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On-line submicellar enhanced fluorometric determination of Se(IV) with 2,3-diaminonaphthalene

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Abstract

The flow injection (FI) analytical determination of Se(IV) with 2,3-diaminonaphthalene (DAN) was assayed employing enhanced fluorometric detection. The different variables affecting the reaction rate between Se(IV) and DAN were studied in batch mode, and the best conditions for the 4,5-benzopiazselenol (Se–DAN complex) formation were found. On this basis, two different FI manifolds able to give optimal sensitivity were designed. Once established the optimal flow conditions for the product formation, a submicellar medium of sodium dodecylsulphate with the addition of β-cyclodextrin was tested to perform enhanced fluorometric detection. A limit of detection of 0.3 $\mu g\,l^{-1}$ of Se(IV), a linear dynamic range ranging between 1 and 500 $\mu g\,l^{-1}$ of Se(IV) and a sampling throughput of 40 samples h^{-1} were obtained. A full discussion on each stage of the optimisation procedure and the validation of the propose methodology is provided.

Keywords: Flow injection; Selenium determination; Fluorometric detection; β-Cyclodextrin; 4,5-Benzopiazselenol

1. Introduction

The beneficial role of micro-amounts of selenium in biological and environmental systems has encouraged the development of analytical strategies for its determination at trace levels. Selenium is a special case since it is essential for living organisms at low levels but toxic at slightly higher ones [1–3]. Thus, the analytical methods for the determination of selenium need to be sensitive and accurate.

Particular attention has been focussed on the determination of Se in natural waters [4–10], where the "window" of useful concentrations is very narrow. In this way, several spectroscopic techniques, mainly inductively coupled plasma mass spectrometry (ICP-MS) and graphite furnace atomic absorption spectrometry (GFAAS) or hydride generation atomic absorption spectrometry (HGAAS) have been reported in the literature [7–18].

The main disadvantages of these techniques are the complexity and cost of the instruments, together with the need of some degree of expertise for their proper operation. Therefore, it is still important to develop highly sensitive methods based on cheaper and easier to operate techniques such as spectrophotometry or fluorometry. Amongst another factors, the high sensitivity obtained with these last techniques depends on the selection of a suitable reaction between the analyte and some kind of complexing agent. In the particular case of selenium, care must be taken with the oxidation state as most reactions involve Se(IV). These reactions permit Se(IV) determination but not total determination unless all forms of selenium are converted into Se(IV).

The reaction of Se(IV) with aromatic diamines has been used as an alternative approach for the determination of selenium at trace levels. Se(IV) reacts with 2,3-diaminonaphthalene (DAN) yielding the complex 4,5-benzopiazselenol (Se–DAN complex) which allows the determination of selenium in different matrices either by spectrophotometry or fluorometry [19–28]. However, the last alternative—which is more sensitive—presents two

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kind of problems. First, the large number of variables that influence the reaction between selenium and DAN and, second, the poor quantum efficiency of fluorescence of Se–DAN in aqueous solutions.

Several works have been devoted to the study of the variables affecting the rate of the reaction between selenium and DAN [21,29]. Se(IV) slowly reacts with DAN at room temperature and a heating stage is usually required to complete the reaction within some minutes. Nonetheless, several discrepancies in the heating programs appear in the literature at the moment of informing the optimal combination temperature/time. The same is true for the pH of the reaction [29].

Regarding the fluorometric detection, the main problem is the poor fluorescence quantum efficiency of Se–DAN in aqueous solution, which drastically reduces the sensitivity of the direct determination. Thus, the extraction of the Se–DAN complex into an organic phase (*n*-hexane, cyclohexane or toluene) is usually required [3,23–27,30].

However, solvent extraction is troublesome and several works aiming to avoid this step have been reported. Rodríguez et al. [31] studied the enhancement of the fluorescence of Se–DAN complex by adding water miscible organic solvents but this is not a practical alternative in flow systems because the separation of phases easily occurs while employing samples with high values of ionic strength.

