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On the use of mixing reactors in flow injection analysis with thermal lens calorimetric detection

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Abstract

In flow injection analysis with thermal lens calorimetric detection, mixing of reagent streams of different composition before the detection cell produces a large background noise. The noise is due to the heterogeneity of the mixture which produces refractive changes in the probe beam direction. Procedures to measure the homogeneity of the mixture of solutions are derived, and used to evaluate and improve the mixing efficiencies of packed bead reactors. Using well-designed reactors, the mixing noise can be reduced below the noise levels due to other sources.

INTRODUCTION

In thermal lens calorimetry a sample is illuminated by a laser pump beam. Absorption of the pump radiation, followed by non-radiative relaxation of the excited molecules, results in the greatest heating of the sample at the beam center, producing a radially symmetric thermal gradient. This gradient, which is associated with a refractive index gradient, is probed with a second laser beam. In liquids which expand upon heating, the derivative of the refractive index with temperature dn/dT is negative, and the gradient increases the divergence of the probe beam. The intensity decrease at the center of the probe beam in the far field is proportional to the concentration of the absorbing species.

Highly absorbing species at ultra-trace concentration levels and weakly absorbing species at relatively low concentrations are determined with thermal lens calorimetry. The technique has been used for detection in high performance liquid chromatography (HPLC) [1–5] and flow injection analysis (FI) [6, 7]. However, compared to the use of thermal lens calorimetry with static samples, when flowing samples are used, sensitivity

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decreases as a consequence of incomplete formation of the thermal gradient and noise increases owing to flow fluctuations [8, 9]. Therefore, in the presence of flow, the extraordinary low limits of detection obtained with static samples cannot be reached.

Several procedures have been proposed to improve the signal-to-noise (S/N) ratio with flowing samples [1, 2, 4, 6–10]. In a previous work [11], a computer-controlled system was developed for the measurement of flow injection peaks in continuous and pulsed flow conditions. Using the pulsed flow procedure, the dependence of sensitivity and noise on the flow, and on the presence of turbulences and irregular pump pulses in the flow, was reduced. The main drawback of the pulsed flow approach is the low measurement output rate, which limits applicability to low flow rates and large injection volumes. Better results and a wider applicability are obtained by reducing the flow rate to a low value just before a peak is to be measured [12].

In the absence of on-line mixing of reagents, flow programming can be very effective in improving the detection limits obtained with both HPLC and FI. However, we have observed that the previous mixing of two streams of reagents, even at low flow rates, produces a very large noise on the thermal lens signal, thus hindering the practical development and use of HPLC and FI procedures with on-line chromogenic derivatization. We address here the reduction of the mixing noise by using improved mixing reactors.

In HPLC and FI applications, an additional mixing device or reactor is usually required after a T or Y union point [13, 14]. Optimization of the reactor parameters, e.g. length and diameter, is performed to achieve a higher sensitivity and a better reproducibility. The S/N ratio increases as a consequence of the improved mixing efficiency. When a slow reaction is present, the S/N ratio also increases due to the additional delay time.

However, when thermal lens detection is compared with other optical detection techniques, an important difference arises. Using spectrophotometric or fluorimetric detection, a simple T or Y union gives reasonably low noise, and the main problem is to increase sensitivity; conversely, with thermal lens detection, sensitivity is usually high and the main problem is always how to reduce the large mixing noise.

In this work, the mechanism by which the mixing noise is produced is discussed, and the use of an He–Ne laser to measure sensitively the homogeneity of a mixture is proposed. Mixture homogeneity measurements are used to establish the relative mixing efficiencies of several reactors. The reactors were built up with small-diameter columns, packed with glass beads, and were used in an FI manifold with thermal lens detection. Mixing efficiencies were correlated with physical parameters of the reactors, with the aim of developing better mixing reactors to be used in HPLC and FI with thermal lens detection.

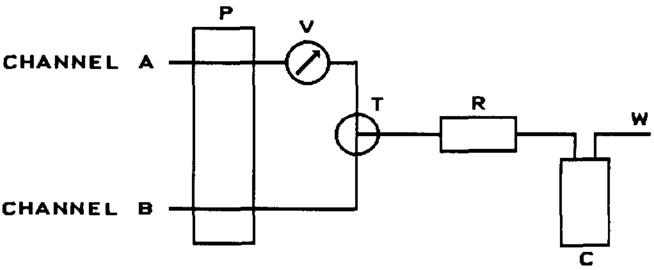

Fig. 1. Flow injection assembly. P, peristaltic pump; V, injection valve; T, T union; R, mixing reactor; C, detection cell; W, waste.

