Application of a Steady-State Flow Analysis to a Long-Period Reaction in a Continuous-Flow System

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(Received May 23, 1994)

A steady-state flow analysis (SSFA) has been developed and applied to the determination of trace amounts of manganese in an unsegmented flow system. Because this method is no longer characterized by the sample zone dispersion and/or the sequentiality, sharp decreases in the analytical speed and sensitivity for long-period reactions can be avoided. This method has been demonstrated to be useful, especially for long-period reactions in continuous-flow systems. Optimizations of a steady-state flow manifold are described in detail, and determinations of sub-nanogram manganese have been carried out in a long-period reaction of manganese-catalyzed malachite green with periodate. As a result, several improvements were made in comparison with an ordinary flow-injection method: The detection limit decreased to ca. one-thirtieth, the analytical speed was ca. 2.5 times, the coil length was one-twelfth, the dispersion coefficient was ca. one-eighteenth, while the reaction time was ca. 2.3 times. Determinations of manganese in reference materials of NIES of Japan were performed, and the results were obtained as follows: Manganese in $\mu g\,g^{-1}$ in sargasso, tea leaves, and rice flour (containing high cadmium content) were 22.0±2.6, 686±19, and 35.7±1.7, respectively. These results correspond well with their reference values.

Recently, the partial or complete replacement of human participation in laboratory processes has been a growing trend. Many automatic batch analyzers have been developed utilizing both microcomputer and robotic technology. However, cost reduction is yet another aim of the growing trend towards automation. Consequently, it has become necessary to develop an automatic continuous analytical system which can correspond to the treatment for traditional chemical concepts and at low cost. Since the continuous-flow approach is the most flexible way to handle solutions, it is worth studying further.

Generally speaking, how to depress or control the dispersion of the sample zone is an important problem throughout the entire development of a continuous-flow analysis. Irrespective of the dispersion in any flow systems, a key to success in a flow-analysis method first requires signals having good producibility. The combination of a steady-state concept and an air-introducing approach has made air-segmented flow analysis (SFA)[1] successful. However, since there were some problems, such as the low throughput rate and complexity, its application was very limited. Since the flow-injection analysis (FIA)[2] concept was proposed, a large number of FIA manifolds have been developed for several analytical purposes. Because the transition from baseline to baseline via a steady-state stage requires more time than that of SFA, a sharp decrease in the sampling speed would lead to a reduction in its usefulness. This is why the steady-state concept was given up regarding FIA.[3] Although FIA signals have a well-controlled dispersion pattern, they depend critically on the physical processes and chemical kinetics of the FIA system, such as the flow rate, sample volume, tube geometry,[4,5] mixing degree of the solution and reaction conditions. Further, since it is a contradiction to increase the sensitivity and the sampling frequency simultaneously in a time-share manner, extremely slow chemical reactions have almost been refused to be used in FIA. Therefore, it is known that FIA cannot deal directly with those subjects concerning steady-state concepts and extremely slow chemical reactions.

Two kinetic processes, physical dispersion and a subsequent chemical reaction, occur simultaneously in any flow system; their interaction is very complicated. Therefore, an exact description of the chemical kinetics which take place in the FIA system is very difficult; this is one of the reasons why so few papers dealing with the theory of chemical kinetics in FIA systems have been published.[6—14]

The stopped-flow FIA (SFFIA)[15] increases only a few fold in sensitivity, whereas the injection of the next sample cannot be effectively carried out during the stop time. It is also important that no parasitic phenomenon, such as dilution caused by a concentration gradient, should occur during the stop time (commonly between 10 and 20 seconds). If this condition is not fulfilled, the FIA mode gives poor reproducibility, and is hence of little analytical interest. Therefore, the SFFIA approach has not been applied to long-period reactions. The sequential-injection analysis (SIA),[16] similar to SFFIA, is also unsuitable for long-period reactions.

Trials of applying the FIA mode to a long-period reaction, such as the determination of chemical oxygen demand (COD), were carried out.[17—19] It was shown that ca. 50-m long coils are required to finish ca. a 30 min reaction. In this instance, an exceedingly slow throughput rate and a significant decrease in the sensitivity would make this mode uninteresting for further development.

The steady-state flow analysis (SSFA)[20] proposed by the present authors can be considered to be a continuous-flow batch method. It is an appropriate method for solving the problems mentioned above. The object of this paper is to consider the further development of the method (SSFA) as well as an application to the catalytic determination of manganese based on a malachite green (MG)-periodate system.

