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Steady-State Flow Analysis. A New Approach to Rapid and Sensitive Analysis for Long-Period Reactions in Continuous-Flow System

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Air-segmented flow analysis, flow-injection analysis and sequential-injection analysis which characterized with sample zone dispersion and/or sequentiality, do not suit to an analysis for long-period reactions because of their sharp decreases in analytical speed and sensitivity. A new approach, based on the combined techniques of a parallel double-line FIA, a stopped-flow FIA and a steady-state determination model, is proposed to develop a rapid and sensitive method for filling a gap of the application to long-period reactions in the present continuous-flow analysis.

The continuous-flow analysis is playing an important role in serial assays, process and laboratory analysis now. Skeggs firstly introduced air-segmented flow analysis (SFA) for minimizing the carry-over of sample zones and made the continuous-flow analysis method practicable in chemistry assays.1) Based on the consideration of dispersion theory, Ruzicka and Hansen developed a flow-injection analysis (FIA).2) Recently, however, the FIA has been hampered by the use of complex flow manifolds so that it has not been entirely acceptable in the industrial environment. Consequently, Ruzicka and Marshall proposed a new method called sequential-injection analysis (SIA) which based on the random walk model.3) For fast reactions, it is worthwhile to lose some analytical sensitivity for compensating analytical speed as that has been done in FIA. However, for long-period reactions, it is not the case. Because of the characterized sample-zone dispersion and/or sequentiality in three methods mentioned above, both of the sensitivity and the sampling rate sharply decrease so that these methods will not be suitable for long-period reactions.

As well known, the reproducible dispersion is a key issue in any flow-injection analyses based on the dispersion control. All of successful examples in FIA have been achieved only in this way that samples or sample zones were designed so as to pass through an identical flow line with the same physical and chemical situations in each measuring cycle. Generally, a long-period reaction with reaction time 20-30 minutes in FIA requires the coil length up to *ca.* 30-50 meters to answer for the finish of the reaction.4) Because the dispersion of the sample zone increases with the square root of the distance traveled through the tubular conduit,5) a sharp decrease in sensitivity and analytical speed is unavoidable. It is very difficult to obtain the reproducible dispersion of the sample zones traveling through parallel flow lines in order, because the mechanical design of a manifold requires that all parallel cavities must exhibit exactly identical flow geometries. This is the reason why the parallel FIA6) designed for long-period reactions is not yet applied to practical assay until nowadays.

On the other hand, the stopped–flow FIA developed for raising the sensitivity of reaction system is inherently unsuitable for long–period reactions, because next sample can not be injected during stop interval. For these reasons, many long–period reaction systems with high sensitivity, such as catalytic and enzymatic reactions *etc.*, are not yet well studied and applied in FIA.

This paper proposes a new approach based on the combined techniques of the parallel double–line FIA, the stopped–flow FIA and the steady–state determination model to solve the problem mentioned above. This method may be called the steady–state flow analysis (SSFA) for its characteristics. It is considered to be possible that this method is applied to long–period reactions in flow–analysis system with high analytical speed and without any loss in sensitivity, and to fill the gap of applications to long–period reactions in the present continuous–flow methodology.

In the present work, at first, a double–line–two–channel manifold was designed to perform the fundamental study about the pulse signal pattern by a steady–state determination manner in a non–reaction system. Figure 1 shows the flow–analysis system. A peristaltic pump with Tygon tubings was used to feed reagent, samples and water. The manifold consists of two valves and PTFE tubes, in which the sampling and the detection are alternatively run. A JASCO UVIDEC–340 double–beam spectrophotometer with an 8–μl flow–cell was employed to measure the changes of absorbance which were recorded.

