

## A Cyclic Flow Injection System for A Determination of Thiamine Using A Sensitive Chemiluminescent Detection Associated with Zero Emissions Research Initiative

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### ABSTRACT

A unique analytical technique going along with a concept of The Zero Emissions Research Initiative(ZERI) announced by The United Nations University in 1995, a cyclic flow injection system with a sensitive chemiluminescent(CL) detection was developed for a determination of thiamine. The present system with much possibility of a recycling for the used CL reagents was consisted of a single manifold and also superior to a sensitivity, a simplicity and an economicity in comparison with a current technique. The CL reaction is initiated by oxidation of thiamine in an alkaline potassium ferricyanide solution and then produces thiochrome of an emitter. The CL was also enhanced until an available level for the determination by the energy transfer to a dye-sensitizer uranine. Analytical figure of merits for the present system was as follows, concerning a use of the standard solution of thiamine. The limit of determination:  $2.0 \times 10^{-5} \text{M}$ , magnitude of the determination: 3, sample frequency: 150 for the 100 ml reagent solution, RSD: ca. 2% in 150 repeated runs of  $2.5 \times 10^{-3} \text{M}$  thiamine, stability of the system: one week at least. The present system was also applicable to a real sample analysis and then could successfully contribute to a concept of the ZERI, too.

**KEYWORDS:** Cyclic flow injection analysis, thiamine, chemiluminescent detection, zero emissions research initiative.

## INTRODUCTION

In 1995, The United Nations University announced all chemists a concept of The Zero Emissions Research Initiative(ZERI) associated with the International Summit Conference for Environments in Rio de Janeiro (Brazil) in 1992 [1]. This new concept essentially recommends to create the most comfortable life style for our future with no damage for the environments. Concerning an establishment of the concept, it may take both of much time and much money, but we must access the proposal for our nice future as soon as possible with our reflections on the damage of the environments during last several decades.

The concept is just now started taking this opportunity for an establishment of unique chemical plant based on the ZERI concept by Du'Pont Co.LTD (USA)[1]. Additionally, these research activities are recently recommended in some chemical fields such as chemical engineering, semiconductor industry or Analytical Chemistry[2-6].

According to the concept, we have continued an attempt in Analytical Chemistry in order to contribute to the research activities of ZERI through studies on a cyclic flow injection analysis(cycFIA)[2,4,7]. The cycFIA has some analytical figure of merits as follows; 1) since the system is essentially closed, it has no waste material directly; 2) since the system is automatically and continuously operated, it is available for its ready supply as seen in an emergency-using; 3) since the system is constructed with both of compact scale and simplified design, it can take both of a low cost and a portable small size to be made; 4) especially by a connection with an analytical technique of chemiluminescent(CL) detection, the system is much advanced in sensitivity and utility with a use of small amount of resources as described in our previous report[7] and so on. In other words, these merits are reflected in a development of unique analytical techniques based on the ZERI, especially in a development of their ones with no loading-damage to the environments, by much reducings of total amounts of the used resources and/or the waste materials.

Some cycFIA systems have been studied on their simplifications and/or emergency uses[7-9], but the research activities based on the ZERI concept have not been yet started until today since the ZERI concept is just new one and it has some difficult problems to be started such as much consumption of money and/or time.

A FIA system for a thiamine determination with a CL detection, which

authors targeted in this research, was reported by Grekas et al.[10]. Since however, that system was constructed with two manifolds having the waste water, the system was not gone along with the ZERI concept.

In this paper, a cyclic flow injection system with a chemiluminescent detection(cycFIA/CL) and its characteristics are presented for a sensitive determination of thiamine associated with the ZERI. Of course, the authors recommend in this paper that a design of the cyclic FIA system contributes to the ZERI concept so much.

## EXPERIMENTAL

### Apparatus

Water purifier(Tokyo Rika Kikai Co., Model RO-10C,Tokyo): employed to get deionized water(purified water).

Zeeman atomic absorption spectrometer(ZAAS; Hitachi, Model Z-8000, Tokyo): used for a check of content for some metals such as copper(II) or lead (II) in the CL reagent and water used.

UV/VIS spectrometer(UV; Shimadzu, Model UV-2200, Kyoto): employed to obtain spectra of some CL reagents.

