

# Flow injection chemiluminescence for determination of dopamine with immobilized reagents technology

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## Abstract

A sensitive flow injection chemiluminescence for dopamine determination was described in this paper. The analytical reagents involved in the chemiluminescence reaction, including luminol and periodate, were both immobilized on the anion-exchange column in a flow injection system. Through water injection, luminol and periodate were eluted from the anion exchange column to generate chemiluminescence, which was inhibited in the presence of dopamine. It was found that the decrease of chemiluminescence intensity was correlated with the logarithm of dopamine concentration in the range from 1.0 to 300 ng mL<sup>-1</sup>. At a flow rate of 2.0 mL min<sup>-1</sup>, the determination including sampling and washing could be performed in 0.5 min with a sampling frequency of 120 h<sup>-1</sup> and a relative standard deviation of less than 3.0%. The flow system was applied successfully to the determination of dopamine in a pharmaceutical injection with recoveries from 94.0% to 104.0%.

**Keywords:** Dopamine; Chemiluminescence; Flow-injection

## Résumé

Nous décrivons dans cet article un système sensible de chimiluminescence à flux continu pour la détermination de la dopamine. Les réactifs analytiques utilisés dans la réaction de chimiluminescence, incluant le luminol et le périodate, ont tous deux été immobilisés sur une colonne échangeuse d'anions dans un système à flux continu. Le luminol et le périodate ont été élués de la colonne échangeuse anionique en injectant de l'eau pour générer la chimiluminescence, qui est elle inhibée en présence de dopamine. Nous avons trouvé que la diminution

de l'intensité de chimiluminescence était corrélée au logarithme de la concentration de dopamine dans la gamme de 1.0 à 300 ng mL<sup>-1</sup>. À un débit de 2.0 mL min<sup>-1</sup>, une détermination qui comprend l'échantillonnage et le lavage pouvait être effectuée en 30 s, avec une fréquence d'échantillonnage de 120 h<sup>-1</sup> et un écart type relatif de moins de 3.0%. Le système d'analyse en continu a pu être utilisé avec succès pour la détermination de la dopamine dans une injection pharmaceutique avec des recouvrements de 94.0% à 104.0%.

## Introduction

Dopamine is a natural catecholamine formed by the decarboxylation of 3,4-dihydroxyphenyl-alanine. It affects brain processes that control movement, emotional response, and the ability to experience pleasure and pain. Imbalanced dopamine activity could cause brain dysfunction and disease, such as the two major Central Nervous System disorder, schizophrenia and Parkinson's disease.

Different methods have been reported for the determination of dopamine, including fluorometry (1), spectrophotometry (2, 3), electroanalysis (4-8), high performance liquid chromatography (9, 10) and capillary electrophoresis (11). For these methods, a detection limit for dopamine determination was normally around the µg mL<sup>-1</sup> level. Chemiluminescence (CL), offering sensitivity, instrumental simplicity, sampling efficiency and reduced consumption, is an attractive alternative for pharmaceutical analysis (12, 13). CL has also been applied to the determination dopamine with different CL systems, including luminol-hexacyanoferrate (14, 15), luminol-chlorate (16), luminol-hypochlorite (17), lucigenin-iron (II) (18) and electrochemiluminescence of luminol (19, 20). However, no CL method has been reported for dopamine determination using a luminol-periodate CL system with immobilized reagents tech-

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nology.

In this paper, a novel CL method for the assay of dopamine was designed using reagent-controlled-released technology coupled to flow injection (FI), which offered simple apparatus, rapid means of detection and reagentless procedure. The proposed method was based on the inhibitory effect of dopamine on the CL intensity between luminol and periodate in alkaline media. The analytical reagents involved in the CL reaction, including luminol and periodate, were both immobilized on the anion-exchange column in the FI system. The decrease of CL intensity was correlated with the dopamine concentration in the range from 1.0 to 300 ng mL<sup>-1</sup>. The determination could be performed in 0.5 min using a flow rate of 2 mL min<sup>-1</sup>, including sampling and washing, hence giving a throughput of 120 h<sup>-1</sup> with a relative standard deviation of less than 3.0%. The method has been successfully applied to the assay of dopamine in pharmaceutical injection and the recovery was from 94.0% to 104.0%.