EXPERIMENTAL

Apparatus

The thermal lens calorimetric setup was constructed using a 4-W Ar^+ laser (Spectra-Physics, Model 2016, Mountain View, CA) and a 7 mW He–Ne laser (Spectra-Physics, Model 105) in a coaxial pump-probe configuration. The 514.5 nm line of the Ar^+ laser (100 mW at the laser head) was modulated at 4 Hz using a chopper (Stanford, Model SR540, Sunnyvale, CA). Other details have been described elsewhere [15]. A PC/AT computer, provided with a DAS-8 interface (Metrabyte Corp., Taunton, MA), was used to acquire and treat the data. Two thermal lens gradients were measured every 2 s and the arithmetic mean was taken. The logic support of the system was the AUTOTLS program [11]. A Hewlett–Packard diode array spectrophotometer, Model 8452A (Avondale, PA), coupled with a Vectra ES/12 computer and HP UV–vis software was also used.

The manifold shown in Fig. 1 was used to measure the efficiency of different laboratory-made mixing reactors. A Minipuls 3 peristaltic pump (Gilson, Middleton, WI) and a 18 μl flow cell (Hellma, Model 178012-QS, Mülheim-Baden, Germany) were used. Rigid PTFE tubes of 0.8 mm inner diameter (i.d.) were used in the FI manifold. Table 1 shows the

TABLE 1

Inner diameter (id), length (l) and dead volume (dv) of the reactors used in this work

Reactor	id/mm	l/mm	dv/μl	Reactor	id/mm	l/mm	dv/μl
1	0.8	23	20.9	5[a]	3.0	0.45	<10
2	0.8	37	37.7	6	3.0	1	<10
3	10.8	77	62.8	7	3.0	2	<10
4	1.5	37	52.3	8	3.0	8	19.2
				9	3.0	37	87.9

[a] Reactor containing only a single layer of glass beads.

characteristics of the mixing reactors studied in this work. Reactors of 0.8 and 1.5 mm i.d. were made with PTFE tubes, and those of 3 mm i.d. were made of flexible polyethylene tubes. Glass beads of 0.45 and 1 mm diameter were tried: the former were chosen as they lead to much more efficient reactors.

To estimate the dead volumes of the reactors, a 3 M HCl solution was standardized against a 0.2 M NaOH solution. The 3 M HCl solution was pumped through the reactors. The reactors were then inserted in the FI assembly (see Fig. 1) and water was pumped through them. The resulting solution was collected and titrated against the 0.2 M NaOH solution.

The glass beads were packed into the tubes in two ways: either introducing the beads in the absence of solvent and compacting them as much as possible by pressing the walls of the column with the fingers, or first filling the tubes with water and then introducing the beads slowly by gravity. In the latter case, the reactors were more compact, because the beads were deposited more slowly with formation of layers; however, this procedure was rejected because the excessive increase of pressure inside the FI manifold loosened the connections of the FI tubes, even at very low flow rates.

Reagents

Analytical grade HCl (Panreac, Barcelona), NaCl, hydroxylammonium chloride, $Co(NO_3)_2 \cdot 6H_2O$ (Probus, Barcelona), HNO_3, $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ (Merck, Darmstadt, Germany), 1,10-phenanthroline and neutral red (Scharlau, Barcelona) were used. All solutions were prepared with nanopure deionized water (Barnstead Sybron Co., Boston, MA).

Procedure A

A stream containing increasing NaCl concentrations and 4×10^{-7} M neutral red was mixed with a stream of water, using a T union, and the resulting mixture was forced through several mixing reactors. At each NaCl concentration, the far-field intensity at the center of the He–Ne laser beam was monitored for 1 min, and the absolute standard deviation of the set of 30 measurements was calculated. These measurements were repeated both in the absence and presence of 100 mW Ar^+ pumping radiation. In all cases, the experiments were repeated three times and the mean was taken.