### Reagents

All chemicals used were in analytical grade and purchased from Wako Chemicals Co.(Osaka). Those were also used directly with no further purification.

Purified water: a concentration check concerning metallic ions such as copper(II) or lead (II) was cleared at  $1 \times 10^{-8}$  M levels with ZAAS.

Stock solutions: 0.02 M potassium ferricyanide ( $K_3[Fe(CN)_6]$ ), 4.0 M sodium hydroxide (NaOH), 0.02 M thiamine (nitrate) and  $2.0 \times 10^{-3}$  M uranine were stored with their stabilizings for a week at least.

$K_3[Fe(CN)_6]$ /NaOH solutions: prepared in wide variety of concentrations by arbitrarily mixing (1+1) in volume between 0.02 M  $K_3[Fe(CN)_6]$  and 4.0 M NaOH; their solutions were stable for a week at least.

### Samples

Standard solutions of thiamine( $1.0 \times 10^{-5}$ M~0.01M): prepared by dilution of the stock solution with purified water; mixed to a (1 + 1) in volume with  $2.0 \times 10^{-3}$  M uranine in every use.

Drug sample(Chocola BB; thiamine nitrate, CBB): prepared as follows, according to the procedure reported by Grekas et al.[10]. At first, 18 tablets of CBB as shown in Table 1 were grained into powder as small as possible with an agate ball mill. Next, the powder was dissolved with purified water of ca. 500 ml at 40°C. Negligible small amount of the insolubles for the determination were excluded by filtration with No.5 filter paper of analytical grade. The filtrated solution was just diluted till 1000 ml in total volume with purified water. Thus obtained thiamine concentration was  $6.6 \times 10^{-5}$  M in the sample solution.

Table 1 Composition of one tablet of drug(Chocola BB) sample

Compound	Content
Vitamin B <sub>2</sub> phosphoric acid ester	38 mg
Vitamin B <sub>6</sub>	24 mg
Vitamin B <sub>1</sub> nitrate	12 mg
Nicotinic acid amide	40 mg
Calcium pantotemic acid	20 mg
Yellow No.5	Small amount

### CL reaction scheme

A CL reaction scheme for thiamine(Fig. 1) was essentially reported by Chernysh et al.[10]. Though there were two pathways in the reaction, thiochrome production by oxidation of thiamine was generally essential in order to occur the CL since a reaction speed for the other scheme was so slow in an alkaline solution environment[11]. As indeed, shown in Fig. 2, the typical CL profile obtained with a batch type CL analyzer in commonly use[12], provided just a sharp slope with so fast reaction speed. However, no other CL responses came during 2 hours at least. These observations suggested that the pathway of thiochrome production was essential with its good reaction selectivity concerning the operating conditions .