## Experimental

### Reagents

All chemicals used were of analytical reagent grade. Water purified in a Milli-Q system (Millipore, Bedford, MA, USA) was used throughout. Dopamine (Sigma) and luminol (Fluka, biochemika) were obtained from Xi'an Medicine Purchasing and Supply Station, China. Potassium periodate was purchased from Xi'an Chemical Reagent Plant.

A standard solution of dopamine (50.0 µg mL<sup>-1</sup>) was stored at 4°C. Working strength solutions were prepared daily from the above stock solution as required. Luminol was used as supplied to prepare a 0.25 mol L<sup>-1</sup> stock standard solution in 0.5 mol L<sup>-1</sup> NaOH in a 1-L volumetric flask. A 0.04 mol L<sup>-1</sup> stock standard solution of periodate was prepared by dissolving the solid in distilled water.

### Preparation of immobilized reagents column

Amberlyst A-27 (1.0 g) was shaken with 50 mL of 0.25 mol L<sup>-1</sup> luminol or 0.04 mol L<sup>-1</sup> potassium periodate for 24 h. Then, the resin was filtered, washed with doubly distilled water. The resin was then dried for 12 hours at 30°C before being stored in a desiccator. The most convenient method to determine the amounts of luminol and periodate immobilized on the resin was to measure the loss of these reagents from the immobilization solutions. The concentration was detected at 360 nm for luminol and at 225 nm for periodate by UV-Vis. The amounts of luminol and periodate immobilized were 1.99 mmol

g<sup>-1</sup> and 1.01 mmol g<sup>-1</sup> resin, respectively. A column with immobilized reagents was prepared by packing a glass column (i.d. 3 mm and total volume of about 0.5 mL) with resins containing immobilized luminol (0.05 g) and periodate (0.10 g). The resins were mixed together, and the column was plugged with glass wool at both ends to prevent the resin from leaking through.

### Apparatus

The FI system used in this work was shown in Fig. 1. A peristaltic pump (Shanghai meter electromotor plant, Model ND-15, 15 r/min) was used for pumping all flow streams at a flow rate of 2 mL min<sup>-1</sup>. PTFE tubing (1 mm i.d.) was used to connect all components in the flow system. 120 µL of eluant water, flowing through the immobilized reagents column and containing quantitatively determined luminol and periodate eluates, was injected into the carrier stream by a six-way valve. Before reaching the CL flow cell, the luminol, periodate, sodium hydroxide and dopamine injections were combined in a mixing tube (10 cm in length). The CL emission cell is a twisted glass tube (2 mm i.d., 15 cm length) in order to produce a large surface area to be exposed to the adjacent photomultiplier tube (PMT) (HAMAMATSU, Model IP28). Extreme precautions were taken to ensure that the sample compartment and PMT were sealed well such that no light can leak inside. The CL signal produced from the flow stream was detected without wavelength discrimination, and the PMT output was amplified and quantified by a luminosity meter (Northwest Non-Ferrous Geology Institute of China, Model GD-1), which was connected to a recorder (Shanghai Dahua Instrument and Meter Plant, Model XWT-206).

### Procedures

The water carrier and the solutions (NaOH, sample and eluant) were propelled at a constant flow rate on each flow line. The pump was started to wash the whole flow system until a stable baseline was recorded. Then, 120 µL of eluant water, flowing through the immobilized reagents column and containing quantitatively determined luminol and periodate eluates, was injected into the carrier stream, and mixed with the sample stream. The mixed solution was delivered to the CL cell, and the peak height of the CL signal was detected using the PMT and the luminometer. The concentration of sample was quantified by a decrease in CL intensity,  $\Delta I = I_0 - I_s$ , where  $I_s$  and  $I_0$  were CL signals in the presence and in the absence of dopamine, respectively.