Procedure B

Two series of experiments were performed in the absence and presence of a chemical reaction. In one series, 100 μl of an aqueous solution containing Co^{2+} and HNO_3 was injected into a carrier stream containing

HNO_3 at the same concentration (channel A in Fig. 1). The carrier was then mixed with a stream of water (channel B), and was forced through the reactors before entering the detection cell. In other experiments, 100 μl of an Fe^{2+} solution, containing 0.044 M hydroxylammonium chloride and 0.036 M sodium acetate, was injected into a carrier of the same composition (channel A), but without Fe^{2+}. This stream was finally mixed with another stream (channel B) containing 1×10^{-3} M 1,10-phenanthroline [6], prepared with the same carrier solution.

The wavelengths of the absorption maxima for the nitric Co^{2+} and Fe(II)-1,10-phenanthroline solutions were 505 and 510 nm, respectively; molar absorptivities at 514.5 nm were 4.8 and 11400 $mol^{-1}\,l\,cm^{-1}$, respectively.

The areas under the FI peaks were measured, as these were more reproducible than the peak heights. In all cases, the mean and standard deviation of a series of eight replicate FI peaks were calculated.

RESULTS AND DISCUSSION

Preliminary observations

It was first observed that the use of worn-out flexible polyethylene suction tubes in the peristaltic pump affected deleteriously the noise of the thermal lens signal. Old tubes did not maintain a stable flow, and thus the composition of the solution obtained after mixing the two streams (A and B in Fig. 1) suffered from fluctuations. It was also observed that the noise was larger and that deformed FI peaks were obtained, when the pump suction tubes were held in place under strain. Tense tubes probably did not dampen the pulses of the peristaltic pump.

The filling of the injection loop was tried with both the peristaltic pump and with a syringe. In the former case, and with an increasing filling time, from 10 s to 120 s, a 50% improvement in reproducibility was attained, which was attributed to an enhanced renovation of the solution in the injection loop. Using a syringe to inject the sample, the reproducibility was similar to that obtained using the peristaltic pump and a long filling time, e.g. 120 s. Consequently, a syringe was used throughout.

The FI peaks with thermal lens detection were observed to be much noisier when the injected solutions and the carrier were prepared separately, compared to experiments performed with the carrier and the injected solutions prepared from the same stock solution. The additional noise observed in the former case was attributed to small differences in the concentration of the more concentrated components, such as NaCl or HNO_3. Therefore, the same stock solution was used to prepare the injected and carrier solutions.

Origin of the mixing noise and evaluation of the mixing efficiency of the reactors

The mixing noise can be produced by changes in the direction of the pump and probe beams, as well as by deformations of the thermal gradient. Changes in the direction of the beams can be produced by refraction through the irregularly shaped liquid–liquid interfaces, which are present in the heterogeneous mixture. Deformations of the thermal gradient can arise both from flow fluctuations which produce irregular forced convection, and from local changes in the absorbance and photothermal properties of the medium, i.e. changes in the derivative of the refractive index with temperature, and in the thermal conductivity. The latter effect should be small when liquids with similar photothermal properties are mixed, as when the solutions are prepared with the same solvent. However, local changes in the absorbance can be produced when a solution containing a dye is mixed with another solution in the absence of the dye, as with the experiments performed here.

The standard deviations obtained following procedure A, performed without reactor and using reactors 1 and 4, are plotted against NaCl concentration in Fig. 2. No significant differences were observed in the