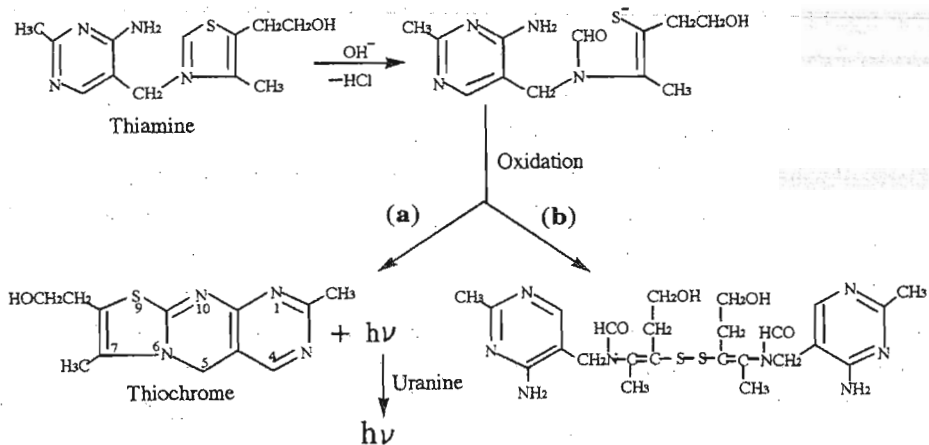


Fig. 1 CL reaction scheme for thiamine<sup>10)</sup>

(a) : Main-scheme, (b): Sub-scheme

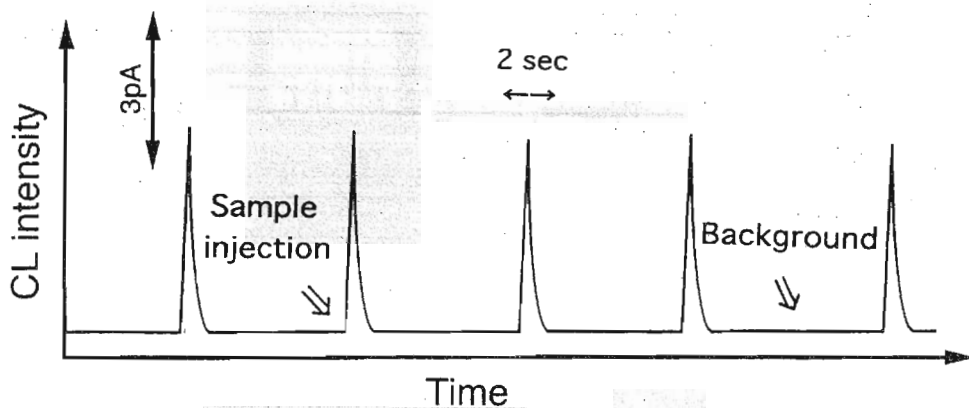


Fig. 2 Typical CL profile obtained with the Batch type CL analyzer

Operating conditions: 4 ml of  $5 \times 10^{-3}$  M  $\text{K}_3[\text{Fe}(\text{CN})_6]$ /2 M NaOH  
 and 0.2 ml of  $1 \times 10^{-3}$  M thiamine.

### Cyclic FIA/CL system

The cycFIA/CL system was designed by use of the following tools as shown in Fig. 3.

P: peristaltic pump(Atto, SJ-1220, Tokyo), S: sample injector(Kusano Kagaku, 10  $\mu$ l Loop Valve, Tokyo), D: detector(Hamamatsu Photonics, R376 photomultiplier tube, Shizuoka), E: electrometer(Advantest, TR-8641, Tokyo), C; computer system(NEC, PC9801RX connected with NEC-GPIB/TNT board of Japan Instrument Co. LTD, Tokyo), R: multi-pens recorder(Rikadenki Kogyo, R-64M, Tokyo). The manifold was made by a 1.0 mm i.d. silicone tubing. A spiral type reaction cell installed in the detector(D) was made by a 0.80 mm i.d., 2.38 mm o.d. and 90 cm long *Tygon*<sup>®</sup> tubing and it was set up in a darken box. A distance between the sample injector and the cell was kept at 10 cm as short as possible since the present CL reaction was so fast.

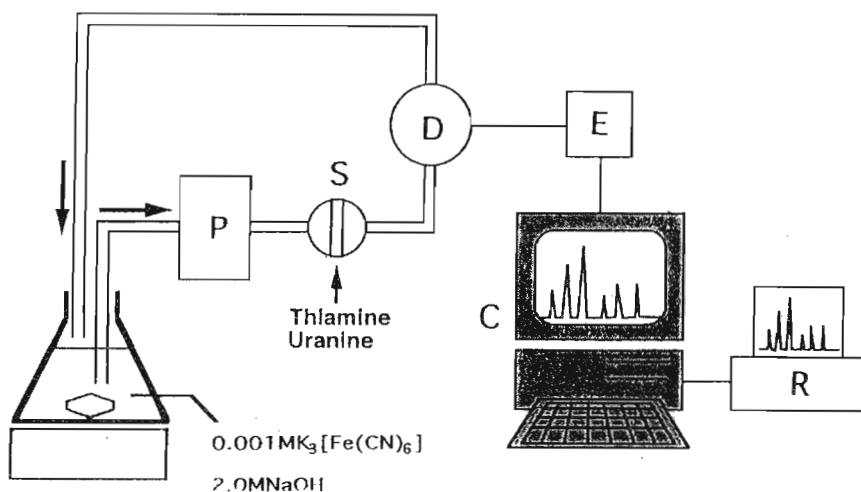


Fig. 3 Schematic diagram of a cyclic FIA/CL system

P: Peristaltic pump, S: Sample injector, D: Detector, E: Electrometer, C: Computer system and R: Recorder; see in the text concerning the detail of the tools.