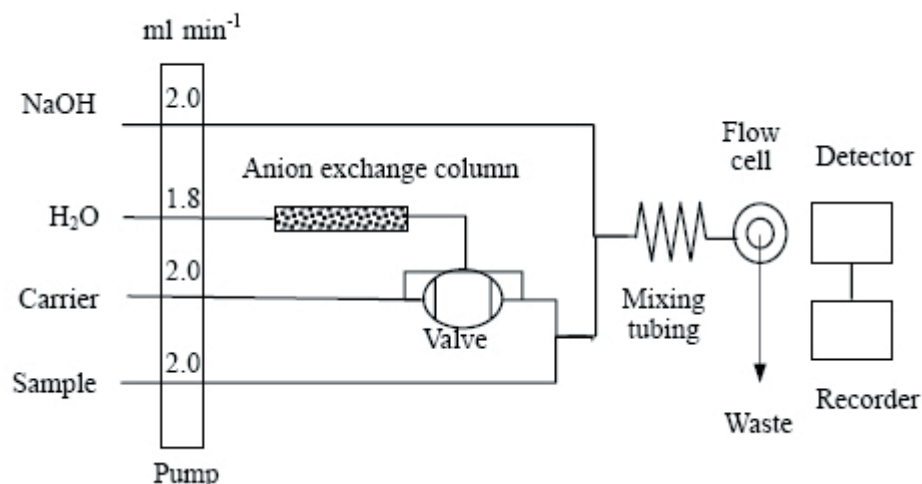


Figure 1. Schematic diagram of the flow injection system for dopamine determination.

## Results and Discussion

### *Time profile of the CL reaction*

The CL intensity-time profile of luminol and periodate reaction, carried out in the presence or absence of dopamine, was examined in the flow system. As Fig. 2 shows, without any special eluant, the mixture of luminol and periodate rinsed by water gave out an evident CL signal. The signal reached a maximum intensity in 5 s and then became extinguished within 15 s after injection. Upon mixing of the sample with the above mixing solution, a decreased CL signal was recorded. The decrease of CL intensity was proportional to the logarithm of dopamine concentration.

### *Designation for the FI-CL system*

The assay was carried out by a continuous-flow mode. Two different manifolds were designed. Through injection of 120  $\mu\text{L}$  water, the reagents on the anion-exchange resin column were eluted and in the presence of dopamine, the CL intensity was inhibited, and the decrease of CL intensity was recorded. When the column containing the immobilized reagents was placed in front of or behind the injection valve, two significantly different results were observed. When the column was placed after the valve, because the immobilized reagents was continuously eluted (including the effect of the water carrier) and there was a high reagent concentration (which results in shorter lifetime of column), the dispersion of the reagent zone was higher, causing the luminescence peaks to become wider. It was also tested, as illustrated by results in Fig. 3, the whole analysis process, including sampling and washing, could be accomplished in 0.5 min

when the column with immobilized reagents was put in front of the injection valve, as in the manifold described in Fig. 1, whereas it took about 2.5 min when the column was put behind the valve. Therefore, the flow system as shown in Fig. 1 was employed for the subsequent work and gave the good precision in the analysis.

### *Selection of eluant*

120  $\mu\text{L}$  amounts of different eluants were injected through the column and released different amounts of luminol and periodate, thus producing the different CL emissions. In this flow system, the characteristics of several eluants, including NaCl,  $\text{Na}_2\text{CO}_3$ ,  $\text{Na}_2\text{SO}_4$ ,  $\text{Na}_3\text{PO}_4$  and  $\text{H}_2\text{O}$ , were evaluated. As Table 1 shows,  $\text{Na}_2\text{SO}_4$  gave a maximum CL emission while  $\text{Na}_2\text{CO}_3$  showed some inhibitive effects on the CL reaction. Nevertheless, it was observed that a continuous flow of eluant through the column resulted in a rather short column lifetime to only a few hours. It was shown that the immobilized luminol and periodate anions on the anion exchange resin undergo dissociation with water, thus releasing trace amounts of luminol and periodate from the column, resulting in an easily observable decrease in CL signal. In this case, the column could be used over 100 h. Higher CL intensity, longer column lifetime, and using water as eluant were considered in subsequent work.