Theoretical

Multi-Treatment Model. Any cross-contamination between samples is usually related to the design and functioning of the flow-analysis manifolds and, particularly, to the sampling rate. This is a major limiting factor in a flow manifold utilizing a time-share process. Since in batch methods every sample preserves its integrity in its own vessel, cross-contamination can only originate from the withdrawal system. Parallel and stopped-flow techniques are mostly expected to be utilized for this purpose, although neither is sufficiently good.

Consequently, a new type of double-line-multi-channel valve (cf. Fig. 1) was designed and used to construct a steady-state flow system. This valve skillfully utilizes the combined techniques of a parallel FIA, a stopped-flow FIA and a steady-state determination model; the injection, storage, and detection of samples are carried out simultaneously in a space-share manner. This system can be considered to have two independent constructions, a reactor and a detector. In the former, the samples are treated until they are aspirated into a detector cell at the final stage. In the latter, a carrier solution flows in the sample zone through a flow-cell. This therefore makes it possible to obtain a high sampling frequency and a high sensitivity, even though the reactions are extremely slow.

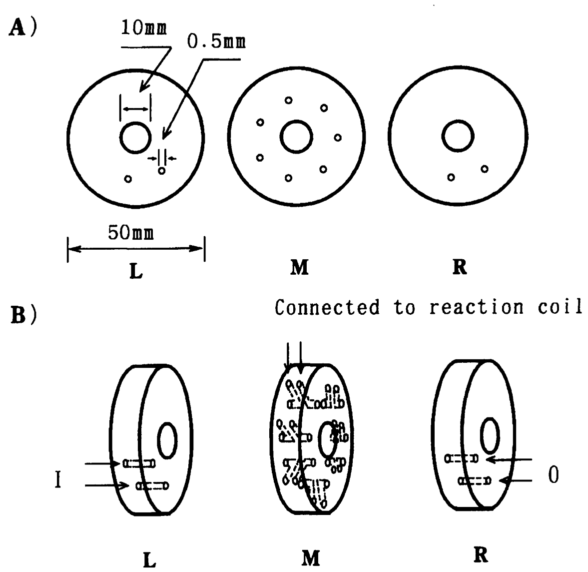

Fig. 1. Schematic drawing of SSFA two-line two-side seven-channel valve. (A) Cross section of the seven-channel valve. L, the left disk of valve; M, the middle disk of valve; R, the right disk of valve. (B) Construction of the seven-channel valve. I, inlet; O, outlet;

Fundamental of Manifold Design. A general relationship between the peak-height signal and the sample volume (S_v) shown in the following equation[3] is used to begin the next discussion:

$$\frac{C^{\max}}{C^{0}} = 1 - \exp(-kS_\mathrm{v}) = 1 - 2^{-n}, \tag{1}$$

where C^0 is the original concentration of the sample, $C^{\max}$ the maximum concentration of the sample at the apex of the peak, $n = S_\mathrm{v}/S_{1/2}$, and $S_{1/2}$ the volume of sample solution necessary to reach 50% of the steady-state value, corresponding to a dispersion coefficient (D) of 2. This D has been defined as the ratio of the concentrations of a sample material before and after the dispersion process has taken place; that is, $D = C^0/C^{\max}$.

By differentiating Eq. 1, we obtain

$$\frac{\mathrm{d}C^{\max}}{C^{0}} = (2^{-n} \times \ln 2)\mathrm{d}n. \tag{2}$$

By substituting Eq. 1 into Eq. 2, one can write

$$\begin{aligned}\frac{\mathrm{d}C^{\max}}{C^{\max}} &= \frac{(n \times \ln 2)}{(2^{n} - 1)} \times \frac{\mathrm{d}n}{n} \\ &= A \times \frac{\mathrm{d}n}{n} = A \times \frac{\mathrm{d}S_\mathrm{v}}{S_\mathrm{v}} \end{aligned} \tag{3}$$

where A represents $(n \times \ln 2)/(2^{n} - 1)$. Equation 3 can be rewritten for relative error yields as

$$\frac{\delta C^{\max}}{C^{\max}} = A \times \frac{\delta S_\mathrm{v}}{S_\mathrm{v}}. \tag{4}$$