Optimal operating conditions: 10  $\mu$ l injection of thiamine/1.0 $\times 10^{-3}$  M uranine sample and 100 ml of 1.0 $\times 10^{-3}$  M  $K_3[Fe(CN)_6]$ /2.0 M NaOH for CL reagents at flow rate of 7.0 ml/min.

## RESULTS AND DISCUSSION

### Design of the cyclic FIA/CL system

The cycFIA system is essential to be constructed with a single manifold, a long period stability of used CL reagents, no interference to the measurement occurred by mixing of prepared CL reagents and some products after the CL reaction as described in our previous report[7]. Since two reactants of  $K_3[Fe(CN)_6]/NaOH$ (reagent) and thiamine (sample) were essential to the present CL reaction, a construction of cycFIA was exactly so easy. As however, shown in Fig. 2, the CL response was very weak at a few pA level in terms of CL current intensity, since a sample injection size was kept at  $10 \mu l$  as small as possible because of both of much less consumption of CL reagents and much less interference to the measurement occurred by some products after the CL reaction. As therefore, some CL enhancement techniques were indispensable to a practical use of the system, the enhancement with just a dye-sensitizer was investigated taking a consideration about a simplification of the system.

Uranine, eosins Y and B in the several tested sensitizers at  $1 \times 10^{-3} M$  levels were available for the system with several hundred times larger CL enhancement in comparison with a case of none sensitizer. Especially, the CL enhancement with uranine was the most applicable to the system with about three times larger CL enhancement compared with that by a next usable sensitizer eosin Y to uranine. However, a long periods-coexistence of uranine with the CL reagents was not acceptable because of providing a consumption of the CL reagents by somewhat a very minor CL reaction among their CL reagents. Therefore, a mixed solution of thiamine(test material) and uranine(sensitizer) was injected from the sample injector into the cycFIA/CL system as shown in Fig. 3.

### Optimisation of operating conditions for the cycFIA/CL system

Concerning an optimisation of the system, concentrations and flow rates of used CL reagents should be investigated on getting the higher CL response. The optimisation was also performed concerning just 100 ml of the CL reagent solution since its case had just a bit waste materials.

A concentration of NaOH solution was also fixed at 2.0 M of the excess in order to proceed the present CL reaction stably according to the report by Grekas et.al.[10], since the minor CL reaction as mentioned above may reduce

NaOH and  $K_3[Fe(CN)_6]$  by itself.

### 1) $K_3[Fe(CN)_6]$ in 2 M NaOH

A concentration of  $K_3[Fe(CN)_6]$  ranging from  $1.0 \times 10^{-4}$  M to 0.01 M was examined to get the most intensive CL intensity. As a result,  $1.0 \times 10^{-3}$  M  $K_3[Fe(CN)_6]$  was obtained to enhance the CL much more although the concentration was different from  $5.0 \times 10^{-3}$  M previously reported by Grekas et al.[10], since our system was modified to have a miniature. Therefore,  $1.0 \times 10^{-3}$  M  $K_3[Fe(CN)_6]$  was used for the following experiments.

### 2)Uranine

According to the usually using CL enhancement techniques such as a surfactant micellar effect[13,14] and/or a dye-sensitisation[14,15], the present CL was examined for the enhancement. Just some dye-sensitizers such as uranine or eosins B and Y, were successfully used to enhance the CL, and uranine was the most usable to do that as described above. The CL response also increased going along with an increase of uranine concentration. Since however, the concentration of  $1.0 \times 10^{-3}$  M was available for the sensitive determination of thiamine and the low concentration was also better for the cycFIA/CL system, the concentration,  $1.0 \times 10^{-3}$  M was used for the following experiments.

### 3)Flow rate

A flow rate of CL reagents ranging from 1.0 ml/min to 10.0 ml/min was examined to get the higher CL intensity. The CL intensity increased exponentially according to an increase of the flow rates, since the CL reaction speed was so fast as shown in Fig. 2. However, we selected the optimal flow rate 7.0 ml/min since the system was overloaded at the flow rate of more than 7.0 ml/min.

