J. Flow Injection Anal., Vol. 16, No. 2 (1999)

EFFECTS OF THE ETHANOL SOLVENT CONCENTRATIONS OF THE SAMPLE AND THE CARRIER IN FLOW INJECTION SPECTROPHOTOMETRIC ANALYSIS. III. IN A CONFLUENCE CONFIGURATION [+]

Shan-jun Liu and Matthieu Tubino*

Department of Analytical Chemistry, Institute of Chemistry
State University of Campinas (UNICAMP), P.O. Box 6154, Campinas, SP, 13083-970, Brazil

Abstract

The effects of the ethanol solvent concentrations of the sample and the carrier in a confluence configuration of flow injection spectrophotometric analysis, not containing chemical reaction, were further investigated. The emphasis of this work is the study of the influence of the ethanol solvent content on the noise. In the confluence configuration, the ethanol fractions in both the sample and the carrier streams have significant effects on the peak height, the dispersion coefficient, the peak-width, and the noise. In comparison with the single line manifold, the confluence configuration diminishes the noise, but it can't eliminate the noise. The pulse of the peristaltic pump may be one of the main sources of noise depending on the combination of the ethanol solvent between the sample and the carrier in the flow injection analysis (FIA). The insertion of a pulse damper into the carrier in the single line FIA manifold can effectively diminish the noise.

Keywords: Flow injection analysis, ethanol, noise, spectrophotometry, rhodamine B, confluence configuration, pulse damper

Introduction

When colourimetric detection is used in FIA the equipment may be sensitive to differences in refractive indices (RI) between

[+] For papers I and II, see references of 4 and 5.

* Corresponding author.

sample and reagent background if the absorbance is low [1]. This RI effect is superimposed on the absorbance peak of the sample and may alter the shape and height of the peak. The RI effect is inherent in FIA when colourimetric detection is used, by virtue of the incomplete mixing of sample and reagent and the formation of concentration gradients [1]. Therefore introducing aqueous samples into a system with relatively high ethanol concentration usually produces spurious signals because of refractive index changes [2]. The origin of the RI effect has been described by Betteridge *et al* [3]. By the use of longer flow cells and the confluence configuration technique, the significance of RI can be minimized [1].

We studied the effects of the various ethanol solvent compositions in both the sample and the carrier in a single line FIA spectrophotometric manifold, rhodamine B and bromothymol blue used as the dyes [4,5]. The following conclusions were obtained with the single line FIA manifold: (1) The ethanol fractions in both the sample and the carrier streams influence the dispersion coefficient, the peak height, the peak-width, as well as the noise; (2) The case where the ethanol solvent content in the sample is equal to that in the carrier is the optimum combination for the sample/carrier, and can achieve higher signal sensitivity and effectively avoid both the peak broadening and the noise; (3) When the ethanol solvent concentration of the sample is less than that of the carrier, noise may be introduced, especially when the carrier contains the higher ethanol content; and (4) The noise mainly depends on the combination of the ethanol solvent concentrations between the sample and the carrier.

Following our previous studies in the single line FIA manifold [4,5], the present work was further to investigate the effects of the ethanol solvent composition in a confluence configuration FIA, incorporated an ordinary T-coupling. Rhodamine B was used as the tracer. Emphasis is given to study the influence of ethanol solvent content on the noise.

Experimental

Reagents

All reagents were of analytical grade. Distilled and deionized water (Mill-Q system, Millipore, Bedford, MA) and 96% of ethanol (Merck) were used for the preparation of the various compositions of ethanol-water solutions (in volume ratio) as the carrier and of rhodamine B-ethanol-water solutions as the sample.

The preparation of the stock solution of rhodamine B was described previously [4]. Working solutions of rhodamine B 1.0×10^{-5} mol l^{-1} were prepared by serial dilution of the stock solution with water and/or ethanol.

All solutions were degassed using a pump prior to use.