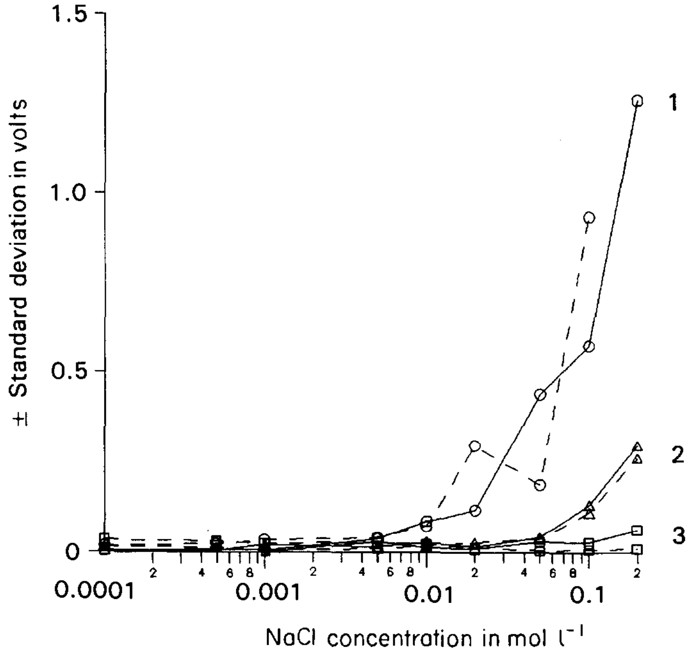

Fig. 2. Standard deviations of a series of 30 measurements obtained by mixing a stream of NaCl solution containing neutral red with a stream of water. Full lines represent absence of pumping radiation; broken lines represent the presence of 100 mW pumping radiation. Curve 1, without reactor; curve 2, with reactor 1; curve 3, with reactor 4.

absence and presence of 100 mW pumping radiation. Therefore, it can be concluded that the predominant contributions to the mixing noise were from the changes in the probe beam direction, rather than from deformations of the thermal gradient.

The standard deviation of the signal obtained using reactor 4 was similar to that of a blank prepared by mixing two streams containing the same NaCl concentration, for concentrations under 0.1 M and with low flow rates. With other reactors, standard deviations were larger.

Some of the experiments, including those performed in the absence of a reactor other than the T union, were repeated using a diode array spectrophotometer as the detector. The absorbance was measured in the 632–220 nm range. No significant differences were observed among the reactors, and without reactor, the standard deviation of the absorbance being ±0.008 in all cases. Therefore, measurement of the homogeneity of the mixture of the two streams, and consequently, of the efficiency of the reactors, was far more sensitive when the He–Ne laser setup was used.

The experiments were repeated in the absence of neutral red, with similar results. Therefore, the proposed procedure was capable of distinguishing composition differences between colorless solutions within the low 10^{-3} M concentration range.

Evaluation of FI peak reproducibility in the absence of a chemical reaction: injection of a Co^{2+} solution

The next experiments, in all cases, were performed in the presence of 100 mW Ar^+ pumping radiation. The reproducibility achieved with the different reactors was studied using an injected absorbing species as a probe, in the absence of a chemical reaction. For this purpose, 3×10^{-3} M Co^{2+} solutions, prepared with increasing HNO_3 concentrations, were used.

First, a 0.15 M HNO_3 solution of Co^{2+} was injected into a carrier stream of 0.15 M HNO_3 (channel A), which was then mixed with a 0.5 M NaCl aqueous solution (channel B). When no reactor was used, and with reactor 1, measurement of the thermal lens signal was not possible, due to the excessive noise. Conversely, with reactors 4 and 9, the signal was much higher than the noise.

In another series, a 0.015 M HNO_3 solution of Co^{2+} was injected into a carrier stream of 0.015 M HNO_3 (channel A), which was then mixed with water (channel B). Without reactor, the signal was still completely buried in noise, but well-shaped FI peaks were obtained using both reactors 1 and 9, the relative standard deviation (rsd) of the series of eight replicate peaks being 19% and 8% respectively.

Table 2 shows the results obtained with several reactors. In this case, a 0.15 M HNO_3 solution of Co^{2+} was again injected into a 0.15 M HNO_3

TABLE 2

Mean areas (S) and relative standard deviations (rsd) of FI peaks (8 replicates) obtained by injection of a 3×10^{-3} M Co^{2+} + 0.15 M HNO_3 solution into a 0.15 M HNO_3 stream, which is then mixed with a water stream

Reactor	S	rsd/%	Reactor	S	rsd/%
No reactor	[a]	–	5	[a]	–
1	3.15	27	6	[a]	–
2	4.06	13	7	2.88	10.2
3	4.05	16	8	3.03	10.1
4	4.49	8.7	9	4.81	10.1

[a] Excessive noise.

stream, which was then mixed with a stream of water. The largest sensitivity and lowest noise corresponded to reactors 4 and 9. Reactors 7 and 8 (same diameter as reactor 9, but different length) gave the same rsd values as reactors 4 and 9, but a somewhat smaller sensitivity. Shorter reactors (5 and 6) lead to very high noise levels, which made measurement of the peaks impossible.

