J Fluoresc (2010) 20:55–65
DOI 10.1007/s10895-009-0521-8

ORIGINAL PAPER

A Gas Diffusion Technique Coupled with Flow Injection Systems. Optimization of the Process in its Application to the Fluorimetric Determination of Ammonium in Water Samples

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Received: 3 March 2009 / Accepted: 8 July 2009 / Published online: 16 July 2009

Abstract A novel flow injection-gas-diffusion (GD-FI) system has been developed for the on-line analysis of ammonium ion in waters with fluorimetric detection, using an acceptor solution containing the Eosin-Bluish (EB) acid-base indicator. This, together with optimization of the process of gas transfer through the membrane, increases the sensitivity of the method to a considerable extent. Under optimum conditions, it is possible to determine the analyte within the 0.02–1.5 mg l^{-1} range, with a limit of detection of 5 μg l^{-1} and relative standard deviations (n=12, [NH_4^+]= 50 μg l^{-1} and 0.05 μg l^{-1}) of 3.4% and 3.0% respectively. The determination rate was 15 samples per hour.

Keywords Gas-diffusion · Flow injection analysis · Ammonium determination · Eosin bluish · Water · On-line process analysis

Introduction

Flow injection (FI) analysis is a powerful flow-through technique that allows simple manipulation of the solution prior to detection [1]. The selectivity of the technique can be improved considerably if on-line separation steps are introduced in the FI procedure. Very often, such separation steps involve processes such as solvent extraction, adsorption, precipitation or membrane based separation [2].

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FI analysis based on gas-diffusion separation (GD-FI) has been used for a variety of analytes [3, 4]. The process, characterized by its high selectivity, promising sensitivity, and excellent precision and speed, has proved useful in determining volatile species and those that can be selectively and quantitatively converted to a gas in complex matrices. In liquid-liquid GD-FI, an analyte in the donor stream is either present as a gas or undergoes some reaction to produce a gaseous analyte that will diffuse through the membrane into the acceptor stream. The membrane prevents other non-volatile species from passing through; thus, to a large extent matrix interferences are effectively eliminated. Procedures involving a liquid donor and a gaseous acceptor stream have also been described [5].

GD-FI methods were first introduced for the determination of carbon dioxide in serum [6], and a similar approach has been suggested for the determination of ammonia [1].

In both cases, selective transfer of the compound to be measured from an ill-defined stream into a carrier stream of well-defined composition takes place. The compound that passes through the membrane causes a protolytic reaction in the acceptor solution and the pH change thus obtained can be detected photometrically or fluorimetrically if a suitable acid-base indicator is present in the solution [4].

Many variables are involved in the above processes, and these variables must be optimized for maximum sensitivity to be achieved. The generation of a signal corresponding to the injection of a sample involves three steps: transfer of the relevant species across the membrane; the effect of this compound on the pH of the acceptor solution, and the change in absorbance or fluorescence caused by this change in pH. Based on a serial tank model, Van de Linden [7]

developed mathematical models, obtaining general expressions for the transport processes in membranes in the same flow unit [8-10]. Their validity has been checked for several analytes with different volatilities and membranes of different compositions. However, for the serial tank model to be able explain the experimental procedure, in all cases it is necessary to work with almost identical flow rates of the donor and acceptor solutions, a condition in which the yield of the gas transfer process through the membrane does not have to be maximum. Other variables, such as temperature or flow stopping, must also be taken into consideration.

The ammonium ion plays an important role in the nitrogen cycle of many biological and industrial processes [11] and is therefore one of the main components in many industrial effluent streams [12]. In general, high concentrations of this ion in waters generate nutrient enrichment, and hence an increase in biological activity, which may lead to the appearance of large amounts of algae and an increase in turbidity, generating problems of toxicity, taste and smell. However, owing to its adverse effect on the oxygen balance in the aquatic environment, the ammonium ion is classified as a List II substance under the European Community Dangerous Substances in Water Directive (76/464/EEC) [13], such that the dumping of wastewaters containing ammonium ion is subject to legislative control. The introduction of increasingly stringent legislation on water quality has enhanced the need for on-line analytical monitoring.