### *Effect of pH on CL and the column lifetime*

The effect of eluant pH on the performance of the column was evaluated. It was found that along with the increase of eluant pH, the CL intensity increased while the lifetime of column decreased considerably (Fig. 4). This phenomenon occurred probably because the quan-

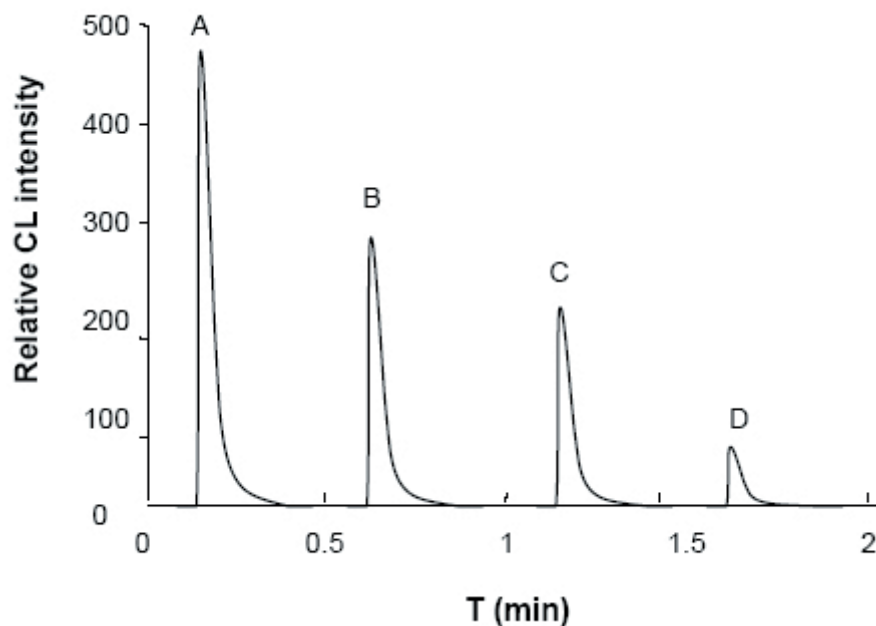


Figure 2. The time profile of CL reaction.

A: CL intensity in the absence of dopamine

B: CL intensity in the presence of dopamine ( $10 \text{ ng mL}^{-1}$ )

C: CL intensity in the presence of dopamine ( $30 \text{ ng mL}^{-1}$ )

D: CL intensity in the presence of dopamine ( $300 \text{ ng mL}^{-1}$ )

HV:  $-750\text{V}$

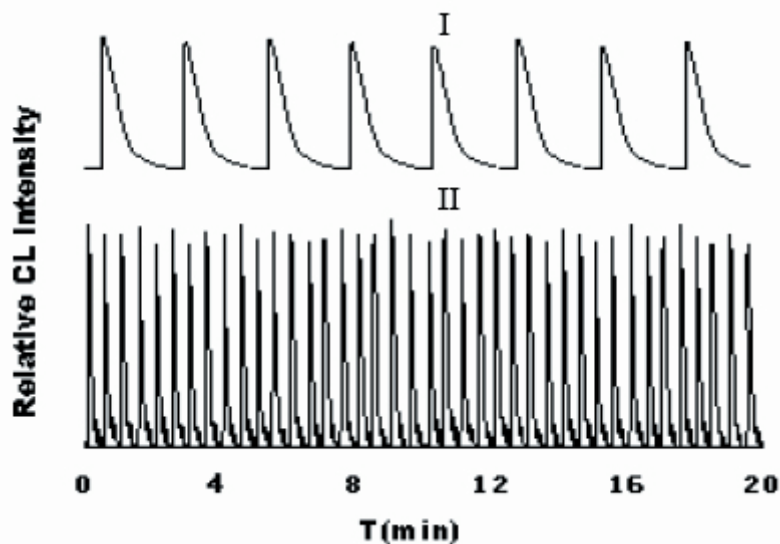


Figure 3. CL signals in two manifolds.

I: The column set behind the valve

II: The column set in front of the valve

The flow rate was  $2.0 \text{ mL min}^{-1}$ .

HV:  $-720 \text{ V}$ .

tities of hydroxide ions in the eluant were increasing. pH 6.5 was then chosen as a compromise between good column lifetime and a sufficient CL intensity. In this case, the column with immobilized CL reagents could be used more than 120 h in a continuous flow mode.

