CL methods. In this work, the structural and catalytic properties of metal ion interactions were used to design a molecularly imprinted polymer with both molecular recognition and CL reaction catalytic properties. A ternary complex, 4-vinylpyridine–Cu(II)–1,10-phenanthroline, was used as the functional monomer and divinylbenzene as the cross-linking agent. The polymerization was initiated by addition of 2,2'-azobisisobutyronitrile as initiator. The prepared polymer was developed as a molecular recognition CL flow-through sensor for the determination of 1,10-phenanthroline.

## Experimental

### Reagents

All chemicals and reagents were of analytical-reagent grade. Water was obtained from a Milli-Q purification system (Japan

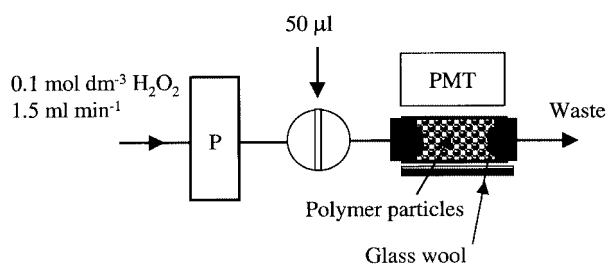
Millipore, Tokyo, Japan). Styrene, divinylbenzene, copper(II) acetate monohydrate  $[(CH_3COO)_2Cu \cdot H_2O]$ , methanol and 4-vinylpyridine (VPy) were purchased from Nacalai Tesque (Kyoto, Japan). 2,2'-Azobisisobutyronitrile  $[(CH_3)_2C(CN)N=N(CN)C(CH_3)_2]$  was a product of Tokyo Kasei Kogyo (Tokyo, Japan). Cobalt(II) acetate  $[(CH_3COO)_2Co \cdot 4H_2O]$ , 1,10-phenanthroline (phen), hydrogen peroxide and other reagents were purchased from Kanto Chemical (Tokyo, Japan). Phen sampling solution was prepared in  $0.01 \text{ mol dm}^{-3}$  NaOH solution. A  $0.1 \text{ mol dm}^{-3}$  hydrogen peroxide solution was prepared by diluting 30%  $H_2O_2$  solution with water. Monomers were freshly distilled prior to use for removing inhibitors.

## Apparatus

The batch method for the CL profile was carried out with a Lumincounter 600 instrument (Microtec NITI-ON, Funabashi, Japan). The flow sensor was an on-line flow system (Fig. 1) which consisted of a peristaltic pump (SJ1211; Atto, Tokyo, Japan), a CL detector (Lumiflow LF-800; Microtec NITI-ON), a flow cell (effective length *ca.* 1.5 cm, 0.5 cm id glass tube packed with the polymer particles) which was placed at the front of photomultiplier tube and a 50  $\mu\text{l}$  loop injector placed near the flow cell. The carrier was  $0.1 \text{ mol dm}^{-3}$   $H_2O_2$  aqueous solution and phen sampling solution was injected into the carrier line from the loop injector. The CL signals were recorded with a Shimadzu (Kyoto, Japan) U-125MN recorder or a Tokken (Chiba, Japan) PC Record System. UV-VIS spectra were recorded with a UV-2200 spectrophotometer (Shimadzu).

## Polymer preparation

The polymer preparation was carried out in two steps. (1) For the formation of the phen-Cu(II)-VPy or phen-Co(II)-VPy ternary complex, 2.00 mmol of copper(II) acetate (399.3 mg) or cobalt(II) acetate (498.2 mg), 2.0 mmol of 1,10-phenanthroline (396.5 mg), 4.0 mmol of 4-vinylpyridine (420.0 mg) and 20 ml of methanol were mixed in a 50 ml borosilicate glass test-tube. After all solid reagents had dissolved, the mixing solvents of the copper and cobalt complexes were deep blue and brown solutions, respectively. (2) For polymerization, to the above solution, 40 mmol of styrene (4.20 g), 40 mmol of divinylbenzene (5.20 g) and 100 mg of 2,2'-azobisisobutyronitrile were added. The polymerization mixtures ( $0^\circ\text{C}$ ) were purged with nitrogen for 10 min, sealed and then heated in a shaking water-bath at  $60^\circ\text{C}$  for 24 h. Seven reference polymers were similarly prepared except for the exclusion of phen [P(Cu-VPy) and P(Co-VPy)], VPy [P(Cu-phen) and P(Co-phen)], phen and VPy [P(Cu) and P(Co)] or all metal ions and ligands [P(blank)] during the polymerization. The resultant bulk polymers were ground to particles. A polymer particle size of 100–300  $\mu\text{m}$  was used to prepare the CL flow-through sensors.



