Practical Approaches to Improve the Determination of Microamounts of Cr(VI) by Spectrophotometric Flow Injection Analysis with a Single Line Manifold

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Abstract

Two experimental approaches to improve the figures of merit of a spectrophotometric flow injection analysis (FIA) of traces for Cr(VI) with 1,5-diphenylcarbazide (DPC) are presented. The characteristics of both systems for trace analysis by means of an appropiate control of dispersion has been evaluated.

The first approach employs a reduced single line flow system (0.25 mm i.d. polyethylene tubing) that, in comparison to other flow injection systems already reported, reduces the sample volume and reagent consumption and increases the sample throughput retaining other figures of merit. The second alternative presented here, incorporates a pre-concentration system with two microcolumns packed with alumina and solid 1,5-diphenylcarbazide for concentration and reaction, respectively. The improvements in detection limit, sensitivity, and sampling throughput are shown.

Keywords: spectrophotometric-flow injection, Cr(VI) trace analysis, reduced-size systems, preconcentration columns, solid reagents, low dispersion.

1. Introduction

It is currently accepted that when low detection limits are required for an analytical technique, Flow Injection Analysis (FIA) may not be a suitable choice, unless some preconcentration stage is included. This argument arises from the loss in sensitivity which is common in most FIA techniques. This reduction in sensitivity is due to the lower peak height of a FIA signal when compared to the signal level for a similar batch procedure, and it is normally ascribed to the global process called dispersion.

Thus, from the point of view of trace analysis, the control and minimization of the dispersion plays an essential role on the optimisation of a given system. Moreover, if a preconcentration step is needed, an inadequate control of dispersion leads to an increase in sample size, which in turn reduces the sample throughput.

Dispersion arises from the boundary conditions imposed by the flow technique. The first cause of dispersion is the mass redistribution undergone by the sample pulse in the carrier stream. If a chemical reaction is involved, the kinetics of mixing of the reactants, as well as the kinetic of the reaction itself, will also contribute to the dispersion process. In this last case, it is obvious that a certain amount of reagent into the sample pulse is necessary in order for the reaction to proceed. This supply of reagents is controlled by the hydrodynamic characteristics of the system, which is associated to the efficiency of mixing. Thus, the way of improving the analytical performance of a system with chemical reaction has been traditionally focussed on the geometry of the manifold. Consequently, single line manifolds have been avoided because of their poorer mixing efficiency, and have been replaced by systems in which the reagent and the sample are merged at a confluence point. However, the better mixing achieved by this systems is counterbalanced by an unavoidable higher degree of dilution of the sample pulse, without yielding significant improvements in sensitivity.

Chalk and Tyson [1] have reviewed the actual influence of the manifold geometry (single, double, and reverse line configuration) on the sensitivity of a given technique. In their work it is shown that under appropriate conditions all the systems show the same sensitivity. The maximun sensitivity, they conclude, is achieved by "minimising the sample dilution and maximising the reagents' concentration".

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It is obvious then, that an appropriate optimisation strategy must be focussed on improving the reagent supply to the sample rather than on the manifold geometry. Based upon this perspective, the improvements achieved by using a microcolumn packed with solid 1,5-diphenylcarbazide (DPC) for the spectrophotometric determination of Cr(VI) has been reported [2] by the authors. A low dispersion system (D≈1) with the same limit of detection (LOD) and sensitivity of the steady-state procedure [3, 4] was obtained keeping the advantages of FIA. Unfortunatelly, the success of this methodology depends on the solid reagent characteristics such as solubility, size and type of particles, etc., and on the possibilities of reducing the column dimensions which is not possible in some practical cases [2].

The reduction of the tube radius was successfully applied to the control of dispersion, but it has been discouraged for routine FIA because of the necessity of more sophisticated instrumentation [5]. Further studies on this topic have been recently reported by several authors [6-8] who analized the pattern of dispersion using capillary tubes. Although dispersion is sharply decreased in systems without chemical reaction, the literature reports a higher degree of dispersion when a chemical reaction is involved, attributable to a poor mixing of rectants. Furthermore, the instrumentation employed looks unpractical when compared to that of typical FI systems.

The work reported here illustrates two approaches for the determination of microamounts of Cr(VI) based on different practical strategies to control dispersion in the spectrophotometric-FI determination of Cr(VI).