The rsd values obtained with reactors 4 and 9 ranged from 5% to 10%. Relative standard deviations within the same range were also obtained when two carriers of the same composition (0.15 M HNO_3) were mixed after injection of the 0.15 M HNO_3 Co^{2+} solution. Thus, under the conditions of the experiment and using reactors 4 or 9, the mixing noise was reduced below the noise levels originating from other sources.

In another series, Co^{2+} solutions in HNO_3 of increasing concentration (0.15, 0.25, 0.35 and 0.5 M) were injected into a HNO_3 stream of the same concentration, which was mixed with a stream of water. Only reactors 1, 4 and 9 were checked. The rsd values increased considerably for reactors 1 and 9 with increasing HNO_3 concentrations. Reactor 4 gave the best reproducibility, with rsd values of 9.3%, 9.8%, 11.4% and 11.8% for 0.15, 0.25, 0.35 and 0.5 M HNO_3, respectively. Therefore, only reactor 4 maintained a high mixing efficiency with increased differences in composition of the solutions to be mixed.

Using reactor 4 and 0.15 M HNO_3 solutions, which are mixed with a stream of water, the flow rate of each stream was increased from 0.44 to 1.8 ml min^{-1}. In order to compare sensitivities at the different flow rates, the areas under the peaks were multiplied by the flow rate. The sensitivity was practically the same, but the rsd values increased with the flow rate, being 7% and 18% for 0.44 and 0.88 ml min^{-1}, and ranging from 25% to 30% for the higher flow rates.

In a similar experiment, performed at 0.44 ml min^{-1} flow rate per channel, a 100 cm coil inserted between the T union and the detector cell was used instead of reactor 4. In this case, the high noise level made the measurement of the peaks impossible. In another experiment, reactor 4 was not replaced, but the length of the tube connecting the injection valve and the T union was increased between 5 and 45 cm. A 40% loss in sensitivity was observed from the shortest to the largest tubes, whereas the noise decreased only slightly. Therefore, an open tube had a very low mixing efficiency and facilitated the free longitudinal diffusion, the results being detrimental. The use of packed reactors is recommended, but, as demonstrated, their diameter and length should be optimized. Furthermore, a coil or a reactor between the injector and the T union is not necessary when sample and carrier solutions have been prepared from the same stock solution, which assures the absence of significant differences in the concentrations of the more concentrated species between the injected and carrier solutions.

Finally, when the injection was performed in the absence of reactor, or using reactors 1, 2 and 3, small peaks appeared before the sample bolus reached the detection cell. This was attributed to the perturbation of the flow produced by the injection. These peaks did not appear when the other reactors were used, probably because of the larger pressure drop along them, which dampened the perturbation produced by the injection. These perturbations were not observed with spectrophotometric detection and, therefore, it further underlines the ability of thermal lens calorimetry to detect very weak physical changes in the flow.

Evaluation of FI peak reproducibility in the presence of a chemical reaction: determination of iron with 1,10-phenanthroline

The experiments above showed that reactor 4 was the best among those used in this work. This reactor was selected to study the reproducibility of the FI determination of Fe^{2+} following chromogenic complexation with 1,10-phenanthroline and thermal lens detection.

The influence of the flow rate was studied by injecting 1×10^{-6} M Fe^{2+} solutions. Using flow rates of 0.44, 0.88 and 1.32 ml min^{-1} at each channel, the rsd values obtained were 2.6%, 10.1% and 11.1%, respectively. These results are similar to those obtained above with reactor 4 by mixing solutions with much higher differences in composition. Here, the only significant difference in composition between the solutions to be mixed is the presence of 1×10^{-3} M 1,10-phenantroline in one of them; therefore, in the experiments performed with Fe^{2+}, mixing is not the predominant source of noise.