Apparatus

A spectrophotometer (Zeiss PM 2D, Germany) was operated at 553 nm, with a glass flow-through cell of 10 mm optical path and of 70 µl volume. The peristaltic pump (ISM 726B, ISMATEC, Switzerland) was used to delivery the solutions. A polytetrafluoroethylene (PTFE) rotary injection valve [6] fitted with a sample loop of 50 µl was employed for the sample introduction. All the connecting tubes were made of polyethylene with an internal diameter of 0.9 mm. The absorbance was continuously monitored on a chart recorder (Linear, USA).

Testing of mixer

The mixing efficiency of a T connector were evaluated in three modes (Fig. 1). Two water carriers of C_1 and C_2 were pumped each at a flow rate of 1.75 ml min^{-1}. A rhodamine B aqueous sample of 50 µl (1.0×10^{-5} mol/l) was injected into the carrier C_2 and dispersed through a 40 cm of straight tube, then the carriers of C_1 and C_2 were brought together in the T-piece (T) in one of the mixing modes. The mixture (M) passed continuously through a 10 cm length of tube into the flow-through cell. The T-piece is made of white PTFE.

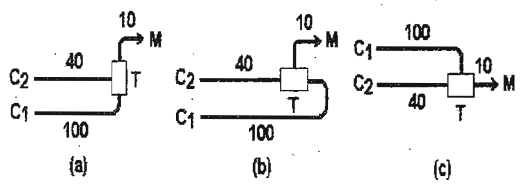

Fig. 1 Three mixing modes of the T-piece. Rhodamine B sample was injected into the water carrier of C_2. The numbers above the line represent the tube length in cm.

Effect of sample volume

The effect of smaller sample volume (16 µl) on the noise was tested in the single line manifold, which is identical to that described previously [4,5]. 60% and 80% of ethanol solutions were respectively used as the carriers, and rhodamine B solutions containing various ethanol fractions were used as the sample.

Pulse damper

A pulse damper described previously [7] was put into the carrier line between the peristaltic pump and the injection valve in the single line FIA manifold [4,5] to study its effect on the noise.

Procedure

Figure 2 shows a flow diagram of the FIA system, which is a confluence configuration. The carriers of C_1, C_2 and the sample stream (S) have the same flow rate of 1.75 ml min^{-1}, giving the whole system a flow rate of 3.5 ml min^{-1}. Injection of 50 μl rhodamine B dye solution (1.0×10^{-5} mol l^{-1}) with various fractions of ethanol solvent as a tracer into the carrier C_2. After the sample was injected, it was dispersed through a 40 cm length of straight tube in the carrier C_2, then it mixed with the carrier C_1, which contained the same composition of ethanol solvent as C_2, in the T-piece in the (c) mode in Fig. 1, and continuously passed a 10 cm length tube into the flow-through cell. The absorbance was measured at 553 nm. Recording of the dispersed sample zone was used to test the effects of the ethanol solvent concentrations of both the sample and the carriers.

A fixed fraction of ethanol solution was used as the carriers of C_1 and C_2, and the dye solutions containing different fractions of ethanol solvent as the samples were determined one by one. Every sample was injected successively in triplicate. After the determinations of one carrier solution were finished, the peristaltic pump and the recorder were stopped, and the composition of the carrier was substituted by another. Then the pump and the recorder were turned on to continue the measurements. All experiments were done at room temperature.

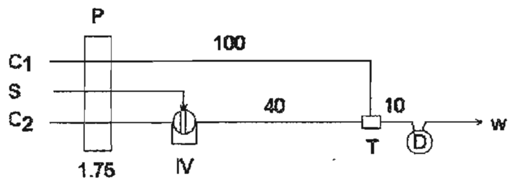

Fig. 2 Flow diagram in confluence configuration. S is the sample solution of rhodamine B, C_1 and C_2 are the carriers, P is the peristaltic pump with indication of the flow rate in ml min^{-1}, IV is the rotary injection valve, T is the T-piece, D is the spectrophotometric detection at 553 nm, and W denotes waste. The numbers above the lines represent the tube length in cm.