Several procedures have been proposed for the determination of ammonium ion using the flow injection (FI) technique [14]. Among them, GD-FI methods have proved useful for the determination of the ion in complex matrices, including Kjeldahl digests [15-18]. In this technique, the sample is injected into, or merged with, an alkaline solution, and the ammonia formed diffuses across a gas-permeable membrane into a recipient stream. The change in the absorbance or fluorescence of the acid-base indicator in the acceptor stream can be measured and related to the ammonium content in the sample.

Here we report the implementation of a procedure for the determination of ammonium using this technique with fluorimetric detection. An important part of the procedure is devoted to optimizing the set of variables that affect yield in the process of gas transfer through the membrane, establishing the optimum experimental conditions for transfer to be maximum. The use of an acceptor solution containing the Eosin-Bluish (EB) acid-base indicator, whose basic form can be monitored fluorimetrically, increases the sensitivity of the method to a considerable extent. The proposed procedure is more versatile, rapid, selective and readily adaptable to routine analyses than other methods described in the literature.

Experimental

Apparatus and materials

Minipuls HP4 (Gilson, France) peristaltic pumps with silicone or vinyl pump tubes. PTFE sample-injection valve (Rheodyne, model 5020). Detection was performed with an RF-5000 spectrofluorimeter (Shimadzu, Japan) fitted with a DR-15 data processor and an FDU-13 data storage unit, to which a sensitization unit was coupled, (Shimadzu, 200-26841-01). A 38-µl flow cell (Hellma, Germany, 176.052) with an optical pathway of 1 cm was employed. A Plexiglas 5020-024 chemifold^{TM} Type V gas-diffusion module and 5000-2875 gas-diffusion membranes (Tecator-Foss) were also used. PTFE tubing of 0.5 mm internal diameter with standard tube fittings and connectors (Upchurch Scientific, Inc.) was used. A Crison 501 potentiometer and a Digiterm 3000542 (Selecta) water bath were also used.

Reagents and solutions

All chemicals used in this work were of analytical grade and were prepared with ultra-high quality deionized water. Ammonium standard solution: a stock solution was prepared by dissolving 0.0376 mg of ammonium chloride (Panreac) in 250 ml of water. Standard working solutions were prepared by diluting the stock solution with water. A 0.012 M solution of sodium hydroxide (Scharlau) was used as the donor stream. Stock indicator solutions of 2.5×10^{-2} g l^{-1} and 4.8×10^{-2} g l^{-1} of EB ($C_{20}H_6Br_2N_2Na_2O_9$), prepared by dissolving the appropriate amount of reagent and adjusted to pH 3.7 with hydrochloric acid, were used as the acceptor stream.

A standard reference solution for water analysis -CRM 409 from the Community Bureau of reference, BCR, (Promochem) was also used.

Flow system

The flow system employed in the experiments is shown in Fig. 1. It consists of three channels driven by two peristaltic pumps with a view to controlling the flow rates of the donor and acceptor solutions. The acceptor solution of 5×10^{-5}M EB at pH=3.60 flows through channel C_1 with a flow rate, F_A, of 0.20 ml min^{-1} for the classic technique (the one in which 408 µl of standard solution or sample containing NH_4^+ was injected) and 0.15 ml min^{-1} for the continuous sampling mode (when the solution of NH_4^+ flows continuously through C_3). The solution of NaOH, at a concentration of 0.012 M, flows through C_2; this is responsible for the transformation of NH_4^+ into NH_3. In the classic technique, water flows through C_3 as a carrier.