The calibration regression straight lines, obtained from a series of triplicate measurements at ten concentrations, within the 10^{-7} to 10^{-5} M Fe^{2+} range, were $S = 1.12 \times 10^6 C + 0.12$ ($r = 0.9992$); $S = 0.57 \times 10^6 C + 0.07$ ($r = 0.9990$) at 0.44 and 0.88 ml min^{-1}, respectively. In the

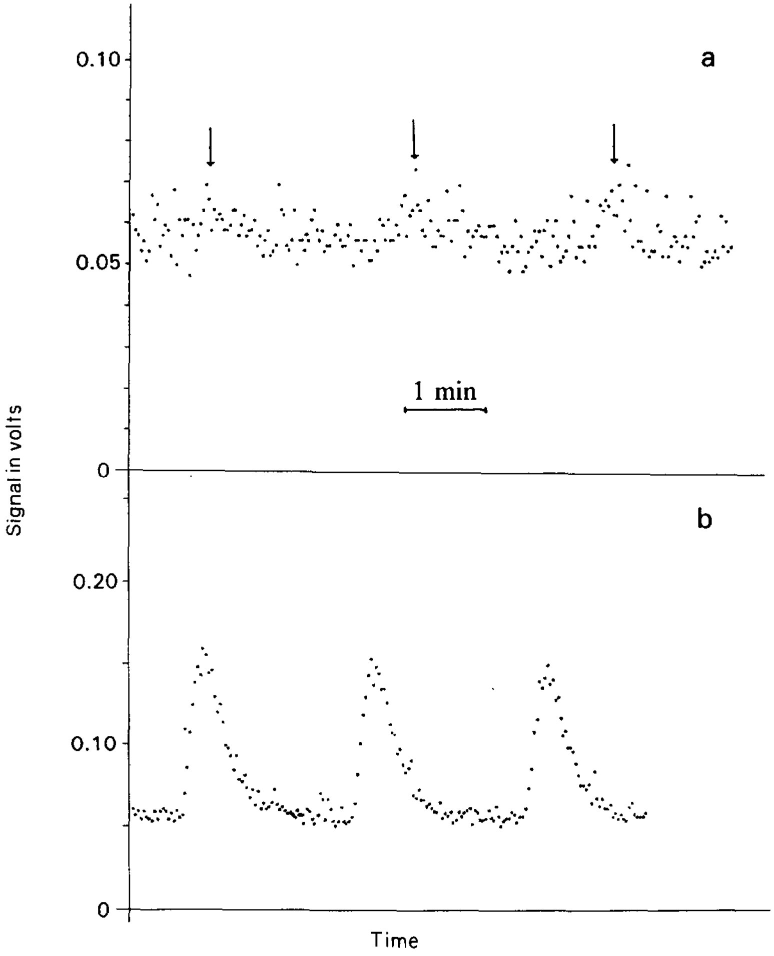

Fig. 3. Typical FI peaks corresponding to the injection of (a) 2×10^{-7} M, and (b) 2×10^{-6} M Fe^{2+} solutions. Flow rate was 0.44 ml min^{-1} at each channel. Reactor 4 was used.

equations, C is Fe^{2+} concentration (mol l^{-1}), and S is the peak area (volt × second). It can be observed that sensitivity, if measured in volt × ml, is approximately the same at the two flow rates. The FI peaks corresponding to the injection of 2×10^{-6} M and 2×10^{-7} M Fe^{2+} solutions are shown in Fig. 3.

The determination of the limits of detection was not possible from the standard deviation of the blanks, because injection of a blank did not produce a peak with a measurable area. Instead, the standard deviation of 8 replicate injections of a 4×10^{-7} M Fe^{2+} solution was taken. This Fe^{2+} concentration gave a small peak of measurable area, which was sufficiently distinguished over the background noise. The results are shown in Table 3; the reproducibility values obtained with 1×10^{-5} M Fe^{2+} are also given.

A similar series of experiments was performed using a diode array

TABLE 3

Limits of detection (LOD) and reproducibility of the FI determination of Fe^{2+} [a]

Concentration	Parameter [b]	Flow rate/ml min^{-1} [c]	
		0.44	0.88
4×10^{-7} M	$S \pm s$	0.54 ± 0.20	0.272 ± 0.061
	rsd%	7.7%	22%
	LOD	1.1×10^{-7}	3.2×10^{-7}
1×10^{-5} M	$S \pm s$	21.35 ± 0.57	9.30 ± 0.33
	rsd%	2.7%	3.6%

[a] Eight peaks were measured at each concentration. [b] S is mean area in volts × second; s is standard deviation; LODs are given in mol l^{-1}. [c] Flow rates at each channel before mixing.

spectrophotometer as the detector, and pumping the solutions at a flow rate of 0.44 ml min^{-1} at each channel. The calibration curve, obtained in the 5×10^{-6}–2×10^{-4} M Fe^{2+} concentration range, was $A = 2483C + 0.004$ ($r = 0.9997$), where A is absorbance at the peak maximum and C is Fe^{2+} concentration (mol l^{-1}). The limit of detection was 8×10^{-7} M, which is eight times higher than the value obtained with thermal lens detection.