**Fig. 1** Schematic diagram of flow injection chemiluminescent system. P = peristaltic pump; PMT = photomultiplier tube.

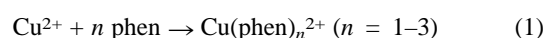
## Procedures

For the batch experiment, to 10.0 mg of imprinted polymer or blank polymer particles, 100  $\mu\text{l}$  of  $0.1 \text{ M}$   $H_2O_2$  solution were added. The CL signals were recorded with a U-125MN recorder. In the flow experiment, prior to sample injection, the carrier solution was allowed to flow for *ca.* 10 min to decrease the high background emission of a newly prepared sensor. A 50  $\mu\text{l}$  volume of phen solution was injected into the carrier stream through the sample valve and the CL signals were recorded.

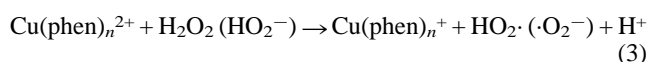
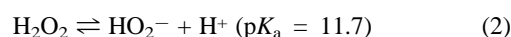
## Results and discussion

### Theory

As an initial attempt, a well-known compound, phen, was used as a model sample for this experiment. The CL of phen was proposed nearly 20 years ago in our laboratory<sup>22</sup> and its CL mechanism was also studied.<sup>23</sup> In the presence of  $Cu^{2+}$  ion, phen forms chelate with the metal ion:

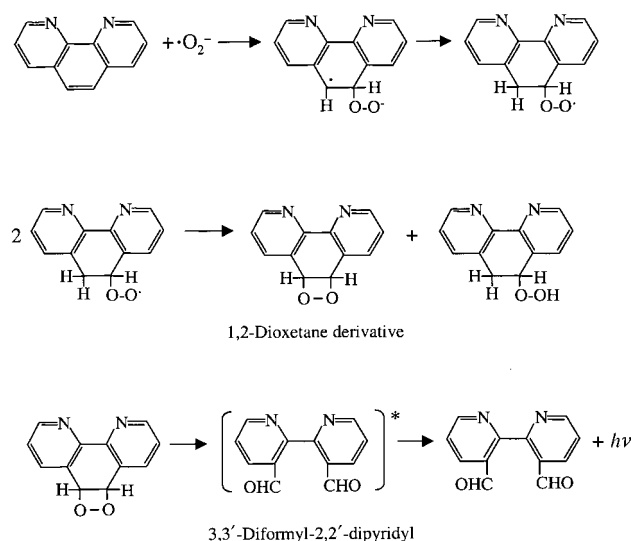


The complex has a strong catalytic activity for the decomposition of  $H_2O_2$  (or  $HO_2^-$ ) solution:



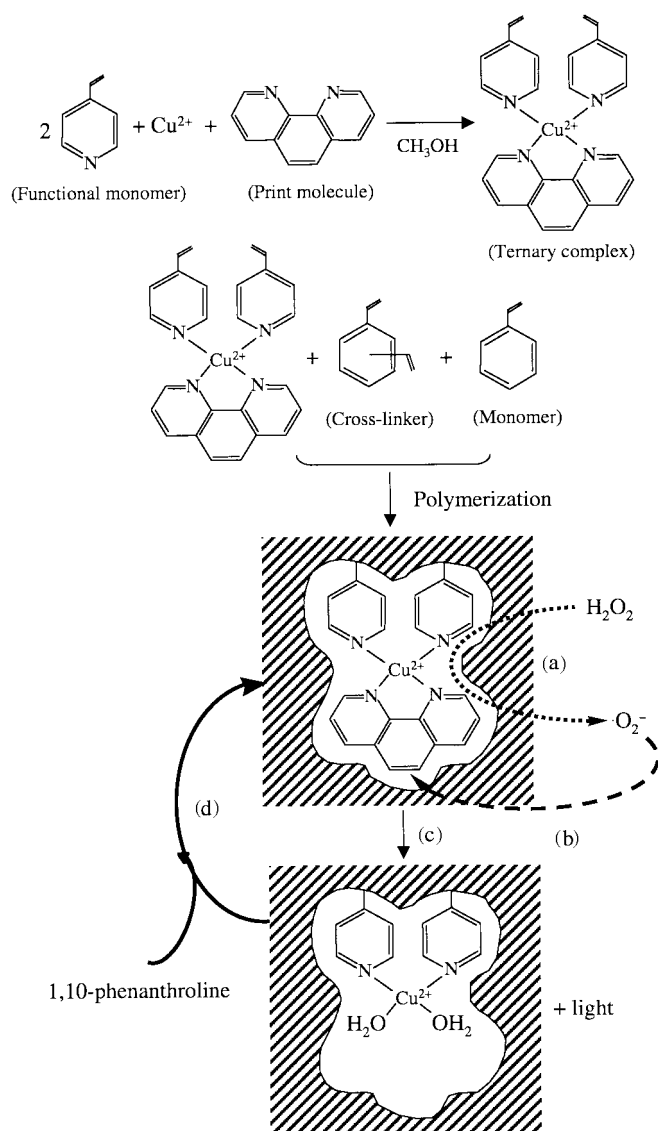
The product, superoxide radical ion ( $\cdot O_2^-$ ), generated from the decomposition of  $H_2O_2$  attacks the phen molecule. A highly energetic molecule, 1,2-dioxetane derivative, is formed.<sup>24</sup> When the unstable 1,2-dioxetane derivative molecule decomposes, excited 3,3'-diformyl-2,2'-dipyridyl is generated and the CL emission with a maximum wavelength in the range 445–450 nm. That is, CL emission resulted from the oxidative destruction of phen during the catalytic decomposition of  $H_2O_2$  by the copper-phen complex. The CL mechanism involving the formation of 1,2-dioxetane is summarized in Scheme 1.