A single-line flow system using tube of a diameter of 0.25 mm i.d. is presented here, which yields the same LOD and sensitivity as the batch procedure. Sample throughput is higher than in other reported FI systems [2, 9, 10]. Sample volume and reagent consumption exhibit the lowest values reported up to date [2, 9, 10].

In order to improve the detection limit beyond that of the steady-state procedure, a double column pre-concentration system, one for analyte retention and the second for reaction, is proposed. Since dispersion is controlled by the column dimensions, reduction of tube radious was not attempted. The results show a decrease in dispersion and an increase in sensitivity, improvement in detection limit, and a better sample throughput when compared to other reports[11, 12].

2. Experimental

2.1. Reagents

Analytical-grade reagents and 18 MΩ water (MilliQ® water systems) were used.

Standard Cr(VI) solution: a stock solution containing 1.000 g l^{-1} of Cr(VI) was prepared by dissolving 2.8270 g of $K_2Cr_2O_7$ (Merck, Darmstadt, Germany) in purified water and diluting to 1.000 l. Other standard solutions were obtained by proper dilution.

The following solutions were also employed: 1% (w/v) solution of 1,5-diphenylcarbazide (Merck, Darmstadt, Germany) in 0.4 mol l^{-1} nitric acid, 2.0 mol l^{-1} nitric acid, 0.1 mol l^{-1} ammonia, and 1 % (w/v) solution of $CoSO_4$ (Merck, Darmstadt, Germany) in water.

1,5-Diphenylcarbazide Column: a mixture of DPC and nitric acid washed silica was prepared as column packaging [2].

Alumina Column: this column contained "Aluminium oxide S acid", active for column chromatography, 70-290 mesh (Riedel-De Han, AG Seegze, Hannover)

2.2. Apparatus

An automatic injection valve (Valco Instruments Co., Austin, TX, USA), a variable speed peristaltic pump Ismatec MS Reglo (Cole-Parmer, Chicago, IL, USA) and PFTE tubing of 0.8, 0.5 and 0.25-mm i.d. were employed. The dimensions and packaging material of the DPC column were the same as reported by the authors in a previous work [2]. The preconcentration column, 2.5-cm length, 1.5-mm i.d., was filled as reported elsewhere [11]. Both columns were made of poly(methyl methacrylate) (PMMA). Spectrophotometric measurements were carried out with a Hitachi U-1100 spectrophotometer equipped with a flow cell of 80-µl, 1.00-cm optical path length or, with a Shimadzu SPD-AV HPLC spectrophotometric detection module equipped with a quartz flow cell of 8-µl, 1.00-cm optical path length.

2.3. Procedure

System without preconcentration

The single-line manifold is shown in Fig. 1. The sample was conditioned by the on-line addition of nitric acid. Two different inner tube diameters were tested: 0.8 mm i.d. (System I) similar to the one reported by De Andrade *et al.* [9], and 0.25 mm i.d.

(System II). The FI variables (reactor length, flow rate, reagents' concentration, sample volume) were selected in order to optimise the detection limit. The physical dispersion of the systems was estimated by employing a 1% $CoSO_4$ solution (λmax= 520 nm) in place of the sample. In order to evaluate the contribution of the detection to the overall dispersion, two different flow cells, 80-µl and 8-µl total volume were used.

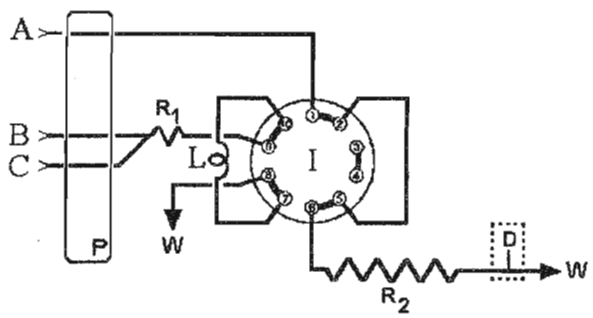

Fig. 1 Configuration of the miniaturised flow System II PFTE tubing 0.25-mm i.d.; quartz flow cell of 8-µl, 1.00-cm optical path length; **A**: 1% w/v DPC in 0.4 M nitric acid; **B**: sample; **C**: nitric acid for on-line sample conditioning. cm; $\mathbf{R_1}$: 20 cm; $\mathbf{R_2}$: 150 cm.