CONCLUSIONS

When FI assemblies with merging of reagent streams and thermal lens detection are implemented, the major source of background noise can be the incomplete mixing of the merged solutions. Mixing noise is predominant when the differences in composition of the solutions which are mixed are of the order of 1×10^{-3} M or higher. The main contribution to the mixing noise does not arise from deformation of the thermal gradient, but rather from changes in the probe beam direction produced by refraction through the irregular liquid–liquid interphases.

When a sample is injected into a carrier and at least one of them, sample or carrier, contains a concentrated species, a large mixing noise may also be produced. Therefore, use of the same stock solutions of concentrated reagents is advisable for the preparation of the injected and carrier solutions. This eliminates the need of inserting a reactor between the injection valve and the T union point.

A simple, sensitivie procedure of measure the degree of homogeneity of a mixture of solutions in flowing conditions, based on monitoring the intensity of an He–Ne laser in the far field, has also been described. The procedure was applied to evaluate the mixing efficiencies of packed-bead mixing reactors. In the FI experiments with thermal lens detection, peak reproducibilities correlated well with the estimated efficiencies of the reactors, which validates the proposed procedure. In the presence of a predominant mixing noise, the FI peak reproducibility can be considered as an indirect way of measuring reactor mixing efficiencies.

Using these sensitive procedures, better laboratory-made packed-bead mixing reactors can be designed. Improved reactors can effectively reduce the mixing noise below the noise levels due to other soruces. In this work, it has been shown that packed reactors are much more efficient than the frequently used open-tube coil reactors. The use of efficient mixing reactors is necessary to develop FI and HPLC procedures with on-line derivatization and thermal lens detection. Otherwise, and in comparison with spectrophotometric detection, the presence of mixing noise will largely limit the main advantage of thermal lens detection, i.e. the ability of reaching very low limits of detection.

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REFERENCES

1 R.A. Leach and J.M. Harris, J. Chromatogr., 218 (1981) 15.
2 C.E. Buffett and M.D. Morris, Anal. Chem., 54 (1982) 1824.
3 M.J. Sepaniak, J.D. Vargo, Ch.N. Kettler and M.P. Maskarinec, Anal. Chem., 56 (1984) 1252.
4 T.-K.J. Pang and M.D. Morris, Anal. Chem., 57 (1985) 2153.
5 S.R. Erskine and D.R. Bobbitt, Anal. Chem. 61 (1989) 910.
6 R.A. Leach and J.M. Harris, Anal. Chim. Acta, 164 (1984) 91.
7 J. Georges and J.M. Mermet, Analyst, 114 (1989) 541.
8 N.J. Dovichi and J.M. Harris, Anal. Chem., 53 (1981) 689.
9 W.A. Weimer and N.J. Dovichi, Anal. Chem., 57 (1985) 2436.
10 Z. Wu and W.G. Tong, Anal. Chem., 61 (1989) 998.
11 E.F. Simó Alfonso, M.C. García Alvarez-Coque, G. Ramis Ramos, A. Cladera Forteza, M. Estela Ripoll and V. Cerdá Martín, Anal. Lett., 25 (1992) 573.
12 R.M. Villanueva Camañas, J.M. Sanchis Mallols and G. Ramis Ramos, unpublished results.
13 I.S. Krull (Ed.), Reaction Detection in Liquid Chromatography, Marcel Dekker, New York, 1986.
14 J.S. Rhee and P.K. Dasgupta, Mikrochim. Acta, 3 (1985) 49.
15 E.F. Simó Alfonso, M.A. Rius-Revert, M.C. García Alvarez-Coque and G. Ramis Ramos, Appl. Spectrosc., 44 (1990) 1501.