Based on the above CL mechanism and the principle of molecular imprinting, the polymer prepared should be a catalyst for the CL reaction and the molecular recognition of phen. The principles of the design and preparation of molecularly imprinted polymers and phen molecular recognition CL



**Scheme 1** Chemiluminescent reaction between 1,10-phenanthroline and superoxide radical anion.

reaction are summarized in Fig. 2. A ternary complex, phen-Cu(II)-Vpy, which is a catalyst for the decomposition of  $\text{H}_2\text{O}_2$ , was used as an imprinted molecule for polymer preparation. After the polymerization of the phen-Cu(II)-Vpy complex with divinylbenzene and styrene, the resultant polymer has a strong, catalytic ability to decompose  $\text{H}_2\text{O}_2$ . When the  $\text{H}_2\text{O}_2$  solution was mixed with polymer particles, superoxide radical ion was generated (Fig. 2, route a). This superoxide radical ion attacked a phen molecule which is a ligand of the copper complex (route b). During the reaction of phen with superoxide radical ion, phen was destroyed to give 3,3'-diformyl-2,2'-dipyridyl and CL was emitted. The reaction product 3,3'-diformyl-2,2'-dipyridyl combined weakly with copper and could easily be replaced by water molecules (route c). The cavity and the two coordination sites for phen molecule remained in the polymer. These cavity and the binding sides were selective for phen. When a phen sampling solution was mixed with the polymer particles, a phen molecule was complexed or drawn into the cavity (route d), and the CL reaction catalyst polymer was regenerated. From route a to d, the polymer could be used cyclically as a catalyst and molecular recognition agent.



**Fig. 2** Schematic representation of the molecular imprinting process and the principle of the molecular recognition chemiluminescent reaction. (a) Decomposition of hydrogen peroxide with phen-Cu(II)-Vpy complex as catalyst; (b) superoxide radical ion attack on phen molecule of copper complex; (c) phen molecule destruction, chemiluminescence emission and recognition cavity remaining; and (d) accepting a new phen molecule into the recognition cavity.

## Preparation of molecularly imprinted polymer

To prepare the polymer with molecular recognition and CL reaction catalysis properties, both the CL reaction mechanism and the polymer properties should be considered. There are two main steps, (1) ternary complex formation and (2) polymerization of the ternary complex with divinylbenzene and styrene. As shown in Table 1, two kinds of transition metal ions, four-coordinate square-planar copper ion and six-coordinate octahedral cobalt ion, were selected. In addition to  $\text{Cu}(\text{phen})_2$ , the  $\text{Co}(\text{phen})_2$  complex has also been used as catalyst in the aqueous phase CL reaction system.<sup>25</sup> Moreover, cobalt ion mediated recognition in molecularly imprinted polymers has been reported.<sup>26,27</sup> Therefore, the comparison of these two complexes was considered necessary.

The formation of the complexes was carried out in methanol solvent. A 2.0 mmol amount of cobalt(II) acetate completely dissolved in 15.0 ml of methanol, but the dissolution of copper(II) acetate in methanol was poor. After adding 2.0 mmol of Vpy and 2 mmol of phen to both copper(II) acetate and cobalt(II) acetate, the colors of the solutions of copper(II) acetate and cobalt(II) acetate in methanol solvent changed from green to deeply blue and from pink to brown, respectively. All residual copper(II) acetate was dissolved after it had formed the complex.