#### *Effect of molar ratio of immobilized luminol and periodate*

To examine the influence of the mixing ratio, resins (0.15 g) with different mixing ratios were packed into a column with the same internal diameter and volume. By

injection of distilled water at a fixed volume of  $120 \mu\text{L}$ , different amounts of luminol and periodate were eluted from the resins and emitted CL signals with different intensity. As Fig. 5 shows, the CL intensity dropped drastically from beginning to the next day, and then it decreased slowly. The most stable CL signal was found with a molar ratio of 1:2 (luminol to periodate) and an average CL intensity was favored for measuring the inhibitory effect of dopamine on CL intensity.

Table 1. Comparison of CL intensities amongst various eluents for dopamine determination<sup>a</sup>.

| Type of analysis condition | Relative CL intensity |      |                                 |                                 |                                 |
|----------------------------|-----------------------|------|---------------------------------|---------------------------------|---------------------------------|
|                            | H <sub>2</sub> O      | NaCl | Na <sub>2</sub> CO <sub>3</sub> | Na <sub>2</sub> SO <sub>4</sub> | Na <sub>3</sub> PO <sub>4</sub> |
| I                          | 226                   | 353  | 19                              | 457                             | 388                             |
| II                         | 205                   | 224  | 102                             | 409                             | 350                             |
| III                        | 21                    | 29   | 7                               | 48                              | 38                              |

<sup>a</sup> The concentration of each solution was  $1.0 \times 10^{-4}$  mol L<sup>-1</sup>.

I: CL intensity in the absence of dopamine

II: CL intensity in the presence of 70.0 ng mL<sup>-1</sup> dopamine

III: The decrease in CL intensity in 70.0 ng mL<sup>-1</sup> dopamine

HV: -700V

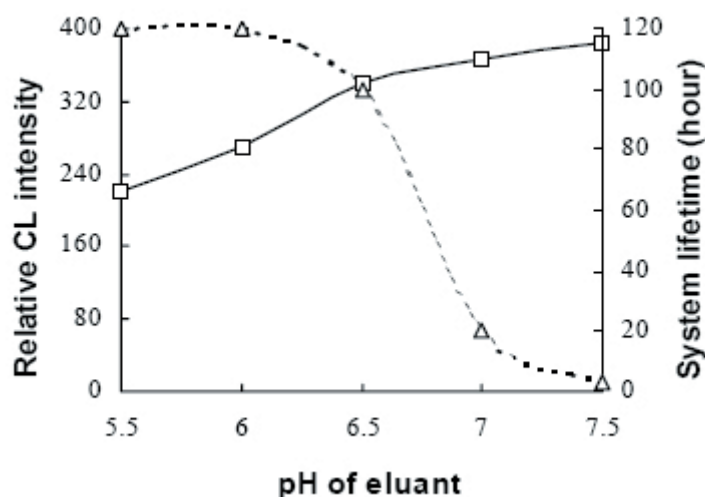


Figure 4. Effect of eluant pH on CL intensity (□) and column lifetime (Δ). HV: -720V

#### Effect of NaOH concentration

Owing to the nature of the luminol reaction, which was more favored under basic conditions, sodium hydroxide was introduced into the manifold through a flow line to improve the sensitivity of the system. As Fig. 6 shows, a NaOH concentration of less than 0.05 mol L<sup>-1</sup> led to an apparent decrease in  $\Delta I$ . The maximum intensity was found with 0.1 mol L<sup>-1</sup> NaOH. Thus, 0.1 mol L<sup>-1</sup> NaOH was selected as an optimal condition.

#### Effect of sampling loop, length of mixing tubing and flow rate

The influence of sampling loop volume of 80, 100, 120, 150, 200  $\mu$ L for the determination of dopamine was tested in the FI system. It was found that the CL intensity varied with volume of sampling loop and that the 120  $\mu$ L loop had the highest CL intensity and sampling frequency. Therefore, the 120  $\mu$ L sampling loop was selected in the experiment. The length of the

mixing tubing was also adjusted to yield maximum light emission in the cell. It was found that a 10 cm of mixing tubing afforded the best results in regards to sensitivity and reproducibility.