Another approach for enhancing fluorescence in aqueous solutions is the use of a (sub)micellar medium. Zheng and Lu [32] studied the effect of different surfactants on the analytical signal of the Se–DAN complex and found that negatively charged surfactant micelles, for example sodium dodecylsulphate (SDS), notably increase the complex signal in aqueous solutions. On the other hand, the use of β-cyclodextrin (β-CD) also showed an improved fluorescence signal of Se–DAN piazselenol. But optimum results have been obtained by the combined use of SDS and β-CD, in a submicellar SDS concentration, which produces a synergistic effect that drastically enhances the fluorometric signal of the Se–DAN complex [32]. It is claimed that micelles or SDS/β-CD "aggregates" provide some kind of cavities where the complex can be inserted thus changing its chemical environment: the lifetime of the excited state is increased and, at the same time, the possibilities of quenching are reduced [32,33]. Nevertheless, the fluorometric method in submicellar media is tedious and time consuming when it is performed in batch mode and automation becomes mandatory for routine analysis.

This work is devoted to the development and optimisation of a flow injection procedure for the determination of Se(IV) in aqueous solutions employing fluorometric detection in a submicellar medium. The optimisation presents three stages: a batch study of the different variables that affect the reaction rate between Se(IV) and DAN, the increase of the quantum efficiency of fluorescence of the Se–DAN complex by the use of SDS and β-CD, and the design of two different FIA systems of enhanced sensitivity based on the previous findings.

2. Experimental

2.1. Materials

Doubly deionised water (DDW, 18 MΩ cm^{-1}) obtained from a Milli-Q water system (Millipore, Bedford, MA, USA) was used throughout the experiments. All the reagents were analytical-reagent grade.

Selenium(IV) stock standard solutions (1.000 g l^{-1}) were prepared by dissolving Na_2SeO_3 (Aldrich, St. Louis, MO, USA) in DDW. Working standard selenium solutions (1–500 μg l^{-1}) were daily prepared by appropriate dilution of the stock standard solutions.

A 0.1% w/v 2,3-diaminonaphthalene solution was freshly prepared each week by dissolving 50 mg of 99% w/w DAN (Spectrum Quality Products Inc., USA) in 50 ml of 0.05 mol l^{-1} HCl (Merck, Darmstadt, Germany), that also contained 0.1 g of hydroxylamine hydrochloride (Merck, Darmstadt, Germany) and 0.1 g of disodium salt of ethylene diamine tetracetic acid (Merck, Darmstadt, Germany) as reducing and masking agents, respectively [19,21,23,27]. The resulting solution was extracted three times with 10 ml of cyclohexane to remove impurities.

A 0.07 mol l^{-1} sodium dodecylsulphate solution was prepared from 90% w/w SDS (Riedel & de Haën, Germany).

The SDS and β-cyclodextrin (β-CD) mixed solutions were prepared by dissolving a suitable weighted amount of 98% w/w β-CD (Sigma, USA) in the volume needed for obtaining a final concentration of 10 mmol l^{-1} of β-CD. The adequate volume of SDS solution was added to the β-CD solution in order to obtain the desired molar ratios of SDS to β-CD.

2.2. Instruments

A spectrophotometer Perkin-Elmer UV-Vis Lambda 20 and a spectrofluorometer Hitachi F2000 equipped with quartz flow cells of 80 and 18 μl, respectively, were used for the on-line measurements of the analytical signals. A personal computer for time recording and data acquisition was attached to each instrument. Conventional 1.00 cm quartz cell were employed for the batch procedure in both instruments (vide infra).

The two manifolds tested for fluorometric flow injection experiments are shown in Fig. 1. An eight-channel Ismatec-IPC peristaltic pump (Valco, Houston, USA), 0.75 mm i.d. PFTE® tubing and VICI-Cheminert C22Z injection valves (Valco, Houston, USA) were employed in all cases.

2.3. Procedure

The product of the reaction between Se(IV) and DAN (i.e., Se–DAN complex) was followed by UV-Vis spectrophotometry. Once the conversion degree of Se(IV) was optimised, the enhancement of the fluorometric signal by the addition of SDS/β-CD mixtures to the Se–DAN complex was tested.

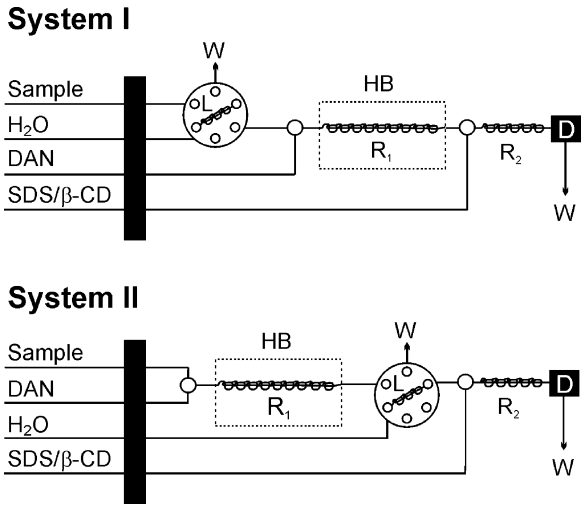

Fig. 1. FI manifolds for the submicellar enhanced fluorometric determination of Se(IV) with DAN: L: sample loop; HB: heated water bath; R_1: knotted reactor 1; R_2: knotted reactor 2; D: detector; W: waste.