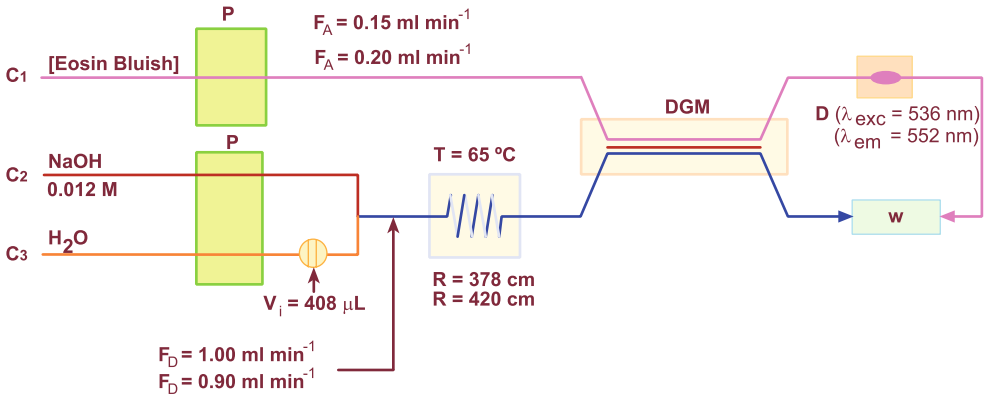

Fig. 1 Flow scheme used for the determination of NH_4^+ after its conversion into NH_3 the collection of the gas in an acceptor solution Eosin Bluish. In the classic technique, the solution of NH_4^+ is injected into the system. In continuous flow mode the solution is passed in continuous sampling mode through C_3. C_1, C_2, C_3: channels, P: peristaltic pumps, R: reactor, T: temperature, DGM: gas diffusion module, D: detector, W: waste

The flow rates of the above solutions through C_2 and C_3 are identical, such that when the solutions merge they have an F_D value=1.00 ml min^{-1} for the classic technique and 0.90 ml min^{-1} for the continuous sampling mode. The donor solution generated from the merging point passes through a reactor of 378 cm in the first case and 420 cm in the second, the reactor being thermostatted at 65 ºC (optimum temperature). Under these conditions, the heating time of the *donor bolus* is 50 s in both techniques. The ammonia generated is transferred through the membrane in the gas diffusion module (DGM) and is collected in the acceptor solution containing EB, giving rise to a change in the fluorescence signal.

Results and discussion

Fluorescence spectra

In preliminary experiments it became necessary to determine the fluorescence behavior of EB. The compound used ($C_{20}H_6Br_2N_2O_9Na_2$), the sodium salt 4',5'-dibromo,2',7'-dinitrofluorescein, is highly soluble in water and its basic form emits fluorescence.

The spectra in aqueous solution showed maximum emission λ_{em}=552 nm, the optimum excitation wavelength being λ_{exc}=536 nm (slit width 5 nm).

Figure 2 shows the excitation and emission spectra at the optimum wavelengths at basic pH (pH=10). The same solution of EB at pH=1 did not emit fluorescence at any excitation wavelength, confirming that the acid form of EB is not fluorescent. The spectra did not change shape with pH, although the intensity of emission decreased as pH became more acid owing to the decrease in the concentration of the basic form of the indicator.

Preliminary studies

To determine the relationship between the concentration of EB and the intensity of fluorescence emission, aqueous solutions were prepared at pH10.58 -the pH at which the basic form of the indicator predominates- containing varying concentrations of the indicator between 1.67×10^{-7} M and 33.3×10^{-7}M, measuring the values of fluorescence emission intensity at 552 nm.

The values of the emitted fluorescence intensity, up to a concentration of 16.7×10^{-7}M, fitted a straight line against the concentration of the indicator, with an equation of I_F= (8±7) + (3.0±0.1)×10^{-8} [EB]; R^2=0.997, for a confidence interval of 95%.

Influence of pH on the concentration of the basic form of EB

With a view to determining the evolution of the concentration of the basic form of the fluorescent tracer with pH, solutions of the dye were prepared, all of them containing a concentration of 13.3×10^{-7}M and having a pH between 3.01 and 11.49. The intensity of fluorescence was measured at 552 nm, maintaining the excitation wavelength constant at 536 nm. Plotting the values obtained against pH revealed that for pH values below 5 a sharp drop in the fluorescence emission intensity occurred, due to the decrease in the concentration of the basic form of the dye. Once the initial concentration of the dye was known, it was possible to deduce the pKa of EB experimentally, this proving to be 3.6.