System with preconcentration

The system described by Pannain and Santelli [11] was tested as **System III. System IV** (see Fig. 2) incorporates a solid DPC reactant column as described previously by the authors [2]. The FI variables (dimension and packing of the column, flow rates, reagent concentrations, sample volume and manifold design) have been chosen in order to improve the detection limit, the sample consumption, and the time for analysis.

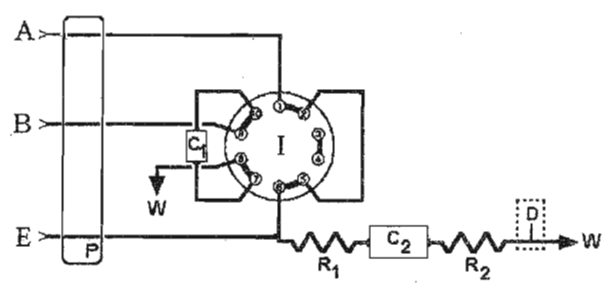

Fig. 2 Configuration of pre-concentration System IV PFTE tubing 0.80 mm. id; quartz flow cell of 80-µl, 1.00-cm optical path length; **E**: nitric acid solution; **B**: sample; **A**: ammonia solution; $\mathbf{C_1}$: acid alumina column 70-200 mesh, 1.00-cm x 0.15-cm i.d.; $\mathbf{C_2}$: DPC-silica column 2.5% w/w, 4.00-cm x 0.15-cm i.d.; $\mathbf{R_1}$: 60 cm; $\mathbf{R_2}$: 30 cm.

The signals for both systems were acquired using a Keithley® DAS-801 data acquisition system and processed with a personal computer. The control of actuated valves and peristaltic pumps was carried out by means of a dedicated tailor-made software. Graphical reports of the signals and storage of data for further calculations were also obtained via this software.

3. Results and Discussion

Table 1 shows the analytical figures of merit for System **I** (replica of the system presented in reference 10) and System **II** (a minimized version) and, the improvement obtained with the latter is clearly seen. A comparison between these systems, regarding economy of samples and reagents, is given in Table 2

Table 1 Comparison of analytical figures of merit. System II (miniature system); System I (replica of the conventional system used in ref.10).

	Sensit. (μg^{-1} l)	LOD ($\mu g\ l^{-1}$)	Samp. Freq. (h^{-1})	Linear Range ($\mu g\ l^{-1}$)
System I	0.019	18	120	18-2000
System II	0.045	1	150	1-800
Improvement	2.2	18	1.25	----

Table 2 Comparison of FI conditions. System II (miniature system); System I (replica of the conventional system used in ref.10).

	Sample volume (µl)	Carrier flow rate (ml min^{-1})	DPC Conc. (g l^{-1})	[HNO_3] Conc. (M)
System I	77	1.2	0.50	0.8
System II	30	0.7	0.20	0.4
Improvement	2.5	1.7	2.5	2

As it is shown in Table 1, the sensitivity for the determination under study is improved more than two times when System II is employed. Moreover, the sensitivity yielded by System II can be considered optimum, since it is very close to the reported value for a steady state procedure (0.049 μg^{-1} l) [3, 4].

As it has been mentioned above, dispersion is a complex process, which in this case includes physical and chemical aspects. Thus, in order to discriminate between both components, a system with no chemical reaction was first studied by

injecting a $CoSO_4$ solution and using water as carrier. Since time is the most important variable from a kinetic point of view, the FI conditions were set up in order to keep the same mean residence time throughout the experiments. The results are shown in Fig. 3, and are in agreement with the pattern already reported by Spence and Crouch [6]. It is clear that the reduction in the volume of the whole system causes an important increase in the peak height, thus decreasing the value of the dispersion coefficient.