Evidence for the complex formation was followed by the visible absorption spectra. Fig. 3(a) shows that the four spectra have different absorbances and maximum wavelengths in the order (1)  $\text{Cu}^{2+}$ , (2)  $\text{Cu}(\text{II})\text{-Vpy}$ , (3)  $\text{Cu}(\text{II})\text{-phen}$  (683.2 nm) and (4) phen- $\text{Cu}(\text{II})\text{-Vpy}$  (661.2 nm). These results clearly indicate that the ternary complex, phen- $\text{Cu}(\text{II})\text{-Vpy}$ , was formed in methanol solvent. However, the spectra of (1)  $\text{Co}^{2+}$ , (2)  $\text{Co}(\text{II})\text{-Vpy}$ , (3)  $\text{Co}(\text{II})\text{-phen}$  and (4) phen- $\text{Co}(\text{II})\text{-Vpy}$  in Fig. 3(b) do not show a similar order. Cobalt(II) acetate and  $\text{Co}(\text{II})\text{-Vpy}$  in methanol solution have almost the same absorption wavelength (518.7 nm) and absorbance. Also,  $\text{Co}(\text{II})\text{-phen}$  and phen- $\text{Co}(\text{II})\text{-Vpy}$  in methanol have a similar maximum absorption wavelength of 464.3 nm. Therefore, we think that the formation of the phen- $\text{Co}(\text{II})\text{-Vpy}$  complex hardly occurred or the combination of  $\text{Co}(\text{II})$  with Vpy is weak in methanol solvent. The complex formation constants of the metal ions with phen at 25 °C in aqueous solution are  $\text{Cu}(\text{II})$ ,  $\log K_1 = 9.1$ ,  $\log K_2 = 6.8$  and  $\log K_3 = 5.0$ , and  $\text{Co}(\text{II})$ ,  $\log K_1 = 7.08$ ,  $\log K_2 = 6.64$  and  $\log K_3 = 6.08$ .<sup>28–30</sup> Copper(II) forms a stable 1:2 complex with phen. The complex  $[\text{Cu}(\text{phen})_2]^{2+}$  is in coordination equilibrium with acetate ion at the two vacant coordination sites.<sup>31</sup> As we do not have the complex formation constants of  $\text{Co}(\text{II})\text{-Vpy}$  in methanol and the combination constants of copper or cobalt with Vpy at polymer at present, the quantitative comparison of the stabilities of phen- $\text{Cu}(\text{II})\text{-Vpy}$  and phen- $\text{Co}(\text{II})\text{-Vpy}$  will be

**Table 1** Imprinted and blank polymers prepared in this study

Imprinted substance	Maximum absorption wavelength/nm <sup>a</sup>	Polymer	Polymer colour
Non	—	P(blank)	White
$\text{Cu}^{2+}$	715.3	P(Cu)	—
$\text{Cu}(\text{II})\text{-Vpy}$	709.6	P(Cu-Vpy)	—
$\text{Cu}(\text{II})\text{-phen}$	683.2	P(Cu-phen)	Light blue
Phen- $\text{Cu}(\text{II})\text{-Vpy}$	661.2	P(phen-Cu-Vpy)	Light blue
$\text{Co}(\text{II})$	519.7	P(Co)	Pinky white
$\text{Co}(\text{II})\text{-Vpy}$	520.7	P(Co-Vpy)	Pinky gray
$\text{Co}(\text{II})\text{-phen}$	468.3	P(Co-phen)	Light gray
Phen- $\text{Co}(\text{II})\text{-Vpy}$	464.3	P(phen-Co-Vpy)	Pinky gray

<sup>a</sup> The wavelengths were measured using methanol as reference. The absorbance of 2.00 mmol copper(II) acetate, cobalt(II) acetate and their complexes in 15 ml of methanol were too strong, so the present results were obtained from their 10-fold methanol-diluted solutions.

discussed elsewhere after these constants have been determined.

Copper(II) acetate, cobalt(II) acetate and their phen and VPy complexes were imprinted in divinylbenzene–styrene copolymers. VPy acts not only as the ligand for the ternary complex but also as the functional monomer for the polymerization. With 2,2'-azobisisobutyronitrile as polymerization initiator, the formation of the polymers could be completed within 24 h at 60 °C. However, the polymerization of the mixture of monomers, cross-linking agent and copper(II) acetate or Cu(II)–VPy could not be carried out at 60 °C with this initiator. The possible reason is that the high concentration of free copper ion inhibited free radical formation even though 2,2'-azobisisobutyronitrile existed in the solvent mixture.