Results and Discussion

The RI effect is inherent in FIA when the colourimetric detection is used. By the use of longer flow cells [1] and confluence configuration technique [1,8], the significance of RI can be minimized. In their confluence configurations, the sample is not injected directly into the reagent, but into an inert carrier stream which is merged into the reagent at a Y or straight connector. The composition of the carrier is made similar to the matrix so that there are no large differences in RI between the two [1].

The continuous mixing of a water stream C_2 with an ethanol solution C_1 was first studied without the dye sample in the (b) and (c) modes in Fig. 1. The confluence stream passed through a 40 cm length tube into the spectrophotometric detector. Large noise was observed when the water stream C_2 was continuously mixed with the ethanol solution stream C_1 (20, 40, 60, 80, and 100% of ethanol) except the case that C_1 was the water stream, and the noise level and the blank value increased as the ethanol concentration of C_1 increased. It is obvious that the noise relates to the incomplete mixing and the difference of refractive index between the water and ethanol solution streams. Thus, in this work the carriers C1 and C2 have the same ethanol composition.

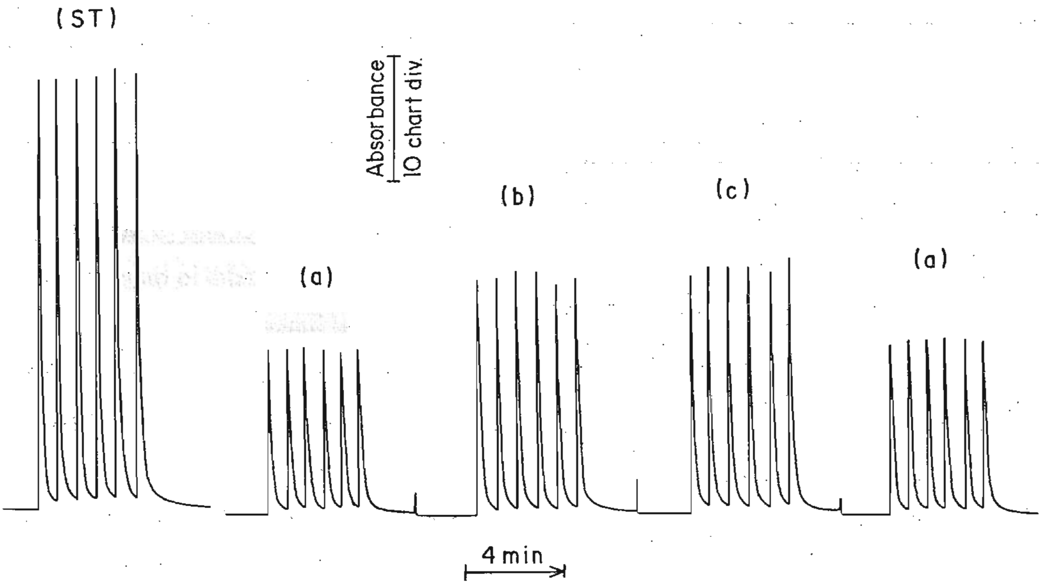

Fig. 3 Effect of confluence mode in the T-piece.
(a), (b) and (c) are identical to the connecting modes in Fig.1. ST represents the single tube FIA manifold. The sample is 50 μl of rhodamine B aqueous solution in 1.0×10^{-5} mol l^{-1}. The carriers C in single line manifold and C_1/C_2 in confluence configuration are distilled water, not containing ethanol.

1. Effect of Mixing Modes

Mixing is an important feature of all flow systems. Three connecting modes of the T-piece were examined in the confluence configuration with rhodamine B aqueous sample and water carriers of C_1 and C_2, not containing ethanol solvent.

The recorded curves (Fig. 3) show that the mixing modes of the T-piece have some influence on the signal sensitivity. The peak heights of (a), (b), and (c) mixing modes in the confluence configuration are respectively 38/43%, 55%, and 56% of that in the single line manifold. The decrease of the sensitivity in the confluence configuration in comparison with the single line manifold is caused by the dilution effect of the mixing of C_1 and C_2. The mixing mode of (a) gives smaller signal relative to the modes of (b) and (c). For the modes of (b) and (c) no significant difference in mixing qualities was found. The (c) mode was used in this work so as to compare with the single line manifold [4,5].