The values of $\delta S_\mathrm{v}/S_\mathrm{v}$ corresponding to the $\delta C^{\max}/C^{\max}$ values of 1 and 0.5%, respectively at different n values or dispersion coefficients have been calculated, and are listed in Table 1. From Table 1, it can be seen that the relative errors of the signals have almost the same level compared with those of the sample volumes in a range from a small dispersion to a large dispersion or more. However, from a small dispersion to the steady state, the signal becomes hardly influenced by the sample volume, the larger is the injected sample volume, the larger is its permissible relative error. When the n value is as high as 5, the relative error of the signal is only 0.5% even if the volume relative error is as high as 4%. The signal at about $n = 10$ is practically equal to the steady-state signal within the measuring precision of the instrument. These relationships suggest that the steady-state is applicable to unsegmented flow systems.

It is also noted that a signal with ca. 97% of C^0 can be obtained, provided that the n value is about 5. Under this condition, the construction of parallel coils can be easily made. Therefore, this system can be operated under two conditions: the quasi-steady state and the steady state, respectively, according to typical n values of 5 and 10. The former is sufficiently good for general analytical purposes. If steady-state operation is required, for example, when a chemical equilibrium concept or a pure chemical kinetics treatment is necessary, the latter can be used.

Table 1. Relationship between $\delta C^{\max}/C^{\max}$ and $\delta S_v/S_v$ at Different n or $C^{\max}/C^0$ Values

n	A	$C^{\max}/C^0$	D	$\delta S_v/S_v$ (%)	
				$\frac{\delta C^{\max}}{C^{\max}}$ (1%)	$\frac{\delta C^{\max}}{C^{\max}}$ (0.5%)
$\to 0$	$\to 1$	$\to 0$	$\to \infty$	$\to 1$	$\to 0.5$
0.05	0.983	3.41×10^{-2}	29.4	1.02	0.509
0.1	0.965	6.70×10^{-2}	14.9	1.04	0.518
0.5	0.837	0.293	3.41	1.20	0.598
1	0.693	0.500	2.00	1.44	0.722
5	0.112	0.969	1.03	8.94	4.47
7	3.82×10^{-2}	0.992	1.008	26.2	13.1
10	6.78×10^{-3}	0.999	1.001	147	73.5
20	1.32×10^{-5}	0.999999	$1+10^{-6}$	75639	37820
$\to \infty$	$\to 0$	$\to 1$	$\to 1$	$\to \infty$	$\to \infty$

Experimental

Reagents. All of the chemicals used were of analytical reagent grade unless otherwise noted. Ultra-pure water was used to prepare solutions or for a carrier throughout. A stock acetate solution (pH 4.4) was prepared by dissolving the sodium acetate and acetic acid in water. A stock solution of manganese (1.0 g dm^{-3}, atomic absorption grade, Wako) was used. Standard manganese solutions were prepared by suitably diluting the stock solution with the acetate buffer solution before being used. Malachite Green (MG as oxalate, Wako) was used without further purification; a stock solution (2.2×10^{-3} mol dm^{-3}) was prepared by dissolving the reagent in water. A potassium periodate solution (2.5×10^{-3} mol dm^{-3}) was prepared by dissolving the reagent in water, and was used within two weeks.

Apparatus. **Multi-Channel Valve and Manifold:** A hand-made double-line-seven-channel valve (Fig. 1) consisted of three pieces of polytetrafluoroethylene (PTFE) disks, in which the left and the right parts had the same construction with two pores of internal diameter 0.5 mm; and the middle disk had seven pairs of pores of the L-type and of the same size. PTFE coils were connected to each pore pair in the middle as well as those pores outside the right and left disks.

A diagram of the steady-state flow system for manganese determination is shown in Fig. 2. A multichannel peristaltic pump with Tygon tubings was used to feed reagent and sample solutions, and another pump with a single-channel was used to flow water as a carrier. The manifold consisted of a seven-channel valve and PTFE tubes. A JASCO UVIDEC-340 double-beam spectrophotometer equipped with an 8-μl flow-cell was employed. A Chromatocorder-12 of (System Instruments Co.) and a (NEC PC-9801 RA) personal computer were used for the recording and data acquisition of the SSFA signals. A Yamato–Komatsu circulating thermostatic bath was used to control the reaction temperature at 40±0.1 °C.