## Characterizations of the proposed system

### 1)Calibration

A calibration curve for the determination of thiamine with the present system was investigated by use of the standard sample solutions(10 species ranging from  $1.0 \times 10^{-5}$ M to 0.010 M). The linearity between a molar concentration of thiamine( $[Y]/M$ ) and a CL current intensity( $[X]/pA$ ) was observed in their logaligm scales as follows.



$$[Y] = 1.01 \times 10^{-5} [X] + 1.0 \times 10^{-6}$$

(Lower limit of the determination:  $2.0 \times 10^{-5}$  M, Upper limit of the determination:  $4.0 \times 10^{-3}$  M, Correlation coefficient in 5 repeated runs: 0.99)

Thus obtained calibration curve was advanced with a figure of merits in the ten times-wider determination range in comparison with the case reported by Grekas et.al.[10].

## 2) Frequency of sample injection

The typical analytical result was shown in Fig. 4 when the standard samples of thiamine were repeatedly fed into the cycFIA/CL system. As shown in the figure, 150 times sample feedings at  $2.5 \times 10^{-3}$  M thiamine were available for the CL reagent solution of 100 ml. On the other hand, the cases of  $2.0 \times 10^{-5}$  M and  $4.0 \times 10^{-4}$  M standard solutions of thiamine provided successful results with 175 and 160 times sample feedings, respectively. Although the results of the two should provide the sample feedings of more than 1000 times at least on their theoretically calculations, contrary to our expectation, they were not revealed well with a gradually increasing of the CL signals after their limitations of the sample feedings. The lower limitations in the latter two cases were dependent on an accumulation of sensitizer uranine in the system, since a change of the uranine concentration corresponded to that of the CL signals as mentioned above. Therefore, either an excluding of uranine from the system and/or its recycling going along with the ZERI concept or a scale up for a volume of the CL reagent solution is indispensable in order to increase the sample frequency much more over the limitation.

## 3) Stability

The CL reagents in the system were available for a restarting of the determinations after an interval for a week at least at the sample feeding point of 10 times before the limitation, although the coexistence of CL reagents and uranine would provide somewhat the minor CL reaction. However, the exclusion of uranine from the system will expand a period of the stability so longer by the same reason as for studies on the sample frequency as above mentioned.

## Analysis of a real sample

Some components in the real sample, vitamins B<sub>2</sub> and B<sub>6</sub>, phosphoric

ester and nicotinic amide have no interference to the present CL as reported by Grekas et.al.[10], but another substances such as calcium pantotenic acid have not been examined concerning the interference. Therefore, the real sample analyses were performed by both methods of a standard addition and a calibration including a test of reliability for our system.