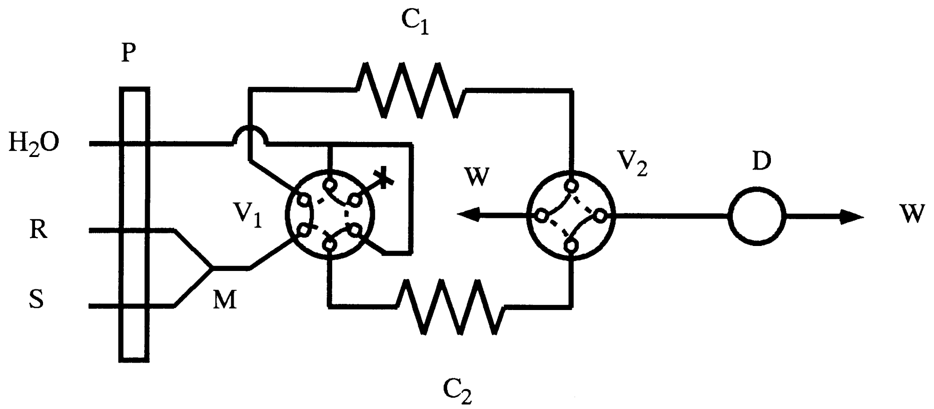

Fig. 1. Two–channel steady–state flow system S=sample; R=reagent; P=pump; C_1,C_2=reaction coils; V_1,V_2=valves; D=detector; W=waste.

The procedure is described as follows. Firstly, the sample is mixed with reagents at the point M, then introduced into coil C_1 through the inlet of the valve V_1, while the front of the solution stream is wasted out through the outlet of the valve V_2. At the same time, the washing water passes through the coil C_2, the valve V_2 and the flow–cell to waste. Secondly, when the positions of the valves are changed at the same time, the previous sample solution is traveled through the flow–cell in which the changes of absorbance are measured, and the sampling is carried out in coil C_2. These operations are repeated until the end of measurements.

Figure 2 shows experimental results obtained. The signals of C^0 (C^0 is the initial concentration of the reagent) are shown in this figure for the comparison with the dispersion. As shown in Fig. 2(A), when the coil length reaches 40 cm, the pulse signals corresponding to non–dispersion steady states are obtained, and their values vary no longer with the increase in coil length up to 100 cm. In this method, a critical volume is defined

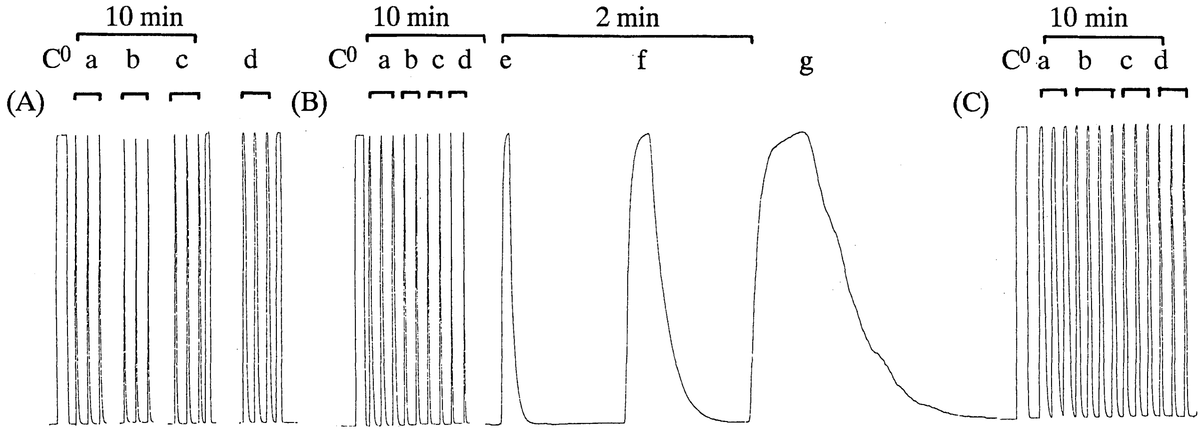

Fig. 2. Pulse signal patterns of steady–state determination model
(A) Profiles of peaks with coil lengths: $C^0=2\times10^{-5}$ mol dm^{-3} (methyl orange); washing speed V_w=4.2 ml/min; coil lengths L (cm): a,40; b,25; c,50; d,100. (B) Profiles of peaks with washing speeds: L=50 cm; V_w(ml/min): a,2.2; b,4.2; c,5.0; d,7.4; e,7.0; f,2.0; g,0.8. (C) Profiles of pulse signals with washing speeds: L=100 cm; V_w(ml/min): a,2.2; b,4.2; c,5.0; d,7.4.