Usually, the resultant molecularly imprinted polymer was pre-treated with solvent before it was used in separation or sensor applications.<sup>2</sup> However, in the present work, the pre-treatment of the resultant polymer, *e.g.*, the extraction of the template from the polymer, was unnecessary. As shown in Fig. 2, after the CL reaction, the decomposition product of phen, 3,3'-diformyl-2,2'-dipyridyl, was weakly combined with Cu(II). It could easily be washed with dilute H<sub>2</sub>O<sub>2</sub> solution. A cavity for the phen molecule was left in the polymer. When the phen solution was injected into the carrier and allowed to flow through the polymer particles, the phen molecule was accepted into the cavity and the CL reaction could be carried out again.

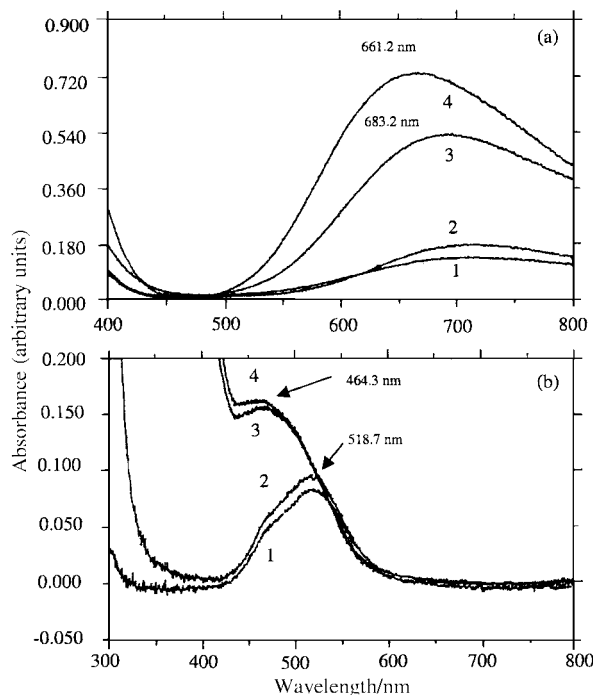
### Comparison of the CL signals for different polymers

In order to compare the CL reaction catalysis and molecular recognition abilities of the polymers, both batch and flow methods were used. Fig. 4 shows the CL signals with batch method. Signals (a)–(g) correspond to the mixtures of 0.1 ml of 0.1 mol dm<sup>-3</sup> H<sub>2</sub>O<sub>2</sub> solution with 10 mg of P(blank), P(Cu–phen), P(phen–Cu–VPy), P(Co), P(Co–VPy), P(Co–phen) and P(phen–Co–VPy) polymer particles, respectively. The poly-

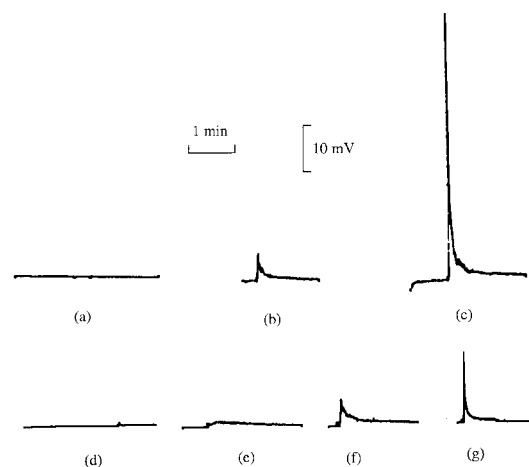
mers containing Cu(II)–phen, phen–Cu(II)–VPy, Co(II)–phen and phen–Co(II)–VPy gave CL emission even though the reactions were carried out under unbuffered conditions. The CL intensities of P(Cu–phen) and P(Co–phen) were almost the same, but that of P(phen–Cu–VPy) was stronger than that of P(phen–Co–VPy). These phenomena could be explained by the fact that 1:1 Cu(II)–phen and Co(II)–phen are only weak catalysts for the decomposition of H<sub>2</sub>O<sub>2</sub> under the unbuffered conditions. The fact that both Cu<sup>2+</sup> and Co<sup>2+</sup> ions are good catalysts for H<sub>2</sub>O<sub>2</sub> decomposition has been known for a long time. Generally, the decomposition of H<sub>2</sub>O<sub>2</sub> started from the hydroperoxide ion. The catalytic activity of Cu<sup>2+</sup> and Co<sup>2+</sup> for H<sub>2</sub>O<sub>2</sub> decomposition is relatively weak in neutral or acidic solution,<sup>32,33</sup> which led to P(Co–phen) and P(Cu–phen) having the same CL intensity. Of course, the polymers prepared without a metal ion have no catalytic ability. The formation of a ternary complex of phen–Cu(II)–VPy or phen–Co(II)–VPy gives a complex with high catalytic activity. However, from Fig. 3(b), the combination of Co(II) with VPy was weak, which means that only a small amount of phen–Co(II)–VPy ternary complex was present in the polymer. The results from both the CL signal and the UV–visible absorbance supported this explanation.