Procedure. The sample was mixed with reagents at the confluence point (M) and then introduced into any one washed coil in the valve, where the front of the solution stream was wasted out through the outlet of the valve. At the same time, the washing water passed through the next coil as well as the flow-cell to the waste. Then, when the valve was rotated again, the previous sample solution was stored into one of the reaction coils immersed in a water-bath at 40±0.1 °C; the sample injection as well as the coil washing were carried out simultaneously. After one rotation period being completed, the reacted sample solution was washed with the carrier and passed through the flow-cell, where the absorbance changes were measured at the required wavelength. These operations were repeated until the end of the measurements.

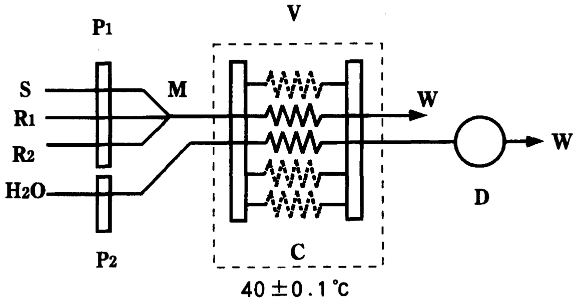

Fig. 2. Seven-channel steady-state flow system for the catalytic determination of manganese. S, samples with Q=0.5 ml min^{-1}; R$_1$, 1.6×10^{-6} mol dm^{-3} MG (containing 0.1 mol dm^{-3} HAc, 0.05 mol dm^{-3} NaAc) with Q=0.5 ml min^{-1}; R$_2$, 2.5×10^{-3} mol dm^{-3} KIO_4 with Q=0.5 ml min^{-1}; H_2O, with Q=1.0 ml min^{-1}; P$_1$, P$_2$, peristaltic pumps; M, confluence point; V, seven-channel valve; C, parallel reaction coils (1.0 mm i.d., 1 m long); D, detector (615 nm); W, waste.

A proposed decomposition procedure[21] was used in this work. We weighed out about 1.0 g of a dried biological sample into a platinum crucible, and then gently heated it on a hot-plate (Iwaki Glass, PC-351). After decomposition, we mixed the ash with 500 mg of a mixture of lithium carbonate–boric acid (1 : 1), and fused it for 15 min at ca. 1000 °C. We then dissolved the cooled melt in 1 mol dm^{-3} of hydrochloric acid with magnetic stirring and diluted to exactly 100 ml with the same acid. We refer to this solution as the master sample solution.

Results and Discussion

Optimizations. Since this method is not related to dispersion theory, the interactions between the dispersion process and the chemical kinetics do not take place. This means that optimization of the parameters for the SSFA system would become rather simple. Two important characteristics of the SSFA system were obtained with a two-channel manifold of SSFA.[20] The first was that steady-state signals of the peak height become independent to the lengths of the coils (an identical internal diameter is assumed), though the peak widths become wider, while the volume is beyond a critical value. The second was that the intensities of those steady-state signals were completely unaffected by any change in the carrier flow rate over a very wide range. These conclusions are still valid for any multi-channel manifolds in SSFA.

Length of Coil: For a new manifold, the length of the reaction coils is the most important parameter to be optimized. Figure 3 shows the relationship between the peak heights and the coil lengths for the manifold used in this work. One meter of coil length, corresponding to the quasi-steady state with a dispersion coefficient of ca. 1.03, was chosen for the determination of manganese. In this case, the dispersion coefficient could be regarded as being a dilution coefficient. In other words, the concentration of the sample solution is hardly diluted under this condition. It is necessary to mention that a dispersion arises while a sample zone flows from a coil to the flow-cell. Because the dispersion increases with an increase in the flow path, the distance from the outlet of the valve to the inlet of the flow cell must be designed to be as short as possible. Here, the distance of the coil (0.5 mm i.d.) was set to be ca. 20 cm long, except for the parts inside of the spectrophotometer.

Flow Rate: The MG, the periodate, and the sample were designed as a group to be fed at a flow rate ratio of 1 : 1 : 1; therefore, their concentration ratio does not change, no matter how the flow rate is changed. Thus, the influences from the characteristic pulsation flux as well as the gradual decrease in the flow rate of a peristaltic pump can be completely compensated. After optimizing the coil length, the flow rates of reagents and sample solutions were minimized to 0.5 ml min^{-1}, which still maintained the same signal intensity, in order to save consumption of the reagents. The carrier line was independent of the sampling line, which was similar to a back-flushing technique in chromatography. Of course, the flow rate of water could be *ad libitum* adjusted, while the carryover between samples was not more than 1%. In this instance, a water flow rate of 1.0 ml min^{-1} was properly selected.