The operating conditions were carried out as described below.

2.3.1. Optimisation of the batch procedure

2.3.1.1. Formation of the Se–DAN complex (UV-Vis detection). Different experiments were performed to evaluate and optimise the degree of conversion of Se(IV) into the Se–DAN complex. Optimal reagent concentration was obtained by adding different volumes (0.25–2 ml) of a 0.1% w/v DAN solution to 1 ml of a solution containing 10 mg l^{-1} of Se(IV), adjusting to pH 2.0 and to 10 ml of final volume, and then, heating at 90 °C during 5 min. The best results were obtained when 1 ml of 0.1% w/v DAN solution was added (see Section 3).

Once fixed the final reagent concentration, a Box-Benhken experimental design [34] was employed for the optimisation of the pH, temperature and time of reaction which ranged between 1.2–3.2, 70–90 °C and 2–8 min, respectively. The experimental procedure is described as follows. A set of testing tubes containing 1.0 ml of a standard solution of 10 mg l^{-1} of Se(IV) and 1.0 ml of a 0.1% w/v DAN solution was prepared. The different pH values were reached by the addition of suitable amounts of HCl (0.1 mol l^{-1}) or NaOH (1 mol l^{-1}). All solutions were made up to 10 ml with DDW and the pH values were measured. The tubes were closed and placed in a conventionally heated water bath for fixed values of time and temperature. Then, the reaction was stopped by cooling down the tubes to room temperature and the absorbance of each sample was measured at $\lambda = 380$ nm.

2.3.1.2. Enhancement of fluorescence intensity of the Se–DAN complex (fluorescence detection). The enhancement of fluorescence intensity of the Se–DAN complex by the addition of the SDS/β-CD solution was studied in batch mode in order to mimic the confluence point of the continuous system, i.e., including the dilution effect.

A Box-Benhken experimental design was employed by modifying the pH (1.2–3.2), the SDS to β-CD molar ratio (SDS/β-CD = 0.4–1.0) and the volume (3–7 ml) of the SDS/β-CD mixtures added to a constant amount of Se–DAN complex. The experimental procedure is described as follows. A set of testing tubes containing 1.0 ml of a standard solution of 5 mg l^{-1} of Se(IV) and 1.0 ml of a 0.1% w/v DAN solution was prepared. The pH value and the volume were adjusted to 2.0 and 8 ml, respectively. Then, the samples were heated at 90 °C for 5 min. The pH was again adjusted at the values of the experimental design and the volume was made up to 10 ml ($V_{Se–DAN}$). The prefixed volumes of SDS/β-CD mixtures ($V_{SDS/β-CD}$) were then incorporated to the previously obtained 10 ml of the Se–DAN solutions, without adjusting the final volumes. Afterward the fluorescence signal of each sample was measured at $\lambda_{ex} = 375$ nm and $\lambda_{em} = 546$ nm.

2.3.2. Optimisation of the FIA system

First, the optimisation of a flow system with spectrophotometric detection was performed to establish the best FIA variables for the formation of Se–DAN complex, i.e., the on-line conversion degree of Se(IV). Second, the fluorometric detection was optimised for two flow injection manifolds shown in Fig. 1. Under both modes of detection, residence time was optimised adjusting the flow rates and reactor length to attain the best signal to noise ratio. Sample volume was modified to maximise the signal to noise ratio without significantly decreasing the sampling rate.

The influence of the residence time and the temperature of reaction over the conversion degree of Se(IV) was tested by merging a 10 mg l^{-1} of Se(IV) solution and a 0.1% w/v DAN solution with a fixed flow rate ratio (equal to the volume ratio stated from the batch procedure), pumping them into a reactor immersed in a heated water bath and, finally, monitoring the product of the reaction by spectrophotometry.