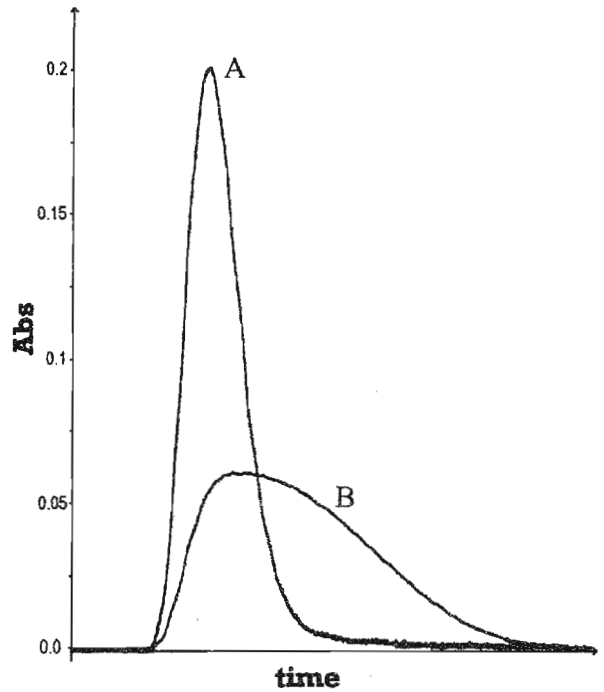

Fig. 3 Comparison of peak profiles for cobalt sulphate solutions. **A**: Miniature System II (tubing radii of 0.25mm i.d., flow cell of 8-µl); **B**: Conventional system I (tubing radii of 0.80-mm i.d., flow cell of 80-µl). (System variables have been chosen in order to obtain the same residence time for both systems).

For practical reasons, dispersion is evaluated at the maximum of the FIA transient. In fact, as there is no unequivocal relationship between the signal profile and sample distribution [13], the practical dispersion coefficient (D) lacks physical meaning. Therefore, despite of its usefulness for analytical purposes, the D value is rather an attribute of the signal profile than a physical characteristic of the sample plug. This lack of distinction between the evaluation of the dispersion by D and the dispersion process itself (which has been already pointed out by the authors in a previous work [14]) usually leads to several misunderstandings. It is commonly found the misconception of the dispersion coeficient as a measure of the degree of dilution undergone by the sample pulse [15]. Although it is true that dilution is a part of the dispersion process, its effect may not necessarily affect the value of D as stated by Narusawa and Miyamae [16].

With the regular FIA conditions the sample pulse is distorted in the axial direction due to a radial velocity gradient. Therefore, a plug flow type transport [17] will depend on the relative contribution of the radial transport of mass (essentially diffusive) respect to the axial one (mainly convective). This means that the only possibilities for optimisation are the reduction of the flow velocity, or the reduction of the tube radius. If there is no good reasons, it is obvious that the only choice is the second one, which is reflected in the results shown above.

It is important to stress that dispersion increases when tubing radius or flow cell volume are increased. If the wider manifold (0.8-mm i.d. tubing) is employed together with a 8-µl cell; the transport process brings about the main contribution to the sample dispersion. When the miniature manifold (0.25-mm i.d.) is attached to a 80-µl flow cell, the cell contributes to the physical dispersion acting, mainly, as a 'dilution factor' since the measurement is carried out in its whole area. Moreover, as the end of the manifold opens out into a broader channel, a reduction in the mean flow velocity into the cell is observed which increases the peak width and asymmetry: the lower the mean flow velocity into the cell the wider the peaks. Note that both Systems I and II show similar mean residence times but, sample throughput is higher for the latter due to the difference in the mean flow velocities into the cells. (See Table 1).

All these results are in agreement with those reported elsewhere. However, the question that still remains unclear is how to optimise the system when a chemical reaction is included. In these cases the kinetics of product formation is governed by two factors: the kinetics of the mixture of sample and reagent and the kinetics of the reaction itself. It must be remembered that, if an axial concentration profile is drawn, the maximun of the sample's profile will be coincident with the minimum of the carrier, which can lead to a poor supply of reagent into the sample pulse. Moreover, the conversion of chemical species (reactants to products) alters the concentration gradients and consequently it may modify the mass transport [18].