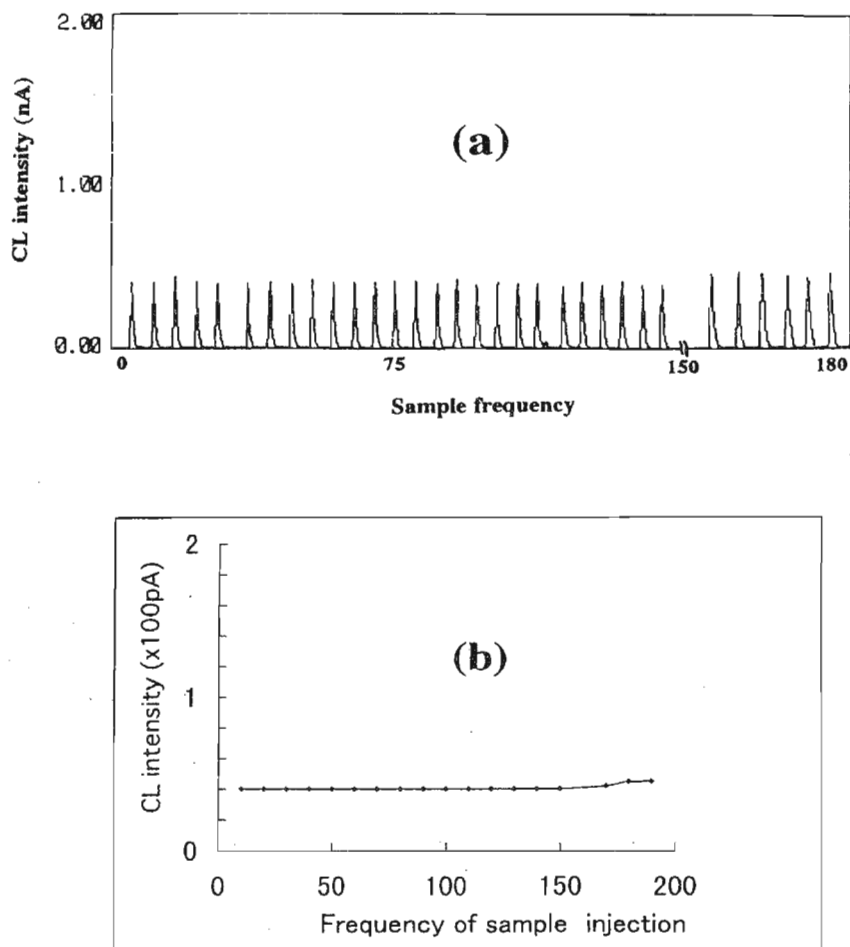


Fig. 4 Typical profile and/or check of the sample frequency concerning the proposed cycFIA/CL system.

Operating conditions: (a),  $2.5 \times 10^{-3} \text{M}$  thiamine/ $1.0 \times 10^{-3} \text{M}$  uranine and  $1.0 \times 10^{-3} \text{M}$   $\text{K}_3[\text{Fe}(\text{CN})_6]$ / $2.0 \text{ M}$  NaOH; (b),  $4.0 \times 10^{-4} \text{M}$  thiamine/ $1.0 \times 10^{-3} \text{M}$  uranine and  $1.0 \times 10^{-3} \text{M}$   $\text{K}_3[\text{Fe}(\text{CN})_6]$ / $2.0 \text{ M}$  NaOH.

The both determined values were  $6.5 \times 10^{-5}$  M vs. the check value of  $6.6 \times 10^{-5}$  M with an almost good agreement. These results suggested the present cycFIA/CL system was highly selective for the determination of thiamine. Additionally, the authors confirmed that an addition of some saccharides such as glucose and lactose, starch, some inorganic substances such as NaCl and  $\text{KH}_2\text{PO}_4$  and EDTA provided no interference to the thiamine determination.

### Evaluation of the present cycFIA/CL system on the ZERI concept

Concerning the ZERI concept, the present cyclic system was compared among the two current ones, i.e., the FIA reported by Grakes et.al.[10] and official method[16]. The result was shown in Table 2.

As seen in the results, the present system was superior to many items listed in comparison with the current one. Especially, total amount of the waste material for the present system was ca. ten- and ca. a hundred-times less for the 100 ml CL reagent solution than those of the two current FIA systems, respectively; in other words, the present system contributed to the minimisation of waste materials going along with one of the ZERI concepts recommended.

### CONCLUSION

The new cycFIA/CL system associated with the concept of ZERI was developed for the sensitive determination of thiamine. The present system was advanced in the sensitivity, the economicity and the simplicity in comparison with the current techniques with the following analytical figure of merits; lower detection limit:  $1.0 \times 10^{-5}$  M, lower limit of the determination:  $2.0 \times 10^{-5}$  M, magnitude of the determination: 3, RSD: ca.2 % in 150 repeated runs of the  $2.5 \times 10^{-3}$  M standard solution of thiamine, sample frequency: about 150 for 100 ml of the CL reagent solution and stability of the system: a week at least.

This present system has not been perfectly zero-emmissionized, but the authors appreciate that this system contributes to the concept of ZERI with a minimisation of total amount of used materials which is one of the concept[3].

As described above, either a separation or an immobilization of uranine from the system is essential in order to provide the recycling system much

Table 2 Comparison of some characteristics of the system among the cycFIA/CL(A), the current FIA(B)<sup>10</sup> and official method(C)<sup>16</sup> concerning the determination of thiamine