Determination of NH_4^+ in continuous flow mode using EB as tracer

Figure 1 shows the flow scheme used for the determination of NH_4^+. In this system, on arriving at the module containing the membrane the NH_3 present in the donor bolus is partially transferred to the acceptor solution, generating an acceptor bolus in which pH rises and, as a consequence, the concentration of the basic form of EB is increased. Accordingly, when the bolus arrives at the flow cell of the fluorimeter, programmed to measure at λ_{exc}= 536 nm y λ_{em}=552 nm, a baseline is obtained with a constant fluorescence intensity that corresponds to the emission due to the concentration of the basic form of EB

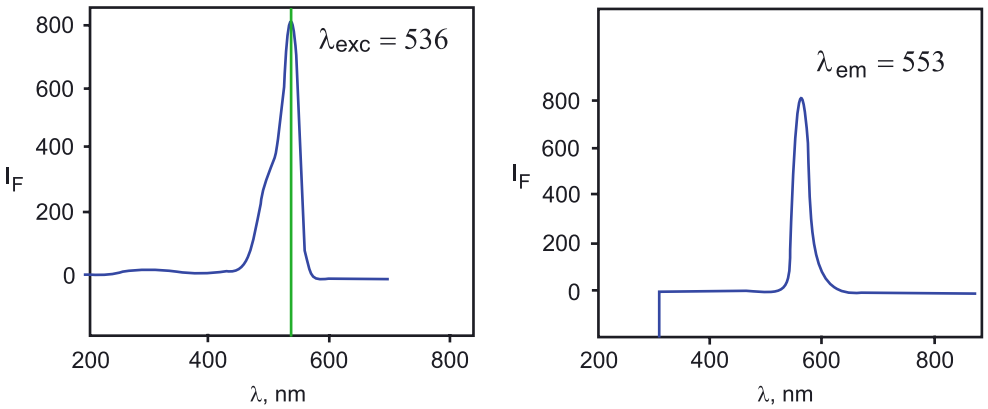

Fig. 2 Excitation (for λ_{em}= 553 nm) and emission (for λ_{ex} =536 nm) spectra of Eosin Bluish at pH=10.0

present in the acceptor solution and a positive analytical signal due to the increase in the basic form of the dye and hence in the fluorescence emission intensity. The analytical signal —a fiagram (Fig. 3)- differs not only in height but also in shape. If the classic technique is employed, in which the solution of NH_4^+ is injected, a typical fiagram is obtained (Fig. 3a), where the increase in emission intensity, ΔI_F, up to the maximum of the fiagram represents the analytical measurement; when the NH_4^+ solution is passed to continuous sampling mode (Fig. 3b), the analytical signal, ΔI_F, is measured by the difference between the emission intensity shown by the signal at its maximum constant value and the emission intensity of the baseline. It may be seen that the value of ΔI_F, for the same solution of NH_4^+, is higher in the continuous sampling mode than in the classic technique.

Optimization of the experimental conditions

Using the proposed flow scheme, in order to determine the influence of the concentration of the solution of NaOH that flows through C_3 standard patterns of ammonium were injected at concentrations of 3 and 10 mg l^{-1}. It was observed that when the concentration of NaOH increased the value of ΔI_F also increased because a greater amount of ammonia had been generated in the donor solution, an almost constant value being reached in both experiments from NaOH=0.006 M and up to 0.06 M, after which value the signal decreased slightly. A value of 0.012 M was chosen for the remaining experiments.

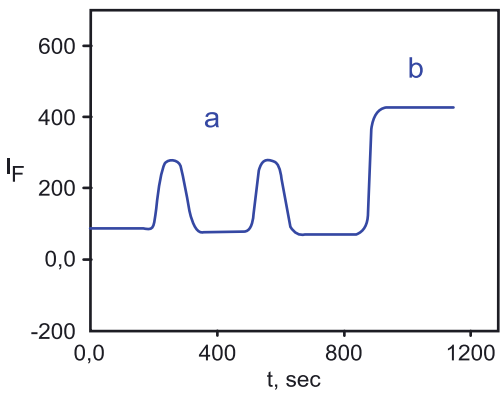

Fig. 3 Fiagrams obtained upon using Eosin Bluish at pH<5.0. **a** Classic technique, **b** Continuous flow mode