Combed with flow injection analysis, the prepared polymer was developed as a molecular recognition flow-through CL sensor. When a 0.1 M H<sub>2</sub>O<sub>2</sub> carrier solution was allowed to flow through the polymer particles, the polymers which contained Cu(II) [or Co(II)] and phen reacted with H<sub>2</sub>O<sub>2</sub>. The baselines of the sensors prepared from three typical polymers, P(blank), P(phen–Cu–VPy) and P(phen–Co–VPy), are shown in Fig. 5(a), (b) and (c), respectively. Similarly to the batch results, the polymers prepared without phen have no background CL signal when H<sub>2</sub>O<sub>2</sub> solution flowed through the sensor. A broad and large CL signal was recorded when H<sub>2</sub>O<sub>2</sub> carrier solution flowed through a new sensor which was prepared from P(phen–Cu–VPy) or P(phen–Co–VPy). Other polymers contained Cu–phen or Co–phen also showed background CL signals, but they were weak.

In order to obtain a stable baseline for the sensor which was prepared from P(phen–Cu–VPy) or P(phen–Co–VPy), a *ca.* 10 min flow of 0.1 mol dm<sup>-3</sup> H<sub>2</sub>O<sub>2</sub> carrier solution at 1.5 ml min<sup>-1</sup> was necessary. This step could be considered as the pre-treatment of a newly formed polymer. The template molecule, phen, was washed or destroyed by H<sub>2</sub>O<sub>2</sub> solution. When a low concentration of phen solution was injected into the carrier solution, the molecular recognition and CL reaction took place at the surface of the polymer particles. Fig. 5(d) shows a typical



**Fig. 3** Visible region spectra of copper(II) acetate, cobalt(II) acetate and their VPy and phen complexes in methanol. The reference was methanol. (a) Spectra 1–4 are copper(II) acetate, Cu(II)–VPy, Cu(II)–phen and phen–Cu(II)–VPy, and (b) spectra 1–4 are cobalt(II) acetate, Co(II)–VPy, Co(II)–phen and phen–Co(II)–VPy, respectively. The concentrations of these compounds are given in the footnote of Table 1.



**Fig. 4** Chemiluminescent profiles in a batch system. The profiles (a)–(g) correspond to the CL reactions of polymers P(blank), P(Cu–phen), P(phen–Cu–VPy), P(Co), P(Co–VPy), P(Co–phen) and P(phen–Co–VPy), respectively. The weight of each without any pre-treated polymer was 10.0 mg and the addition of 0.1 mol dm<sup>-3</sup> H<sub>2</sub>O<sub>2</sub> solution was 100 µl.



CL signal for the determination of phen using the sensor prepared from P(phen-Cu-VPy) polymer. The detection limit of phen with this sensor was  $4 \times 10^{-6} \text{ mol dm}^{-3}$  (signal-to-noise ratio = 3). The sensor prepared from P(phen-Co-VPy) had the same CL emission for phen analysis, but its sensitivity was low. The detection limit of the sensor prepared from P(phen-Co-VPy) polymer particles was only  $6 \times 10^{-5} \text{ mol dm}^{-3}$  phen. The sensor prepared from P(Co-VPy) also had a small CL emission when  $1 \times 10^{-3} \text{ mol dm}^{-3}$  phen solution was injected. This result may be due to part of the phen molecules being sorbed on the P(Co-VPy) polymer and the Co(II)-VPy complex also has a weak catalytic ability to decompose  $\text{H}_2\text{O}_2$  to form superoxide radical ion.