Reaction Time and Sampling Rate: The relationship among the reaction time, rotating speed of the valve, and channel numbers of the valve have been discussed in detail.[20] An approximate equation suited for long-period reactions was used in this case of determination of manganese,

$$R_s = (N_c - 2)/T_r, \qquad (5)$$

where R_s is the sampling rate or the revolution rate of the valve (in samples h^{-1}), N_c the channel number of the valve and T_r the reaction time (in hour). From Eq. 5, we can obtain a sampling rate of 20 samples h^{-1} and a reaction time of 15 min, while rotating one channel every three minutes.

Of course, the sampling rate can be increased by two methods: Increasing the revolution speed if a reaction is faster, or increasing the channel number of the valve. In this experiment, the peak returned to the baseline completely within 30 s (cf. Fig. 4, A or B). This means the sampling rate could increase to at least 120 samples per hour, if there were enough channel numbers. It was

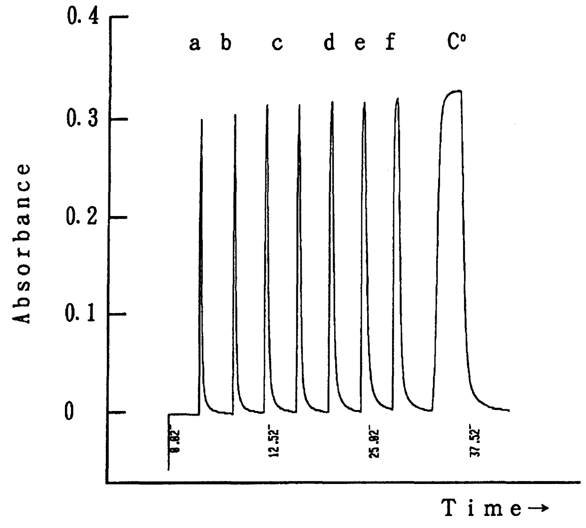

Fig. 3. Relationship between the peak heights and the coil lengths. Coil lengths: a) 0.5 m, b) 0.7 m, c) 1 m, d) 1.2 m, e) 1.5 m, f) 2 m; Dispersion coefficient: a) 1.092, b) 1.076, c) 1.044, d) 1.039, e) 1.035, f) 1.030; C^0: 5×10^{-7} mol dm^{-3} MG with Q=0.8 ml min^{-1}; Carrier flow rate: 0.6 ml min^{-1}.

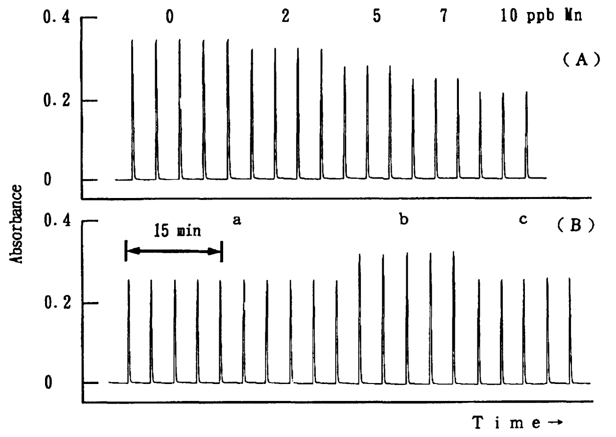

Fig. 4. Recordings obtained for manganese determinations under the conditions recommended. (A) Calibration recording obtained for manganese standard solutions. (B) Peaks obtained for the analysis of NIES reference materials. a) Tea leaves (1000 fold dilution); b) Sargasso (100 fold dilution); c) Rice flour (high level cadmium) (50 fold dilution).

Table 2. Comparison between SSFA and Conventional FIA[22] for Manganese Determination

Detection limit	Sampling speed	RSD	Reaction temperature	Length of coil	Reaction time	Dispersion coefficient	Method
ppb	$\mathrm{S\,h^{-1}}$	%	°C	m	min	$C^0/C^{\max}$	
2	8	2[a)]	50	12	6.5	18	FIA
0.07	20	3[b)]	40	1	15	1.03	SSFA

a) 5 determinations in 30 ppb. b) 8 determinations in 1 ppb.

easy to control the carry over of sample zones less than one percent within a 15 s interval, if the flow rates of the carrier and reagents solutions were increased. Thus, a sampling rate of 240 samples $\mathrm{h^{-1}}$ would be possible.