2. Effect of Ethanol Solvent Concentrations in Confluence Configuration

Keeping the dye concentration of the sample constant (1.0×10^{-5} mol l^{-1}) the effect of various compositions of ethanol solvent in both the sample and the carriers on the signal was examined in the confluence configuration. The recorded curves are shown in Fig. 4. The amounts of ethanol in both the sample and the carriers have a significant influence on the peak height, on the peak-width, as well as on the noise.

The peak height relates to the ethanol solvent contents in both the sample and the carriers (Fig. 5). The absolute values of absorbance, in comparison with the single line manifold, become smaller due to the dilution effect, although the peak height may be higher by adjusting the attenuator of the recorder. In addition, the tendency of the peak heights to be smaller and almost constant in the single line manifold when the ethanol solvent concentration in the sample is higher than that in the carrier, isn't obvious in the confluence configuration. The ethanol contents of the carrier and of the sample also influence the peak-width in time unit and thus the analytical frequency.

The ethanol concentrations of the sample and/or the carriers have also significant influence on the dispersion coefficient in the confluence configuration. The dispersion coefficient D values for the rhodamine B system given in Fig. 6 and Table 1 are in the range of medium dispersion (D=3-10). Since the variation of the ethanol contents in the sample and in the carriers produces the changes of the solution physical properties (viscosity, density, and refractive index, etc.) [9], which may affect the dispersion behaviour of the solute in FIA. For example, Brooks *et al* [10] observed that the dispersion coefficient increases with both an increase in carrier stream viscosity and as the difference in viscosity between the injected sample and the carrier stream increases. Both the increased difference in viscosity between the injected sample and the carrier and the absolute viscosity increase ensure a decrease in mass transfer in the radial direction in the reaction coil [10].

Another significant influence of the ethanol solvent introduced in flow injection spectrophotometric system is the noise (see Fig. 4). The noise depends on the ethanol solvent concentration of the sample and the carriers, as well as on the relative ethanol contents between the sample and the carriers. When the ethanol solvent concentration in the carrier is less than 40% (v/v), there is no significant noise observed even though the sample is 100% of ethanol solution; when the ethanol concentration of the carrier is more than 60% (v/v), the noise may be introduced depending on the ethanol solvent concentration of the sample: the case where the ethanol content in the sample is less than that in the carrier produces significant noise, and the other case where the ethanol concentration of the sample is equal to or higher than that of the carrier doesn't produce noise.

An aim of using the confluence configuration is to avoid or minimize the noise observed in the single line FIA manifold [4,5]. The results in Fig. 4 show that the noise cannot be completely avoided, although in comparison with the single line manifold [5] the noise was reduced when the confluence configuration was employed (see Table 1). In this confluence configuration, the sample was injected into the carrier C_2, similar to the single line manifold, but the carrier C_2 containing the dispersed dye sample was merged with other carrier C_1 at the T connector. The composition of the carrier C_1 was made the same as the carrier C_2 (e.g. sample matrix) so that there were no large differences in refractive index between C_1 and C_2. Merging after injecting the carrier into other stream produced a stream of fairly uniform composition and again with minimal difference in refractive index along its length. Hence the noise was improved in the confluence configuration. The noise begins to appear in the confluence configuration only when the ethanol concentration of the carriers is equal to or higher than 60%, in comparison with the single line FIA manifold, 40% [5].