The influence of flow rate on signal-to-noise ratio was investigated under different flow rates. The signal-to-noise ratio decreased at a higher flow rate because the higher flow rate affected the rate of contact of sample molecules with the ion-exchange resin. A lower flow rate caused peak broadening and slowed down sampling rates. Nevertheless, the high flow rate could lead to an unstable baseline and shortening of the system lifetime. Therefore, a rate of 2.0 mL min<sup>-1</sup> was then chosen for pumping each of all flow streams as a compromise between good precision and lower reagent consumption.

#### Performance of the system for measurements of dopamine

A series of standard solutions were injected into the

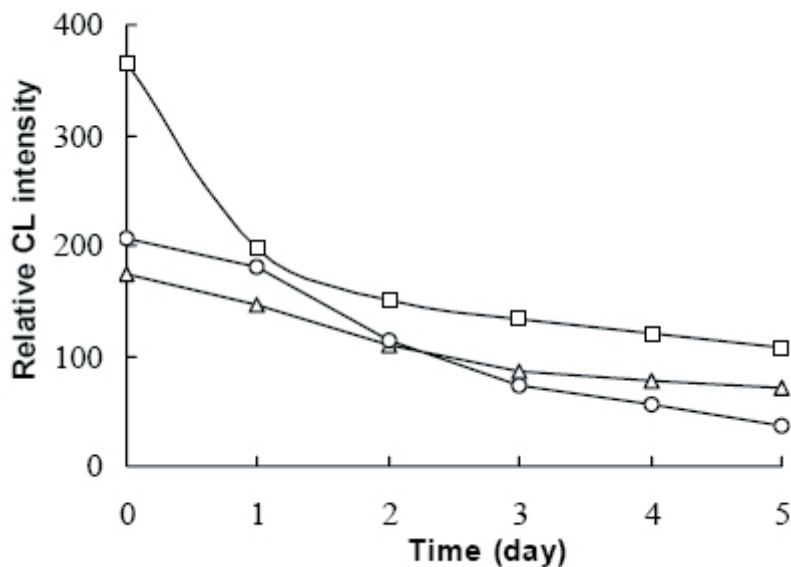


Figure 5. Effect of molar ratio on CL intensity and column lifetime.  
luminol to periodate: □: 1:1; ○: 2:1; △: 1:2.  
HV: -720V

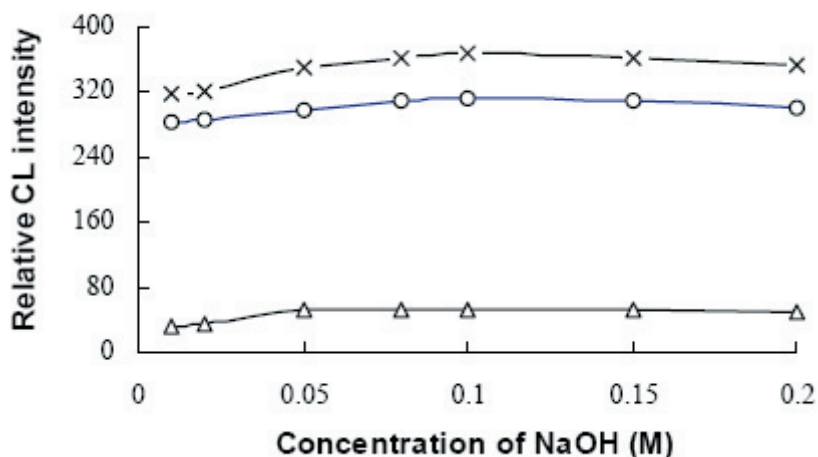


Figure 6. Effect of concentration of NaOH on CL intensity.  
×: CL intensity in the absence of dopamine ( $I_0$ ).  
○: CL intensity in the presence of dopamine ( $I_s$ ).  
△: The decrement of CL intensity ( $\Delta I$ ).  
HV: -720V

manifold depicted in Fig. 1 to test the linearity of dopamine concentration. It was found that the inhibition of CL intensity was linear with the logarithm of dopamine concentration, and the linear range is from 1.0 ng mL<sup>-1</sup> to 300.0 ng mL<sup>-1</sup> and the regression equation is:

$$\Delta I = 145.01 \log C_{\text{dopamine}} + 54.512, R^2 = 0.9982$$

At a flow rate of 2.0 mL min<sup>-1</sup>, the determination of a sample could be performed in 0.5 min, including sampling and washing, giving a throughput of about 120 times per hour with a relative standard deviation of less than 3.0%. The relative standard deviations of five determinations were 2.70, 0.85 and 0.31% with dopamine concentration of 3.0, 30.0 and 300.0 ng mL<sup>-1</sup>, respectively. The limit of detection was 0.3 ng mL<sup>-1</sup>.