Reaction Temperature and Others: Although the manganese-catalyzed MG-periodate system involves a reaction of temperature-acceleration, a high temperature would cause some trouble, such as vaporization of water, which would interfere with the spectrophotometric measurement. The reaction temperature was appropriately selected to be 40 °C. Naturally, it becomes unnecessary to use a back-pressure coil, which is often used in a rather higher-temperature system in FIA.

The pH of the buffer solution was still selected to be 4.4,[22] and the used concentrations of MG and of the periodate solution were slightly smaller than those of the FIA method.[22]

Under the reaction conditions mentioned above, a very developed fading near to the chemical equilibrium was observed.

Consumption of Reagents and Sample: The consumption of the sample is at the same level as that of FIA with the pump-type injected manner; it is larger than those of FIA with a cylinder-type process and SIA. However, the consumption of reagents is not always larger than that of FIA. Since the sampling rate of SSFA is larger than that of FIA in the slow-reaction case, consumption of reagents would decrease. The longer is the reaction time, and/or the higher is the sampling frequency, the better is the effect for saving reagents.

Comparison with Conventional FIA: A comparison with the reported FIA method[22] is arranged in Table 2. As can be seen from Table 2, the results were obtained as expected. By using the SSFA manifold, the dispersion coefficient decreased to ca. 1.03. This means that there was little loss in sensitivity. Thus, a great increases in the sensitivity as well as analytical speed were obtained. Besides the detection limit, the reaction temperature and coil length were also improved. Of course, those advantages of FIA, simplicity in operation and strictness in time-control and so on, still remained.

Determination of Manganese. Manganese was determined as a function of the decrease in the absorbance. Since the absorbance of water at the required wavelength was taken as a baseline in the proposed procedure, the positive peaks were recorded instead of those negative peaks.[22] The decreases in the peaks against the peak of no manganese-containing standard solution under the same reaction conditions allowed the calibration of manganese to be constructed. The calibration curve (Fig. 4, A) was linear up to 10 ppb. Also, the relative standard deviation (R.S.D.) was found to be less than 3% for eight determinations of 1 ppb manganese.

This method was satisfactorily applied to the determination of manganese in NIES reference materials of sargasso, tea leaves, and rice flour. Because of the extremely high sensitivity in this procedure, these master sample solutions had been diluted to the required range of concentrations before they were analyzed. Their recordings are shown in Fig. 4, B. The analytical results are given in Table 3 along with the reference values. The relative standard deviation was 2.8% for ten determinations of tea-leave sample solutions containing ca. 7 ppb manganese.

The present method is selective for a manganese determination, since very few ions interfere, as pointed out in the literature.[23] Iodine is removed as an evaporating component in the process of ashing. Iron and aluminum would not interfere, because their contents in the biological reference materials of NIES are not very high compared with manganese.[24—26]

Conclusion

A pulse signal, which may be one of the pursuits in any unsegmented continuous-flow analysis, has been achieved by using this steady-state flow-analysis approach. This method is no longer characterized by sample zone dispersion and/or sequentiality, and succeeds regarding most of the advantages of FIA as well as automatic batch analyzers. The SSFA approach was proved

Table 3. Manganese Determinations in NIES Reference Materials

Samples	Mn in samples ($\mu\mathrm{g\,g^{-1}}$)	
	Found	Reference value
Sargasso	22.0±2.6	21.2±1.0
Tea leaves[a)]	686 ±19	700 ±25
Rice flour (containing high level Cd)	35.7±1.7	34—56

a) R.S.D. was 2.8% for 10 determinations of sample solution containing ca. 7 ppb of manganese.

to have a useful potential for a long-period reaction. The method can be used for routine analysis, since it provides a high sample throughput and analytical sensitivity, even if the reaction is too slow to be used in conventional continuous-flow methods. It also allows one to use techniques based on the steady-state concept directly in flow systems, and to study chemical kinetics without dispersion. Of course, it is applicable to fast chemical reactions without any change in the manifold.

The authors thank Nomura Micro-Science Co. for providing ultra-pure water. They also thank Mr. Tsutomu Fukumoto, Rikkyo University, for his assistance in part of the experiments.

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