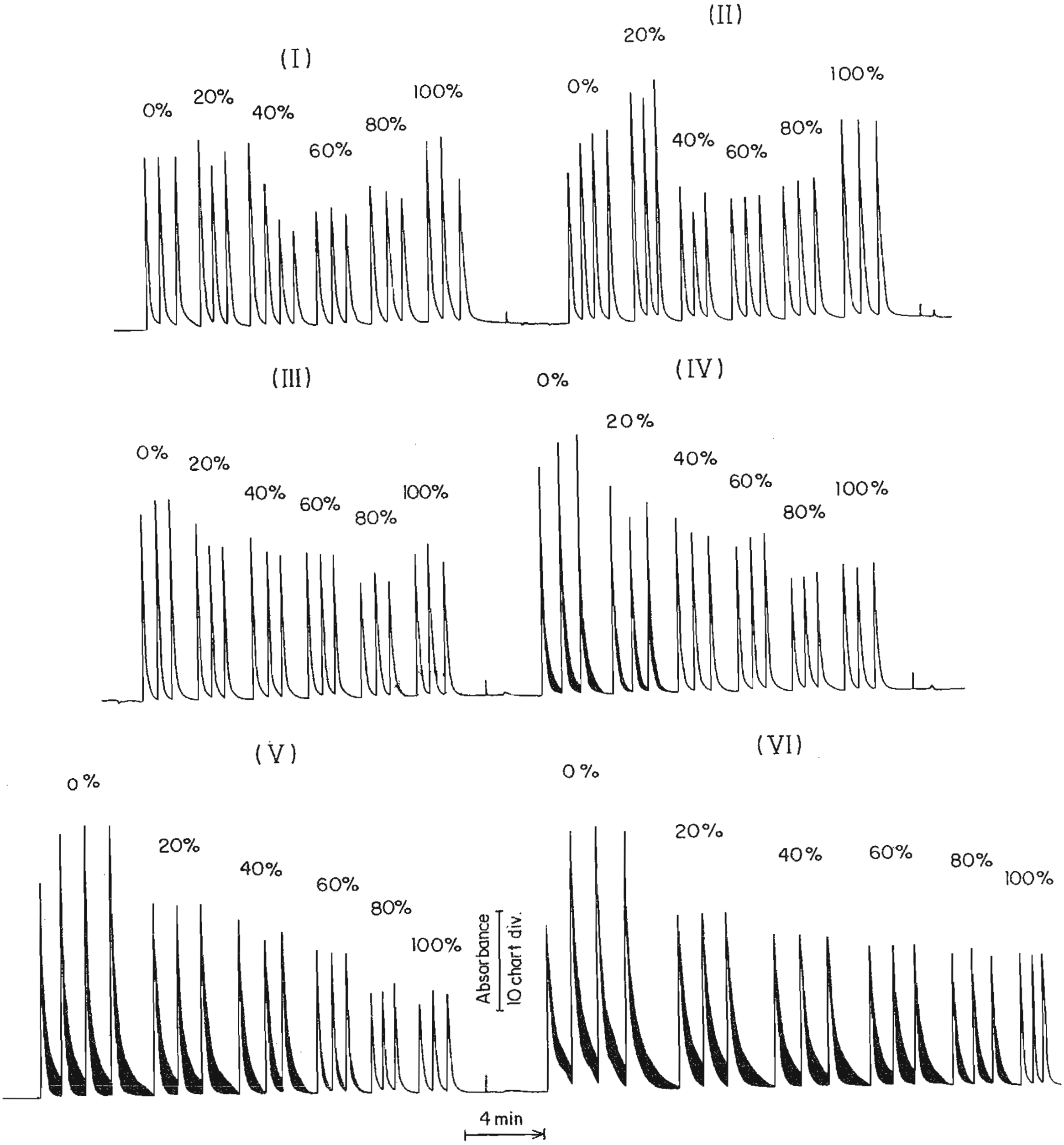

Fig. 4 Recorded graphs for the rhodamine B system in confluence configuration.

Roman numerals of I, II, III, IV, V and VI represent the ethanol solvent concentrations of the carrier: 0%, 20%, 40%, 60%, 80% and 100% (v/v). The percentages on the recorded curves are the ethanol solvent concentrations of the sample in % (v/v). The carriers of C_1 and C_2 were the same ethanol solutions, and the sample added more rhodamine B of 1.0×10^{-5} mol l^{-1}.