After the optimum conditions for the on-line chemical conversion of Se(IV) to Se–DAN complex were set up as described above, the influence of the SDS to β-CD molar ratio on the intensity of fluorescence was tested. In this last case, SDS/β-CD, carrier and DAN solution flow rates ($Q_{SDS/β-CD}$, Q_{H_2O} and Q_{DAN}, respectively) were kept constant. The optimum value obtained for SDS to β-CD molar ratio (SDS/β-CD = 0.6) was then used for the selection of the best ratio between the $Q_{SDS/β-CD}$ and $Q_{H_2O} + Q_{DAN}$.

3. Results and discussion

3.1. On the optimisation of the batch procedure

3.1.1. Formation of the Se–DAN complex

The experimental results show that a maximum degree of conversion of Se(IV) to Se–DAN complex is attained from

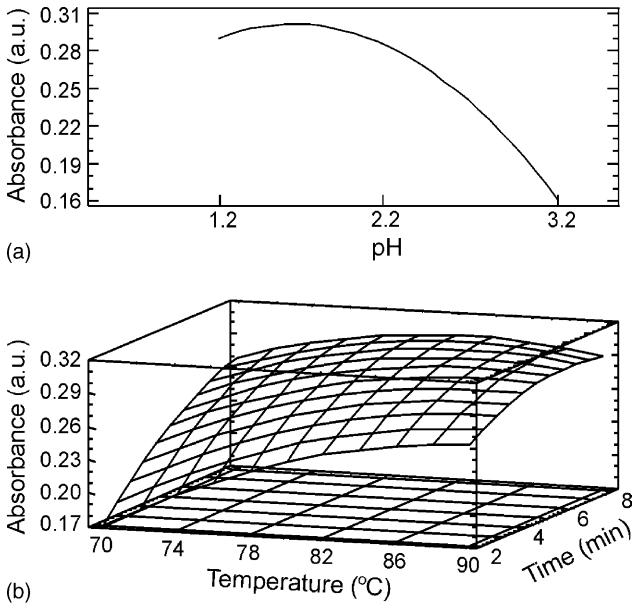

Fig. 2. Effect of the temperature, residence time and pH of reaction over the formation of the Se–DAN complex ($\lambda = 380$ nm).

approximately 50-fold DAN to Se molar ratio. This can explain the addition of 1.0 ml of a 0.1% w/v DAN solution as an optimal value in the batch procedure. Regarding the other variables, the experimental design shows that the three factors (temperature, time and pH) are significant at the 95% confidence level. The model explains 98.1% of the variability.

No correlation between pH and the other variables was found. The dependence of the degree of conversion of Se(IV) into Se–DAN on the acidity is shown in Fig. 2a. A maximum analytical signal (absorbance) is found at pH values ranging between 1.2 and 2.2. Because the change of Se(IV) conversion is below 5% in this range, a pH close to 2 was selected as optimum.

Conversely, temperature and time are highly correlated as expected (Fig. 2b). It is obvious that the lower the temperature the higher the time required to obtain the best yield. Nonetheless, as the Se–DAN complex decomposes at high temperatures, it was observed that heating at 90 °C for 5 min yields the best results.

At this point, some comments should be made on the optimal conditions found in this work. Mostly, studies devoted to classical or enhanced fluorometric determination optimise variables by following the intensity of fluorescence of the Se–DAN complex [23,26,27,33]. However, since several steps are involved in this determination, there is not a clear understanding of the effect of each variable on the ultimate response. To overcome this problem, we have used UV-Vis spectrophotometry to study the factors that influence only the chemical reaction and, based on these findings, we have worked separately on the fluorometric signal. Thus, the optimum DAN concentration is at least 50-fold lower than that already reported by other studies [26–28] probably because the several steps involved in the fluorometric determination over-dimension the quantity needed for a good reaction yield. In this work, it was observed that the increase of DAN concentration above a given value leads to the development of turbidity when mixing with the submicellar media. This fact produces a considerable increase of the blank signal which decreases the signal to noise ratio [19,26–28,30].

The effect of pH on the degree of conversion of Se(IV) also deserves particular attention. While some authors recommend pH values ranging between 1 and 2 [21,25,26], others mention that no strict control is needed in the range 1–3 [27,30]. However, the control of pH seems to influence markedly the efficiency of extraction of the Se–DAN complex with cyclohexane and a value of 1.8 (±0.05) is strongly advised [25,26]. Cukor and Lott [29] found that the chemical species of Se and DAN that participate in the reaction are the undissociated selenous acid (H_2SeO_3) and the monoprotonated form of DAN ($DANH^+$). According to the pK_a values of both acids (H_2SeO_3: 2.46 and 7.31 [35], $DANH_2^{2+}$: 0.50 and 2.11 [29]), the maximum concentration of reacting species is restricted to a narrow range of pH, particularly due to the $DANH^+$ concentration. As a matter of fact, the monoprotonated form of DAN is important at pH values close to 1.5 ($1/2(pK_{a1}+pK_{a2})$) and its concentration sharply decreases at lower and higher values of pH. Thus, it is clear that under no strict pH control, an increase on the reagent concentration needs to be used in order to reach a suitable excess of $DANH^+$. This can explain why reagent concentration reported in the literature is several orders of magnitude higher than the optimum value found in this work.