As discussed above, the combination of Co(II) and VPy was weak, and Co(II) ion complexed on the polymer was easily removed by the carrier solution. However, we found that the sensor prepared from blank polymer, P(blank), has a strong and stably CL emission for  $1 \times 10^{-5} \text{ mol dm}^{-3}$  phen solution. The blank polymer was synthesized from styrene-divinylbenzene, which has a stronger hydrophobic interaction with phen. The long lifetime of superoxide radical ion and the concentration effect of phen in a hydrophobic medium made the blank polymer act as a micelle, as had been discussed in previous papers.<sup>22,23</sup> The polymers that contained VPy show only a very small CL emission for phen. Because of the decomposition of  $\text{H}_2\text{O}_2$ , the affinity or concentration of phen and the CL reaction of  $\text{H}_2\text{O}_2$  with phen on the surface of the polymer are complicated, more detailed research is needed to explain all these phenomena in the future. The CL intensities of the different polymers indicated that the polymer imprinted with phen-Cu(II)-VPy has a relatively high selectivity for the phen molecule. In order to compare the molecular recognition of the sensor prepared from P(phen-Cu-VPy), three substances ( $1 \times 10^{-4} \text{ mol dm}^{-3}$ ), 2,9-dimethyl-1,10-phenanthroline, 2-chloro-1,10-phenanthroline and 1,7-phenanthroline, which have similar chemical structures to phen, were injected into the carrier

solution. No CL emission was observed. These results indicated that phen with a methyl or chloride group was difficult to accept into the cavity of the P(phen-Cu-VPy) polymer. 1,7-Phenanthroline not only could not emit CL during its reaction with  $\text{H}_2\text{O}_2$ , but also has two different nitrogen binding sites from the phen molecule, so it is impossible to combine with Cu(II) in the polymer. Both the selectivity of the CL reaction and polymer recognition make the prepared sensor highly selective for the determination of phen.

## Conclusions

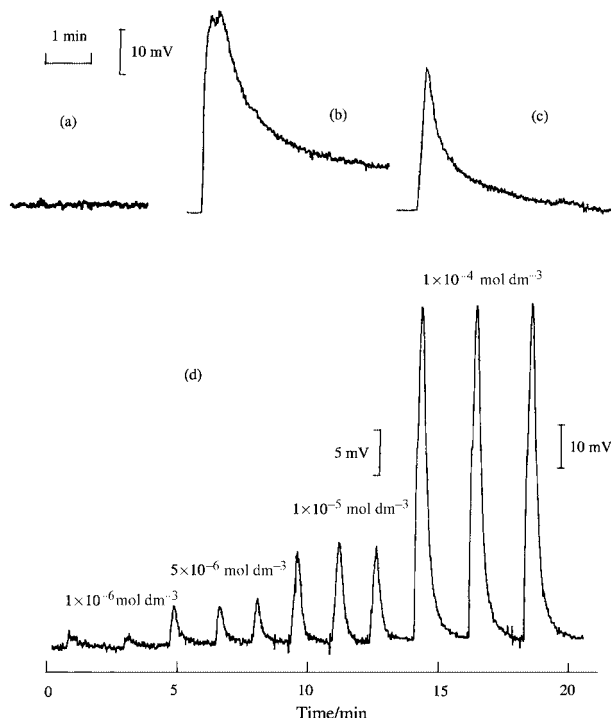
As a first attempt to use molecularly imprinted polymers as a catalyst and molecular recognition medium in CL, the results evidenced that P(phen-Cu-VPy) polymer was a catalyst for the CL reaction and molecular recognition for phen. Phen was selectively combined into the cavity (Fig. 2, route d) owing to the advantageous cavity shape and the metal interaction with the polymer. The catalytic function of the polymer comes from the formation of a Cu(II) ternary complex in the presence of phen and pyridine groups. In this work, a major shortcoming of CL reactions, the destruction of the reactants during the CL reactions, was put to good use to prepare a molecular recognition CL flow-through sensor. Even though in this work the results with the prepared CL flow-through sensor are not satisfactory enough to compare with established versions, we believe that in the near future, combined with CL or other highly sensitive detection methods, molecular imprinting will develop as a convenient, highly sensitive and selective method in the analytical field.

## Acknowledgement

This work was partially supported by a grant-in-aid for scientific research from the Ministry of Education, Science and Culture of Japan, which is gratefully acknowledged.