Influence of the pH of the acceptor solution

To deduce the optimum pH of the acceptor solution, under the conditions indicated in Fig. 1, a standard solution of NH_4^+ at 0.35 mg l^{-1} was injected (408 µl) or passed in continuous mode, and the pH of the EB solution was modified from 3.30 to 4.10; this solution containing a total analytical EB concentration of 5.0×10^{-5}M. The results obtained, ΔI_F, upon performing each experiment in triplicate are shown in Fig. 4. It may be seen that the evolution of the analytical signal, ΔI_F, is the same, regardless of the technique employed and the only difference is that the magnitude was greater in the continuous sampling mode.

For both techniques, as the pH value surpassed a value of 3.30 the analytical signal increased because so did the concentration of the basic form of the dye, maximum values being reached around pKa values between 3.60 and

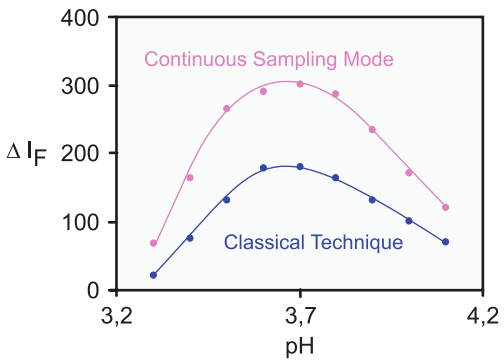

Fig. 4 Determination of ammonium by gas diffusion in continuous mode using Eosin Bluish as the fluorescent indicator. Influence of pH of acceptor solution on the analytical signal. Experimental conditions: λ_{exc}=536 nm, λ_{em}=552 nm, slit width: R_{exc}=5 nm, R_{em}=5 nm, [EB]= 5.0×10^{-7}M, [NH_4+]=0.35 mg l^{-1}

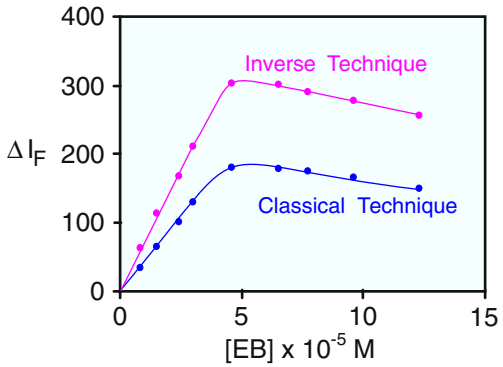

Fig. 5 Determination of ammonium by gas diffusion in continuous mode using Eosin Bluish as the fluorescent indicator. Influence of EB concentration in the acceptor solution on the analytical signal. Experimental conditions: λ_{exc}=536 nm, λ_{em}=552 nm, R_{exc}=5 nm, R_{em}=5 nm, [NH_4^+]=0.35 mg l^{-1} , pH=3.60

3.70. Less acid values than these led to a decrease in the analytical signal because the basic EB approached its zone of predominance and hence the acid, non-fluorescent, form of the dye decreased.

Logically, the basic form emits more fluorescence as the pH of the acceptor solution rises, but this does not affect study of the ΔI_F value, measured up to the maximum of the analytical signal.

A pH value of 3.6 was selected for the rest of the experiments carried out.

Influence of the EB concentration

To study how the concentration of EB affected the final analytical signal, ΔI_F, the pH of the acceptor solutions was fixed at 3.60 and the concentration of the indicator between 0.80×10^{-5} and 12.3×10^{-5}M. In all cases, a standard solution of NH_4^+ of 0.35 mg l^{-1} was used for injection (408 µl) or passage in continuous sampling mode (inverse technique). The other variables were kept at the values indicated in Fig. 1. The values of ΔI_F obtained after three experiments with each concentration of EB are shown in Fig. 5 *vs.* the reagent concentration.