Several discrepancies are also found regarding the heating programs [19,26–28,33]. In most papers, temperature is fixed at a certain value and sufficient time is given for the reaction to reach equilibrium. However, the whole process involves two consecutive reactions, the formation and the decomposition of the Se–DAN complex, both favoured by temperature. Therefore, excess of heating may lead to a decrease in the analytical signal as observed when temperatures close to 90 °C are kept for more than 10 min. Furthermore, side reactions as those involving the reagent decomposition may be favoured bringing about several problems (e.g., turbidity, increase of the background).

3.1.2. Enhancement of fluorescence intensity of the Se–DAN complex

Once the best conditions for the chemical reaction were established, the enhancement of the fluorometric signal was tested taking into account the influence of the SDS/β-CD molar ratio and the pH of the resulting solution. The results of the experimental design show some degree of correlation between pH and SDS/β-CD molar ratio (Fig. 3). A range of 0.5–0.7 SDS/β-CD molar ratio was selected, being the optimum range of pH 1.9–2.8 as shown in Fig. 3a. Moreover, the batch experiment revealed that the addition of a volume approximately equal to 5 ml of a solution of SDS/β-CD = 0.6 to 10 ml of sample containing the Se–DAN complex (i.e.,

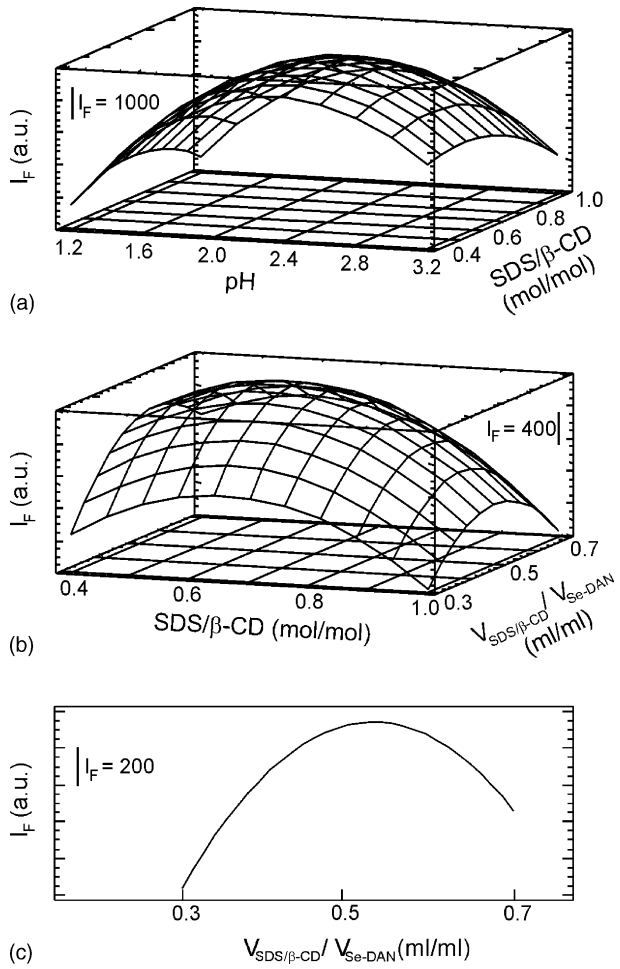

Fig. 3. Effect of the pH, the SDS to β-CD molar ratio and the volume of SDS/β-CD mixture added to a constant amount of Se–DAN complex ($V_{Se–DAN}$ = 10 ml) on the fluorescence signal (λ_{ex} = 375 nm, λ_{em} = 546 nm).

$V_{SDS/\beta\text{-CD}}/V_{Se–DAN} = 0.5$) was optimum (Fig. 3b and c). Note that the signal in Fig. 3 does not include the correction of the dilution effect, and then, allows us a more realistic comparison with the fluorometric FIA systems, as we will see later.