### Selectivity Studies

The selectivity study of the proposed method for dopamine was focused on some common metal ions and organic compounds. The tolerable excess concentration of other species which caused no interferences (relative error < 5% in the presence of 100 ng mL<sup>-1</sup> dopamine) were: 500 μg mL<sup>-1</sup> for NH<sub>4</sub><sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>, Zn<sup>2+</sup>, Ni<sup>2+</sup>, Mn<sup>2+</sup>, Cr<sup>3+</sup>, borate, oxalate, tartrate, citrate, methanol, ethanol, urea, glucose, sucrose, gelatine, starch, and dextrin; 100 μg mL<sup>-1</sup> for mannitol; 50 μg mL<sup>-1</sup> for 8-hydroxyquinoline, 10 μg mL<sup>-1</sup> for Cu<sup>2+</sup>.

### Operational stability and life time of the column

120 μL of eluant (water, pH 6.5) was flow-injected

through the column and the CL intensity ( $I_0$ ) was recorded to test the operational stability and the lifetime of the immobilized column. As Fig. 7 shown, the CL signal of the blank was rather stable in a short time (20 min). The total experiment lasted for eight days and the column was tested for over six hours per day. The CL intensity in the first day fluctuated and was unstable. This was possibly due to the swelling of the resin in water. With increased soaking time, the interaction between water and resin seemed to equilibrate and the CL signal ( $I_0$ ,  $I_s$  and  $\Delta I$ ) became steady. Therefore, the column could be used with stability for over five days with a relative deviation of less than 3.0%.

## Application

### Determination of dopamine in pharmaceutical injection

The proposed method was applied to the determination of dopamine in dopamine injections. The injection was diluted by an appropriate factor and then determined directly by the proposed method followed by the procedure introduced in the experiment section without any pretreatment. In order to evaluate the validity of the proposed method for the determination of dopamine in pharmaceutical injections, standard addition methodology was implemented to test the recovery. The results are listed in Table 2. The recoveries for the different concentration levels varied from 94.0% to 104.0% with a relative standard deviation of less than 3.0%.

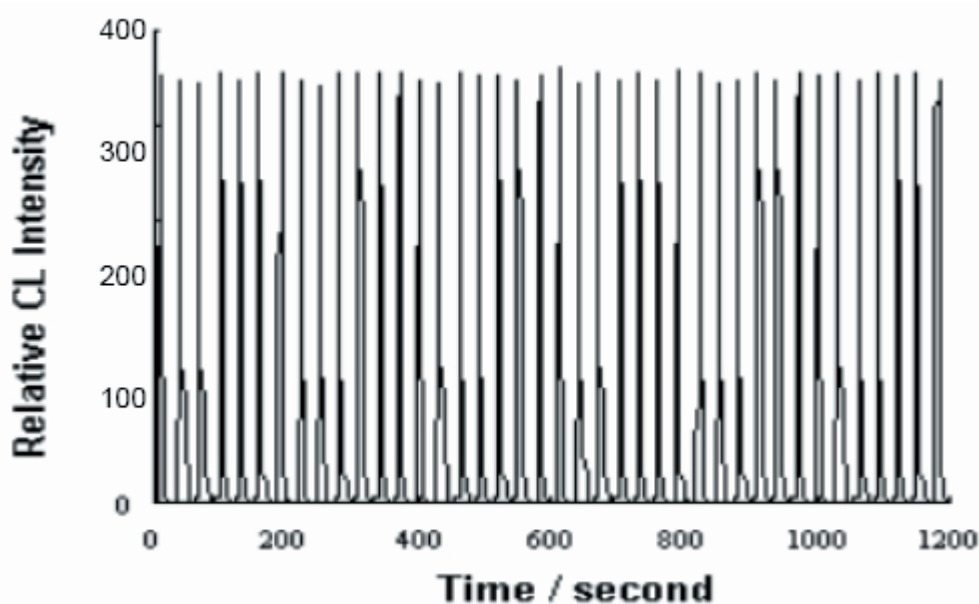


Figure 7. CL signals of the system in the absence of dopamine. HV: -720V

Table 2. Results of dopamine in a pharmaceutical injection<sup>a</sup>.