It may be seen that the qualitative behavior observed with both flow techniques is similar, the analytical signal, ΔI_F, being higher in the case of the inverse technique for all the concentrations of EB. When the EB concentration increased, the value of the signal, ΔI_F, also increased owing to the increase in the concentration of the basic form of the dye. A maximum value for the concentration of the reagent used of between 4.5 and 5.0×10^{-5}M was obtained. Higher concentration values generated analytical signals that decreased with the concentration of reagent owing to the quenching phenomenon, previously observed in the studies carried out in discontinuous mode.

Influence of temperature

One of the most important variables to be studied was temperature, which affects the yield of the diffusion process through the membrane. From the theoretical point of view, the highest yield in the gas transfer process should be reached when the difference in temperature between the donor and acceptor solutions is highest. The higher the temperature of the donor solution, the lower the solubility of the gaseous NH_3 in it, and the lower the temperature of the acceptor solution, the greater the solubility of the gaseous species, thus favoring the diffusion process.

Influence of reactor length on fluorescence intensity at 60 ºC

Using the flow scheme shown in Fig. 1, the following experimental conditions were fixed: [EB]=5×10^{-5}M, F_A= 0.40 ml min^{-1}, [NaOH]: 1.0 mg l^{-1}, V_i=408 µl, F_D= 0.80 ml min^{-1} (C_2=C_3 =0.40 ml min^{-1}), and thermostatting temperature: 60 ºC, modifying the reactor length between 1 and 4 m.

The greater the reactor length, the greater the contact time between the solution of NH_4^+ injected or passed in continuous sampling mode and the thermostatted bath, such that the temperature of the donor solution will increase.

Table 1 shows the values of ΔI_F obtained for each reactor length together with the contact times (t_c), calculated theoretically, during which the *injected bolus* was heated on passing through the thermostatted reactor. The table also indicates the appearance times (t_a) of the fiagram for each experiment. In all the experiments, the time taken by the *heated NH_4^+ bolus* to pass through the 7.5 cm of membrane was approximately 70 s, although the greater the reactor length, the greater its dispersion, and the passage time may increase, although only slightly. The time of passage was calculated theoretically taking into account the volume injected, the value of F_D (0.80 ml min^{-1}), and the volume of the lower chamber of the model (10 µl).

It may be seen that as the length of the reactor was increased, so did the value of ΔI_F, because a greater amount

Table 1 Thermostatting of reactor R. Influence of reactor length on the value of ΔI_F, at 60ºC. Classic technique with injection of 408 µl of NH_4^+ (1.0 mg l^{-1}). F_D=0.80 ml min^{-1}, F_A=0.40 ml min^{-1}, t_c=time of residence of the ammonium bolus in the thermostatted bath; t_a=time of appearance of fiagram

R, cm	ΔI_F	t_c , sec	t_a , sec
100	75.2	15	98
200	130.7	29	112
300	175.8	44	128
400	182.7	59	143

of gaseous NH_3 had been transferred to the acceptor solution. The longer the length of the reactor, the greater the increase in temperature reached by the injected bolus, and hence the lower the solubility of the gaseous NH_3 generated, which was therefore transferred in greater amounts.

As from a reactor length of 300 cm, ΔI_F tended towards a constant value, because so did the amount of NH_3 transferred. This constant value would be that corresponding to the NH_3 transferred when the donor solution reached 60 ºC and when the acceptor solution was at 22 ºC. From the practical point of view, a length of 300 cm was taken as optimum, since higher lengths would have slightly increased the yield of the gas diffusion process, but would have increased the time of appearance of the fiagram to a considerable extent. In all experiments, the values of Δt -the width of the base of the fiagram- was constant at 250 s.

Influence of reactor thermostatting temperature

With a reactor length of 300 cm and the rest of the variables kept at the values indicated above, 408 µl of a standard solution of NH_4^+ was injected (in triplicate) and the thermostatting temperature was varied between 25 and 90 ºC.

The ΔI_F values obtained for each temperature are shown in Fig. 6. As can be seen, these values increased linearly with the thermostatting temperature up to 65 ºC: $\Delta I_F = (0.6 \pm 0.3) + (2.92 \pm 0.01)$ TºC; R=0.9998. The values obtained above 65 ºC departed from linearity and tended towards a constant fluorescence value.