The use of a (sub)micellar medium is very important at the moment of optimising the fluorometric determination of selenium at trace levels. As it is well known, the addition of surfactants or organic solvents increases the intensity of luminescence of many compounds, particularly organo-metallic complexes [31–33,36,37]. This enhancement is produced by changes in the viscosity and micropolarity of the tested solution, which contribute to stabilise the excited state (singlet) of the fluorescent molecules reducing their collisional deactivation. Inside this group of "stabilising or protecting" compounds, cyclodextrins are very well known: in aqueous solutions they tend to form hydrophobic cavities where non-polar compounds can be located [38–40]. This type of interaction restricts the mobility of the molecules, and thus we can expect an increase of the intensity of fluorescence of some compounds. Especially, it has been reported a synergistic influence on this enhancement when a solution of the β-CD is added to a surfactant below its modified critical micellar concentration (cmc), i.e., under submicellar conditions [32].

The synergistic effect over the fluorescence quantum efficiency of 4,5-benzopiazselenol after using a surfactant and β-CD mixture have been previously reported by Zheng and Lu [32]. They found that, the intensity of fluorescence of the Se–DAN complex in aqueous solutions could be increased 30 times for the addition of a SDS/β-CD solution. Our results are similar to those reported by Zheng and Lu [32]: an enhancement of two orders of magnitude in the fluorescent signal was achieved by the co-addition of SDS and β-CD (SDS/β-CD = 0.6), and only a poor increase of the signal was observed when either SDS or β-CD were used separately. The maximum signal enhancement was obtained for a concentration of SDS below the cmc of SDS modified by the presence of β-CD. Once the maximum is reached, a decrease of the signal is observed, because the modified SDS cmc have been exceeded. In this way the highest signal was found for a final concentration of SDS equal to 6 mmol l^{-1} (lower than the modified cmc) and a final concentration of β-CD equal to 10 mmol l^{-1} (see Fig. 3b).

Finally, the figures of merit for the batch fluorometric determination of Se(IV) were calculated for the optimal experimental conditions (pH = 2, SDS/β-CD = 0.6, $V_{SDS/\beta\text{-CD}}/V_{Se–DAN} = 0.5$) and are reported in Table 1. A good analytical performance in terms of limit of detection (LOD) is observed when compared the use of the SDS/β-CD signal enhancer with the classical organic extraction procedure (LOD = 1 μg l^{-1}) [25–27,30,33]. However, manual operation is laborious, particularly when a large number of samples needs to be handled. Thus, a flow injection automatic strategy was developed in order to apply the technique to routine analysis.

Table 1
Figures of merit for the batch and FIA fluorometric determination of Se(IV) using SDS/β-CD mixture as signal enhancer

Parameter	Batch system	FIA system[a]	
		I	II
Detection limit, 3 s (μg l^{-1})	3[b]	0.3[c]	0.3[d]
Linear dynamic range (μg l^{-1})	10–500	1–500	1–500
Precision, R.S.D., n = 10, 20 μg l^{-1} (%)	3.7	0.3	0.7
Sampling frequency (samples h^{-1})	10	40	60[e]

[a] Dispersion coefficients for the optimised systems lower than 1.1.
[b] Calculated on the basis of three times the standard deviation for 10 replicate measurements of the blank.
[c] Calculated on the basis of three times the baseline noise.
[d] Calculated on the basis of three times the standard deviation for 10 replicate measurements of the blank.
[e] Calculated on the basis of the reaction time.

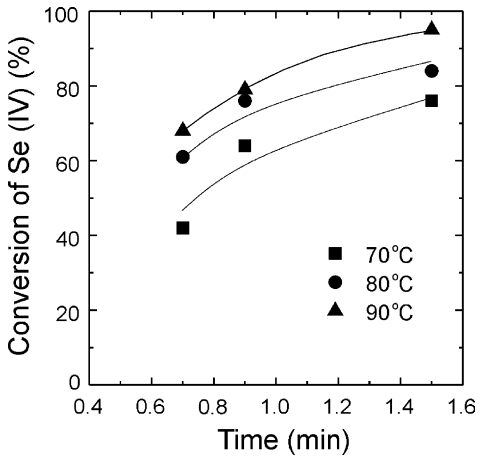

Fig. 4. Degree of conversion of Se(IV) to Se–DAN complex as a function of the residence time at different temperature values using a FIA system with spectrophotometric detection.