Confluence and/or merging zones FI systems contribute to a more efficient mixng between reagents, but the sample is diluted. When a single line manifold is employed, the kinetics of mixture becomes relevant. Most studies increase physical dispersion, i.e. enlarge the manifold, in order to improve the degree of mixing between reactants. Nevertheless, the mass redistribution along the flow system should be accompanied or not by the mixture of the molecules. So for lowering dispersion in single line systems, the key is to confine the plug in order to allow a more effective interaction with the carrier and thus favor diffusion. Therefore, a crucial step to enhance sensitivity is to mix reagents keeping the "identity" [14] of the sample plug as it was proposed by Chalk and Tyson [1] who state that the maximum sensitivity is obtainable by maximising the reagents concentration and minimising the sample plug dispersion. The best case is that of the solid reagent [2]: the sample is not diluted and maximum sensitivity is achieved for a fast reaction. For solutions, the plug behavior of the sample must be favored by lowering the carrier (reagent) flow rate and/or dimminishing the radial distances. Lowering the tube radius should be a better choice since the optimal flow rate decreases, the reagent consumption is reduced, and the sample thorughput is kept constant (see Tables 1 and 2). Sample volume must be reduced accordingly as higher injection volumes could produce a splitting of the FIA peak. Concerning reagents concentration, the optimum nitric acid and DPC concentrations for the miniature system (see Table 2) were found to be about a half of the employed in previous works [9, 10] in apparent discrepancy with the reported necessity of increasing reagents concentration to enhance sensitivity [9]. This can be explained considering that the actual driving force for the diffusion is the radial concentration gradient rather than the concentration itself, and the radial concentration gradient can be increased either by reducing the tube radius and/or by increasing the reagents concentration. Other factor related to this point is that an increase in the solution viscosity yields a decrease on the diffusion process. Under these considerations, it is possible to explain the choice of HNO_3 instead of H_2SO_4 as used by De Andrade *et al.*[9].

Regarding the sensitivity value, the main reason for not reaching the steady-state's lies on the dilution undergone by the sample before the injection. This step, introduced to match the sample matrix with the carrier, could be avoided or replaced by a manual step. However, in the first case a spurious signal (Schlieren effect) affects LOD and the second option is discarded as manual operations are not compatible with automated systems.

From these results it might be concluded that the reduction of manifold volume (tubing + cell) in System II is accompanied by a more efficient mixing sample/reagent resulting in an improvement of the figures of merit.

Other practical advantages could also be mentioned:

- sample and reagents consumption are significantly reduced,
- a conventional peristaltic pump can be employed as it would provide the suitable flow pattern for the working conditions.

Therefore, the use of standard propulsion, injection and detection systems enables the implementation of this technique without practical problems.

Although a further tube radii reduction would reduce the sample and reagent's consumption, a more sophisticated FIA instrumentation should be used in this case.

The improvement of LOD beyond the capabilities of the miniature System II requires pre-concentration.

Miniaturisation showed several practical problems when the optimisation of the pre-concentration system (System IV) was attempted: the use of the small tube radius increases the inner pressure of the system, which is further increased when a packed column is used. This impairs the use of a peristaltic pump and complicates the instrumentation. Therefore, the minimisation of dispersion was achieved by applying the solid reagent strategy.

Table 3 Comparison of analytical figures of merit*. System IV (solid DPC reactant column); System III (replica of the pre-concentration system used in ref.11)

	Sample Volume (ml)	Sensit.* (μg^{-1} l)	LOD ($\mu g\ l^{-1}$)	Samp. Freq. (h^{-1})
System III	5	0.033	3	40
System IV	5	0.065	0.1	50
Improvement	-----	2	30	1.25

*Based on a 5 ml sample volume.

The results obtained for Systems III and IV are given in Table 3. The analytical performance of System III was similar to what has been reported by Pannain and Santelli [11]. For the solid reagent system (System IV) a sharp increase in the sensitivity is obtained. Limit of detection and sample throughput are also improved. As it has been discussed previously, the way in which the reagent is

added to the sample has a strong influence on the overall dispersion, thus the flow dissolution of the reagent causes a reduction of dispersion [2]. The optimisation of the column characteristics (size, concentration of DPC, etc) allowed an increase in the sampling frequency.

4. Conclusions

Reduction of the dispersion is the main goal in the optimisation of any FI technique for trace analysis. Regarding this goal, two experimental approaches to improve the figures of merit in the spectrophotometric flow injection analysis of traces of Cr(VI) with 1,5-diphenylcarbazide are presented in this work with systems with and without a preconcentration step. These approaches allow reducing the detection limit, increasing the sensitivity, and decreasing the sample volume as well as reagent consumption and increasing the sample throughput.

The improvement of sensitivity in a system with chemical reaction hinges upon the control of the physical dispersion of the sample and the strategy used for mixing the reagents. For doing so, the reduction in the tube radius is a suitable strategy, as it allows to enhance sensitivity without affecting the sampling frequency. In addition many practical advantages are achieved, such as the reduction in sample and reagent consumption keeping the simplicity of the instrumentation.

The flow dissolution of the solid reagent is another suitable way for minimising dispersion, particularly, when tube radius reduction is not a practical advantage.

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