Items	System		
	(A)	(B)	(C)
<b>Analytical aspects</b>			
Sample size	10 $\mu$ l	Continuous flow	15 ml
Analytical technique	Closed FIA	Opened FIA	Spectrophotometry
Sample frequency	150/100 ml	ca.75/100 ml	ca.2/100 ml
Limit of the determination	2.0x10 <sup>-5</sup> M	2.00x10 <sup>-5</sup> M	sub-mg
Reproducibility(RSD)	ca. 2 %	< 2 %	ca. 2 %
Analytical time required/1 sample	A few sec.	ca.32 sec	several minutes
Others	Energy transfer	-----	Use of hazardous compound(HCN)
<b>Characteristics of the system<sup>a)</sup></b>			
CL reagent solutions			
Volume	100 ml	100 ml	100 ml
Component(concentration)	NaOH(2 M) K <sub>3</sub> [Fe(CN) <sub>6</sub> ](1x10 <sup>-3</sup> M) Uranine(1x10 <sup>-3</sup> M)	NaOH(2 M) K <sub>3</sub> [Fe(CN) <sub>6</sub> ](5x10 <sup>-3</sup> M)	NaOH(1→10M) HCl(1x10 <sup>-3</sup> M) CNBr(ca.0.01M)
Sample species	Standard/Drug	Standard	Standard
Sample volume	10 $\mu$ l	2.9 ml/min	5 ml
Sample frequency	150	ca.75	ca. 2
Waste water			
Total volume/150 samples	100 ml	ca.430 ml	ca.4000 ml
Residues after 150 repeated analyses			
i )Component			
	NaOH K <sub>3</sub> [Fe(CN) <sub>6</sub> ] Uranine K <sub>4</sub> [Fe(CN) <sub>6</sub> ] Thiochrome	NaOH K <sub>3</sub> [Fe(CN) <sub>6</sub> ] K <sub>4</sub> [Fe(CN) <sub>6</sub> ] Thiochrome	Unknown
ii )Total amount/150 samples	< 9 g	< 40 g	>> 500 g
iii) Possible CL reagents for the recycling	Uranine	none	none

a)The value for the two current methods was estimated to 100ml of the reagent solution used.

more close to the ZERI concept. Today, we have achieved the separation of uranine from the waste water using the ultrafiltration membrane technique applicable to the recycling with a few successful data. Studies on the immobilization technique of uranine attached to the reaction cell are also in progress. The advanced cycFIA/CL system connected with these techniques will be soon presented in this next coming report.

The cycFIA/CL technique is one model of analytical techniques based on the ZERI concept. The authors expect many analytical fellows will join the research activities going along with the ZERI concept, since we are sure the research activities are available for the flow analyses such as both chromatographies of Liquid and Gas, too.

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#### REFERENCES

- 1) Tokyo United Nations University: The 1st International Congress for The Zero Emissions Research Initiative promoted by The United Nations University, Abstracts of Papers, Tokyo, 1995/April 6th-7th, p.1.
- 2) M. Ishii: J. FIA, 12(2), 143(1995); S. Motomizu: Bunseki, No.10, 865 (1997).
- 3) M. Ishii: Proceedings of the 46th Annual Meeting of the Japan Society for Analytical Chemistry, Tokyo, October 1997, p.363.
- 4) M. Ishii & N. Kambara: Clean Technology, 6(3), 45(1996).
- 5) M. Ishii & M. Itoh: Optronics, No.172, 151(1996).
- 6) M. Ishii & M. Sakurai: J. Display, 2(2), 46(1996).

- 7) M. Ishii, M. Yamada & S. Suzuki: *Bunseki Kagaku*, **36**(5), 316(1987).
- 8) M. Yamada & S. Suzuki: *Anal. Chim. Acta*, **193**, 337(1987).
- 9) J.L. Burguera & M. Burguera: *Anal. Chim. Acta*, **214**, 429(1988).
- 10) N. Grekas & A.C. Calokerinos: *Talanta*, **37**, 1043(1990).
- 11) G.P. Chernysh, R.P. Poponina, V.I. Buntushkin: *J. Anal. Chem. USSR*, **31**, 1475(1976).
- 12) M. Ishii, T. Takahashi & S. Babasaki: Proceedings of the 52nd Symposium of the Japan Society for Analytical Chemistry, Obihiro, May 1991, p.415.
- 13) M. Ishii, M. Yamada & S. Suzuki: *Bunseki Kagaku*, **35**(4), 373(1986).
- 14) M. Ishii & M. Yamada: *Bunseki(Reviews)*, No.6, 452(1994).
- 15) M. Ishii & M. Shirai: *Bunseki Kagaku*, **41**,125(1992).
- 16) *Pharmacopoea Japonica*: "Official method for thiamine hydrochloride", p.424, Hirokawa, Tokyo, 1986.

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