3.2. On the optimisation of the FIA system

FIA systems for trace analysis require a strict control of the dispersion of the sample to keep the sensitivity and LOD of the steady-state procedure. However, low dispersion is not easily achieved in our reacting system because large residence times are unavoidable for obtaining, first, a high degree of conversion and, second, an efficient mixing of the reaction product (Se–DAN) with the fluorometric signal enhancer (SDS/β-CD). Additionally, heating may produce noise, which contributes to impoverish LOD.

Since the reaction between selenium and DAN is not fast, a single line configuration was discharged [41] and a flow manifold where sample and reagents merge in a confluence point was chosen. The flow rate of each merging reagent (DAN and the SDS/β-CD mixture) was lowered in order to obtain minimal dilution of the sample without affecting the stability of the flow pattern. A practical criterion of 8 to 1 for the sample to reagent flow ratio at the confluence point was selected while the DAN concentration was 0.1% w/v in order to produce an excess of the reagent (vide supra), being the pH of the out coming solution stream equal to 2.

Several experiments were performed to optimise on-line the conversion of selenium into the Se–DAN complex. Using a FIA system with spectrophotometric detection, the curves of the degree of conversion versus the residence time at different temperature values were obtained and are shown in Fig. 4. It is relevant to remark that bubble formation was observed at temperatures higher than 90 °C. Therefore, the temperature of the reaction was fixed at 90 °C and the time of residence into the reactor was kept close to 60 s. Under these conditions, the degree of conversion achieved for Se(IV) was approximately 80%. Larger residence times produce an increase of the dispersion of the sample plug; consequently the expected increase of the sensitivity for higher degrees of conversion would be lost and an additional decrease of the sample frequency would be obtained.

It must be mentioned that the degree of conversion of Se(IV) as a function of the residence time for a given temperature is higher in the flow system than in the batch procedure (compare Figs. 2b and 4), because the transfer of heat to the solution is more efficient in the FIA system as the diameter of the reactor (0.75 mm) is much lower than that employed in a testing tube (15 mm) in the batch procedure.

With respect to the optimisation of the FIA variables for the fluorometric detection in the system I (see Table 2), a knotted reactor of 200 cm (R_1) was employed and the flow rate of the carrier plus of the DAN solution ($Q_{H_2O} + Q_{DAN}$) was adjusted to 0.9 ml min^{-1} to obtain a residence time in the reactor R_1 of 60 s. A sample loop of 100 cm was used because higher values do not significantly increase the signal but decrease the sampling rate.

Another confluence for the introduction of the SDS/β-CD solution was connected at the end of the manifold and the optimisation of the fluorometric signal was performed. A 60 cm knotted reactor (R_2) was employed to obtain a good degree of mixing between the reaction product and the solution of the fluorometric signal enhancer. Shorter reactor lengths lead to a reduction on the peak height and an increase on the baseline noise, i.e., a decrease in the signal to noise ratio.

The molar ratio of the SDS/β-CD was 0.6 before the dilution at the confluence point, which is approximately the same value found as optimal in the batch experiments (compare Figs. 3b and 5). Fig. 6 shows the fluorometric signal as a function of the ratio of the flow rates of SDS/β-CD to carrier plus DAN solution. It is clear that for $Q_{H_2O} + Q_{DAN} = 0.9$ ml min^{-1}, the maximum signal is achieved for $Q_{SDS/\beta\text{-CD}}$ approximately equal to 0.4 ml min^{-1}. A higher flow rate for SDS/β-CD solution increases the dilution of the sample, and the signal starts to decrease (Fig. 6) which was also observed for the batch procedure (Fig. 3c).

The analytical performance of the FIA system I is shown in Table 1: a good LOD is found and the method can be applied over a wide range of concentrations. Although the sampling frequency is relatively low considering conventional

Table 2
Optimised chemical and FIA parameters

System[a]	Sample loop length (cm)	R_1 length (cm)	R_2 length (cm)	Q_{DAN} (ml min^{-1})	$Q_{SDS/\beta\text{-CD}}$ (ml min^{-1})	Q_{H_2O} (ml min^{-1})	SDS/β-CD molar ratio
I	100	200	60	0.1	0.4	0.8	0.6
II	50	200	60	0.1	0.4	0.8	0.6

[a] FI manifold configuration are shown in Fig. 1.