## References

- 1 G. Vlatakis, L. I. Andersson, R. Müller and K. Mosbach, *Nature (London)*, 1993, **361**, 645.
- 2 *Molecular and Ionic Recognition with Imprinted Polymers*, ed. R.A. Bartsch and M. Maeda, ACS Symposium Series 703, American Chemical Society, Washington, DC, 1998.
- 3 G. Wulff, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 1812.
- 4 S. A. Piletsky, T. L. Panasyuk, E. V. Piletskaya, I. A. Nicholls and M. Ulbricht, *J. Membr. Sci.*, 1999, **157**, 263.
- 5 K. Haupt and K. Mosbach, *Chem. Rev.*, 2000, **100**, 2495.
- 6 L. Schweitz, L. I. Andersson and S. Nilsson, *J. Chromatogr., A*, 1998, **817**, 5.
- 7 T. Takeuchi and J. Haginaka, *J. Chromatogr., B*, 1999, **728**, 1.
- 8 V. T. Remcho and Z. J. Tan, *Anal. Chem.*, 1999, **71**, 248A.
- 9 L. I. Andersson, *J. Chromatogr., B*, 2000, **739**, 163.
- 10 A. Roda, M. Pazzagli, L. J. Kricka and P. E. Stanley, *Bio-luminescence and Chemiluminescence Perspectives for the 21st Century*, John Wiley & Sons, Chichester, 1999.
- 11 J.-M. Lin, M. Ishii and M. Yamada, *Bunseki*, 1998, 865.
- 12 L. J. Blum, *Bio- and Chemi-luminescent Sensors*, World Scientific, Singapore, 1997.
- 13 J. W. Birks, *Chemiluminescence and Photochemical Reaction Detection in Chromatography*, VCH, New York, 1989.
- 14 S. Tsukada, H. Miki, J.-M. Lin, T. Suzuki and M. Yamada, *Anal. Chim. Acta*, 1998, **371**, 163.
- 15 C. A. M. Garcia, W. R. G. Baeyens and Y. Zhao, *Anal. Chem.*, 1997, **69**, 83A.
- 16 J.-M. Lin, H. Goto and M. Yamada, *J. Chromatogr., A*, 1999, **844**, 341.
- 17 J.-M. Lin, H. Arakawa and M. Yamada, *Anal. Chim. Acta*, 1998, **371**, 171.
- 18 J.-M. Lin and M. Yamada, *Anal. Chem.*, 1999, **71**, 1760.



**Fig. 5** Baselines of  $\text{H}_2\text{O}_2$  carrier solution flowing through newly prepared sensors and typical CL profiles for phen determination. Signals (a)–(c) are the baseline profiles of sensors prepared from polymers P(blank), P(phen-Cu-VPy) and P(phen-Co-VPy), respectively. Signal (d) is the CL profile of phen injected into the carrier after the baseline became stable. Conditions for (d): flow rate of  $0.1 \text{ mol dm}^{-3}$   $\text{H}_2\text{O}_2$  carrier solution  $1.5 \text{ ml min}^{-1}$  and sampling volume  $50 \text{ ml}$ . Phen sampling solution was injected into the carrier after the sensor showed a stable baseline.

- 19 X. Hu, N. Takenaka, M. Takasuna, H. Bandow and Y. Meada, *Anal. Chem.*, 1993, **65**, 3489.
- 20 I. B. Agater and R. A. Jewsbury, *Anal. Chim. Acta*, 1997, **356**, 289.
- 21 J.-M. Lin and M. Yamada, *Anal. Chem.*, 2000, **72**, 1148.
- 22 M. Yamada and S. Suzuki, *Anal. Lett.*, 1984, **17**, 251.
- 23 M. Ishii, M. Yamada and S. Suzuki, *Bunseki Kagaku*, 1986, **35**, 373.
- 24 O. S. Fedorova, S. E. Olkin and V. M. Berdnikov, *Z. Phys. Chem. (Leipzig)*, 1982, **263**, 529.
- 25 K. Watanane, T. Yamasaki and M. Itagaki, *Bunseki Kagaku*, 1996, **45**, 897.
- 26 J. Matsui, I. A. Nicholls, I. Karube and K. Mosbach, *J. Org. Chem.*, 1996, **61**, 5414.
- 27 J. Matsui, I. A. Nicholls, T. Takeuchi, K. Mosbach and I. Karube, *Anal. Chim. Acta*, 1996, **335**, 71.
- 28 T. Yokoyama, T. Akamatsu, K. Ohji and M. Zenki, *Anal. Chim. Acta*, 1998, **364**, 75.
- 29 R. M. Smith and A. E. Martell, *Critical Stability Constants*, Plenum Press, New York, 1975, Vol. 2.
- 30 P. E. Bell, Y. Chen, W. E. Potts, R. L. Chaney and J. S. Angle, *Biol. Trace Elem. Res.*, 1991, **30**, 125.
- 31 C. M. Harris, T. N. Lockyer and H. Waterman, *Nature (London)*, 1961, **192**, 424.
- 32 S. Hanaoka, J.-M. Lin and M. Yamada, *Anal. Chim. Acta*, 2000, **409**, 65.
- 33 S. Hanaoka, J.-M. Lin and M. Yamada, *Anal. Chim. Acta*, 2001, **426**, 57.