| Sample | Recovery Results             |                              |              |      | RSD (%) | Found (mg mL <sup>-1</sup> ) | Labelled (mg mL <sup>-1</sup> ) |
|--------|------------------------------|------------------------------|--------------|------|---------|------------------------------|---------------------------------|
|        | Added (ng mL <sup>-1</sup> ) | Found (ng mL <sup>-1</sup> ) | Recovery (%) |      |         |                              |                                 |
| 1      | 10.0                         | 9.4                          | 94.0         | 1.14 | 10.3    | 10.0                         |                                 |
| 2      | 10.0                         | 9.7                          | 97.0         | 1.55 | 10.5    | 10.0                         |                                 |
| 3      | 5.0                          | 5.2                          | 104.0        | 2.09 | 9.9     | 10.0                         |                                 |
| 4      | 5.0                          | 4.9                          | 98.0         | 2.51 | 9.5     | 10.0                         |                                 |

<sup>a</sup> The average of five determinations

## Conclusion

Due to its low detection limit and operational stability, the FI system developed offers good selection, precision and recovery in dopamine determinations. Compared to other methods, the proposed method has advantages of instrumental simplicity, operational convenience and low reducing reagent consumption.

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## References

1. Wang H. Y., Hui Q. S., Xu L. X., Jiang J. G., Sun Y., *Anal. Chim. Acta*, **497**, 1-2, (2003).
2. Maminski M., Olejniczak M., Chudy M., Dybko A., Brzozka Z., *Anal. Chim. Acta*, **540**, 1, (2005).
3. Abdulrahman L. K., Al-Abachi A. M., Al-Qaissy M. H., *Anal. Chim. Acta*, **538**, 1-2, (2005).
4. Othman A. M., Rizka N. M. H., El-Shahawi M. S., *Anal. Sci.*, **20**, 4, (2004).
5. Ravi S. D., Uehara N., Kato, T., *Anal. Chim. Acta*, **478**, 2 (2003).
6. Olivia H., Sarada B. V., Shin D., *Analyst*, **127**, 12, (2002).
7. Yue M. E., Jiang T. F., Shi Y. P., *J. Sep. Sci.*, **27**, 4, (2005).
8. Glod B. K., Stanczak K. I., Wozniak A., *J. Chromatogr. Sci.*, **43**, 4, (2005).
9. Cannazza G., Di Stefano A., Mosciatti B., *J. Pharmaceut. Biomed.*, **36**, 5, (2005).
10. De Toledo R. A., Santos M. C., Cavalheiro E. T. G., Mazo L. H., *Anal. Bioanal. Chem.*, **381**, 6, (2005).
11. Chen Y. L., Yuan J. H., Wang X. Z., Tian C. X., *Anal. Sci.*, **20**, 12, (2004).
12. Roda A., Guarigli M., Michelini E., Mirasoli M., Pasini P., *Anal. Chem.* **75**, 21, (2003).
13. Kricka L. J., *Anal. Chim. Acta*, **500**, 1, (2003).
14. Nalewajko E., Ramirez R. B., Kojlo A., *J. Pharmaceut. Biomed.*, **36**, 1, (2004).
15. Wang S. H., Du L. Y., Wang L. Y., Zhuang H. S., *Anal. Sci.*, **20**, 2, (2004).
16. Sun Y. Y., Tang Y. H., Zheng X. H., Yao H., Xu Z., *Anal. Lett.*, **37**, 12, (2004).
17. Nozaki O., Iwaeda T., Kato Y., *J. Biolum. Chemilum.*, **11**, 6, (1996).
18. Zhang L. H., Teshima N., Hasebe T., Kurihara M., Kawashima T., *Talanta*, **50**, 3, (1999).
19. Guo Z. H., Dong S., *Electroanal.*, **17**, 7, (2005).
20. Li F., Pang Y. Q., Lin X. Q., Cui H., *Talanta*, **59**, 3, (2003).