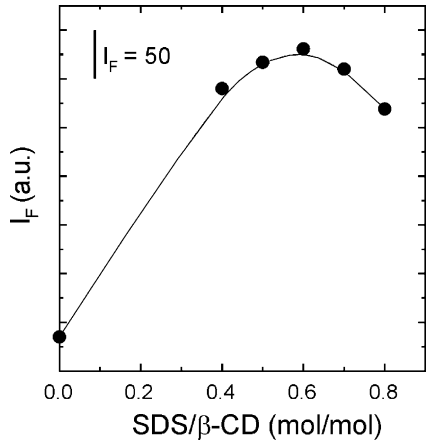

Fig. 5. Fluorescence signal vs. the SDS to β-CD molar ratio using the FIA system I. Experimental conditions: $T = 90\,^{\circ}C$; $Q_{H_2O} = 0.8\,ml\,min^{-1}$; $Q_{DAN} = 0.1\,ml\,min^{-1}$; $Q_{SDS/\beta\text{-}CD} = 0.4\,ml\,min^{-1}$ ($10\,mmol\,l^{-1}$ of β-CD).

FIA systems, it must be noticed that the performance of the batch procedure is markedly improved.

Along the experiments performed with system I, a browning of the tubing located after the heated water bath was observed. This browning, which is attributed to the slow decomposition of the reagent with time (probably a polymer of DAN [29]), promotes an increase of the base line after several days of continuous work. Since this process can occur inside the flow cell and can affect the performance of the fluorometric detection, the alternative FI manifold (system II) shown in Fig. 1 was tested. In this approach, the sample loop is fed with a solution where the Se–DAN complex is already formed and thus, the contact between the reagent DAN and the cell is minimised. Under these conditions, the optimisation of this new FIA system is based upon keeping the dispersion of the injected plug and the optimised parameters are reported in Table 2. The resulting analytical performance of the system II is shown in Table 1 and is similar to that obtained with the previous one. Although the peak width obtained from the system II is significantly lower than the one resulting from system I, sampling rate is about the same in both cases as it is controlled by the reaction time in the first reactor (R_1).

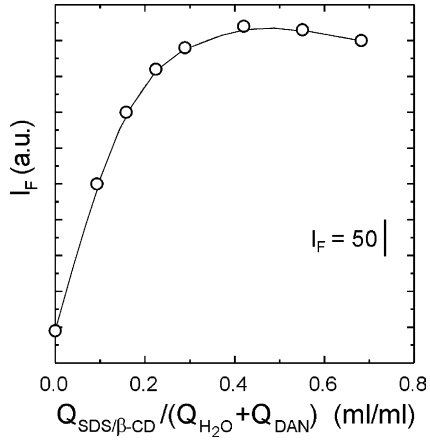

Fig. 6. Fluorescence signal as a function of the flow rate of SDS/β-CD holding constant the flow rate of the carrier plus DAN solution (FIA system I). Experimental conditions: $T = 90\,^{\circ}C$; $Q_{H_2O} = 0.8\,ml\,min^{-1}$; $Q_{DAN} = 0.1\,ml\,min^{-1}$; SDS/β-CD = 0.6 ($10\,mmol\,l^{-1}$ of β-CD).

Table 3
Analytical results of spiked tap water samples[a]

Selenite added ($\mu g\,l^{-1}$)	Selenite found ($\mu g\,l^{-1}$)		
	Fluorescence		Atomic absorption
	System I	System II	FIA–HGAAS
5	5.1 ± 0.3	5.1 ± 0.4	5.1 ± 0.3
10	9.8 ± 0.4	10.2 ± 0.6	10.4 ± 0.3

[a] Mean and 95% confidence limits for four measurements.

The accuracy of both FIA systems, I and II, was tested by spiking a tap water sample with two different concentrations of Se(IV) (5 and $10\,\mu g\,l^{-1}$) and using another FI manifold with hydride generation and atomic absorption detection as a reference method [13]. Table 3 shows the excellent recovery results in both employed methodologies with fluorometric detection. Thus, for the concentration levels that foreign ions are present in drinking tap water, selectivity problems were not detected in our work.

4. Conclusions

The use of SDS/β-CD mixtures as fluorometric signal enhancers of the Se–DAN complex for the determination of selenium allows the effective determination of Se(IV) with similar analytical performance to those obtained with the classical organic-phase extraction procedure, but with the advantage of avoiding an extraction step. On this basis, it have been showed that under optimised conditions, the on-line submicellar enhanced fluorometric determination of Se(IV) with DAN is a very simple, rapid and reliable method for the determination of low levels of selenite in drinking waters. Additional work is currently being performed in relation to the inorganic speciation of selenium in more complex matrix samples.

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