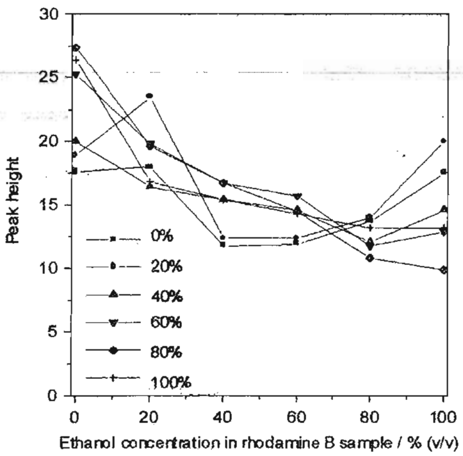

Fig. 5 Peak heights as a function of the ethanol fraction in the sample and in the carriers in confluence configuration.

The percentages on the curves represent the carrier ethanol concentrations. Peak height in the unit of chart-division. The conditions were the same as in Fig. 4.

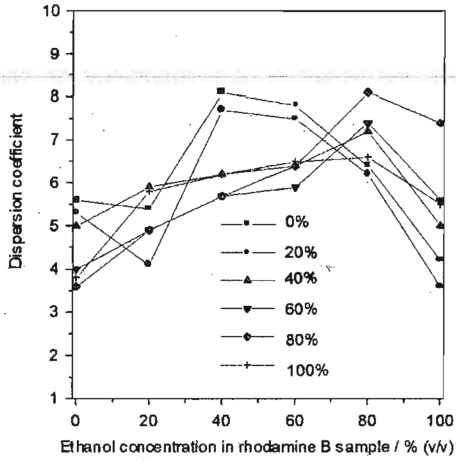

Fig. 6 Influence of the ethanol compositions of the sample and of the carriers on the dispersion coefficient in confluence configuration.

The percentages on the curves represent the carrier ethanol concentrations. The experimental conditions were the same as in Fig. 4.

Table 1 Effect of the ethanol compositions of the sample and of the carriers on the dispersion coefficient in confluence configuration

		Ethanol in carrier / % (v/v)					
Dispersion coefficient *D*		*0*	*20*	*40*	*60*	*80*	*100*
	0	*5.6*	*5.3*	*5.0* †	*4.0* †‡	*3.6* †‡	*3.8* †‡
Ethanol	*20*	5.4	*4.1*	*5.9* †	*4.9* †‡	*4.9* †‡	*5.8* †‡
in sample	*40*	8.1	7.7	*6.2*	*5.7* †	*5.7* †‡	*6.2* †‡
/ % (v/v)	*60*	7.8	7.5	6.4	*5.9*	*6.4* †‡	*6.5* †‡
	80	6.4	6.2	7.2	7.4	*8.1*	*6.6* †‡
	100	4.2	3.6	5	5.6	7.4	*5.5*

† and ‡ respectively represent the noise in the single line and in the confluence manifolds.

When 60% of ethanol solution is used as the carriers the noise is not significant in the confluence configuration when the sample ethanol concentration is more than 40%, in comparison with the single line manifold, 60% of ethanol. It is necessary to further investigate the elimination of the noise observed.

3. Sample Volume Influence in Single Line FIA Manifold

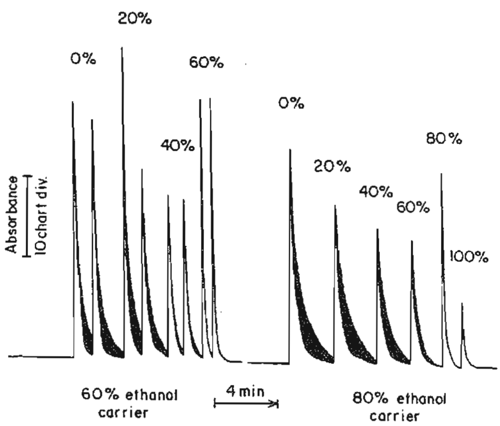

Fig. 7 Smaller sample volume influence in single line FIA manifold.
(a) 60% and (b) 80% of ethanol solution as the carrier. Sample volume is 16 μl.

In order to study the effect of sample volume on noise, a smaller sample volume of 16 μl relative to 70 μl of flow-through cell was injected into the carrier of 60% or 80% of ethanol solution in the single line FIA manifold. The results are shown in Fig. 7 and illustrate that there is not significant improvement on reducing the noise in comparison with 50 μl of sample volume [5].