as a necessary minimal volume of sample for yielding a steady–state signal under the same analytical condition. It is worth noting that even if the loaded sample volume exceeds its critical value, the peak heights become independent to the lengths of coils (identical diameter is assumed), but just the peak widths become different. Moreover, it is shown in Fig. 2(B) that signals are entirely not affected by the change of the flow rate in the range from 0.8 to 7.4 ml/min. In addition, increasing in the washing speed of water can allow the base line to reach rapidly, thus it brings the increase in the sampling frequency. It can be seen from Fig. 2(C) that the results have, virtually, a real steady–state signal pattern of continuous–flow analysis. The air bubble trapped between sampling procedures do not bring any problems, because it always flows to waste and does not stay in the reaction coils. Moreover, the air–segmented effect of air bubble is skillfully utilized to avoid the carry–over between samples in the load stage. Besides, any influences from pulse–flow of peristaltic pump have not been found. Certainly, it is possible to heat reaction coils for accelerating the reactions, if necessary.

Figure 3 shows, generally, the principle of this method. A new type of rotary valve with double–line–double–side multichannel was designed instead of that with the two–channel in Fig. 1. It is with this valve that the injection, reaction and detection of samples can be carried out independently. The relationship between the reaction time, rotating speed of valve and channel numbers of valve is determined by the following equation,

$$T_r = T_{th} + (N_c - 2) / R_v \qquad (1)$$

where T_r is the reaction time, T_{th} the traveled time from mixing point to detector, N_c the channel numbers of valve and R_v the revolution numbers of valve (in channels/h). And the sampling rate, of course, is equal to the revolution number. For fast reaction systems with reaction time less than T_{th}, a two–channel manifold is enough for getting a considerable analytical speed. In long–period reation case, the T_{th} is negligible as compared with T_r, that is the following equation holds,

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

where R_s is the sampling rate (in samples/h). For example, if taking channel number as 12, for the reaction time 5, 10 and 20 min, the sampling rates are equal to 120, 60 and 30 samples/h, respectively.

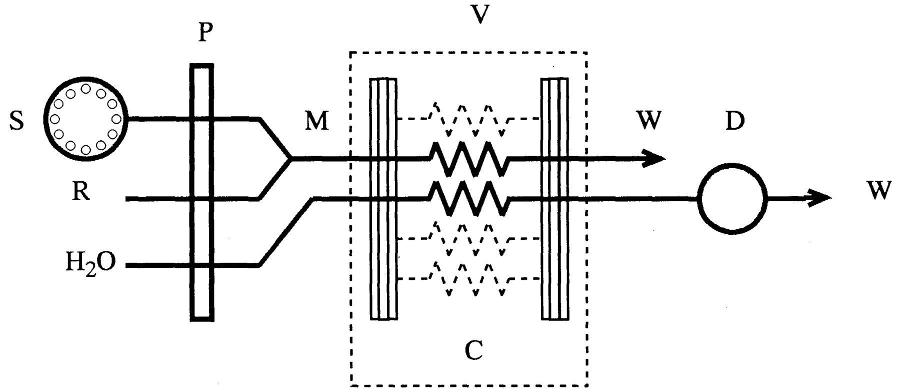

Fig. 3. Multichannel steady–state flow system
S=sample; R=reagent; P=pump; M=mixing point;
V=valve; C=reaction coils; D=detector; W=waste.

In principle, this method is no longer related to the dispersion theory. Because SSFA is operated in a non–dispersion steady state, reproducibility, sensitivity and analytical speed have a great increase. Without the dispersion control, the design of manifolds, the optimization of operating conditions and the automatization of the apparatus *etc.*, will become easier than SFA and FIA. Once an appropriate manifold (including a feed–speed program) is established, it can be widely adapted to different reaction systems only by changing the rotating speed of valve without reconstructing the manifold.

Finally, it needs to mention that SSFA also suits to fast–reaction systems in continuous–flow analysis, although it is developed for the application to long–period reactions. It is believed that SSFA will develop in the present continuous–flow analysis with the progress of hardware including the double–line–double–side multichannel valve.

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