4. Pulse Damper in Single Line FIA Manifold

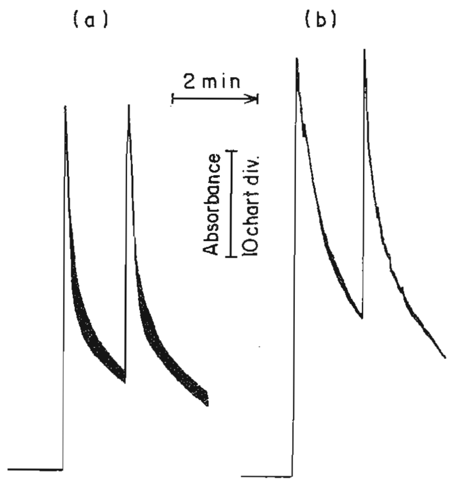

Fig. 8 Effect of pulse damper in single line manifold.
Carrier: 100% of ethanol solution. Sample: 1.0×10^{-5} mol l^{-1} of rhodamine B water solution. (a) and (b) use without and with the pulse damper, respectively.

The injection pulse damper is recommended when flow injection confluence systems are employed for analysis of very dilute samples [8]. The effect of the pulse damper on noise was tested in the single line FIA manifold (Fig. 8). The noise is significantly diminished when the pulse damper was used in the carrier line of the flow system. The spectrophotometric signal is due not only to the absorbance but also to the refraction of the sample [11]. It is obvious that the noise observed in the single line and confluence manifolds was mainly caused by the pulse of peristaltic pump due to the difference of refractive index between the sample and the carrier. In addition, the signal is smaller without the pulse damper due to the pulse of peristaltic pump increases the dispersion of dye sample.

Conclusions

The use of the ethanol solvent in the sample and the carriers in the confluence configuration FIA affects the analytical characteristics. The compositions of ethanol solvent of both the sample and the carriers have a significant influence on the dispersion coefficient, the peak height, the peak-width, and the noise in a flow injection spectrophotometric system. The effects depend on the relative ethanol solvent concentrations between the sample and the carriers. Thus the calibration solutions must be carefully matched to the sample with respect to the ethanol contents. The pulse of the peristaltic pump may be one of the main sources of noise depending on the combinations of ethanol compositions in the carrier/sample, due to the difference of refractive index between the sample and the carrier. The sample contains equal to or higher ethanol fraction than the carrier may eliminate the noise. The use of the pulse damper can effectively diminishes the noise due to the introducing of ethanol solvent into the flow injection spectrophotometric system.

Acknowledgements

The authors are grateful to Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support. S.J.L. acknowledges FAPESP for a post-doctoral fellowship.

References

1. G. Ham: *Anal. Proc.*, **18**, 69 (1981).
2. C. Silfwerbrand-Lindh, L. Nord, L.-G. Danielsson, F. Ingman: *Anal. Chim. Acta*, **160**, 11 (1984).
3. D. Betteridge, E.L. Dagless, B. Fields, N.F. Graves: *Analyst*, **103**, 897 (1978).

4. S.-J. Liu, M. Tubino: *J. Flow Injection Anal.*, **15**, 67 (1998).
5. S.-J. Liu, M. Tubino: *J. Flow Injection Anal.*, **16**, 55 (1999).
6. M. Tubino, F.G. Barros: *Quím. Nova*, **14**, 49 (1991).
7. S.-J. Liu, M. Tubino: *Anal. Chim. Acta*, **366**, 5 (1998).
8. H. Bergamin F°., B.F. Reis, E.A.G. Zagatto: *Anal. Chim. Acta*, **97**, 427 (1978).
9. *"CRC Handbook of Chemistry and Physics"*, 70th ed., p. D-221–D-223 and D-230 (1989–1990), (CRC Press).
10. S.H. Brooks, R.N. Williams, J.D. Dorsey: *Anal. Lett.*, **21**, 583 (1988).
11. L. Anderson: *Anal. Chim. Acta*, **110**, 123 (1979).

(Received October 22, 1999)
(Accepted December 8, 1999)