A similar experiment carried out with F_D=1.572 ml min^{-1}, using a reactor of 650 cm afforded similar results as regards the effect of temperature on the yield of the process of gas diffusion through the membrane.

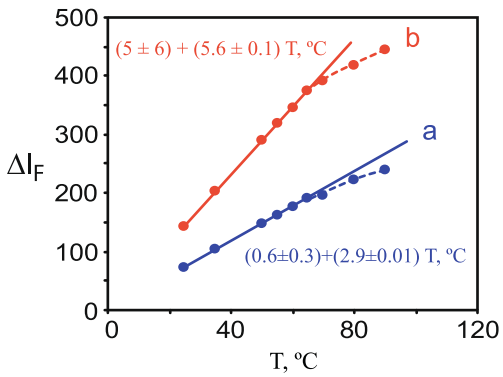

Fig. 6 Influence of thermostatting temperature reactor, R, on the value of ΔI_F. Classic technique with injection of 408 µl of NH_4^+ (1.0 mg l^{-1}). F_A=0.40 ml min^{-1}. **a** F_D=0.80 ml min^{-1}, R=300 cm, F_D/F_A=2.0. **b** F_D=1.57 ml min^{-1}, R=650 cm, F_D/F_A=3.9

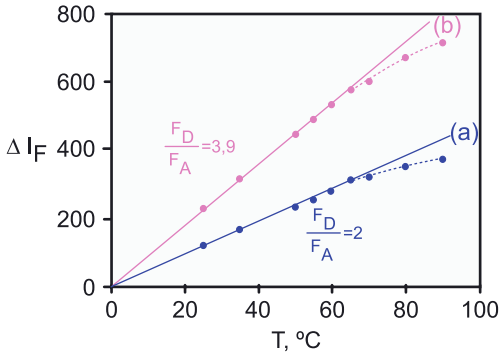

Fig. 7 Influence of thermostatting temperature reactor, R, on the value of ΔI_F. Continuous flow mode. $[NH_4^+]$=1.0 mg l^{-1}. **a)** Reactor of 300 cm; F_D/F_A=2.0; **b)** Reactor of 650 cm; F_D/F_A=3.9. **a** $\Delta I_F = (4 \pm 8) + (8.5 \pm 0.1)$ TºC ; R^2=1. **b** $\Delta I_F = (17 \pm 7) + (4.6 \pm 0.1)$ TºC ; R^2=0,9969

The ΔI_F values obtained for the different experiments are also shown in Fig. 6. To make this part of the study comparative with the previous one, because the value of F_D was higher, it was necessary to use a reactor of 650 cm so that the contact time between the bolus and the thermostatted bath would be similar (44 s). The qualitative result obtained was similar: as the thermostatting temperature increased, so did the difference between the temperatures of the donor and acceptor solutions, the yield of the diffusion process increasing.

The ΔI_F values obtained were higher in this second experiment. This can only be attributed to the fact that the F_D/F_A flow rate ratio was greater, which could have favored the gas diffusion process through the membrane, although without overlooking the fact that in this case, since the time of passage of the donor bolus through the lower part of the membrane was shorter (35 s), the NH_3 diffused was collected by an acceptor bolus of less than 233 µl, pointing to the existence of a preconcentration step.

Up to 65 ºC, in this experiment the values of ΔI_F plotted against temperature fitted a straight line with an equation of $\Delta I_F = (5 \pm 6) + (5.6 \pm 0.1)$ TºC, with a steeper slope than in the previous experiment, in which the F_D/F_A ratio was lower. The linear regression coefficient was 0.9983 for a confidence level of 95%.

In this second experiment the time of appearance, t_a, was shorter, and the width of the base of the fiagram was also smaller: Δt=160 s.

On comparing the equations of both experiments, it may be deduced that although the ratio between the diffusion yield and temperature (up to 65 ºC) is linear the slope increases with the increase in the F_D/F_A ratio. The fact that the ordinate at the origin is independent of the F_D/F_S value suggests that when the temperature of the acceptor and donor solutions is the same this yield does not depend on that ratio.

The departure from linearity is favored by the fact that since the vapor pressure rises at high temperatures the

Table 2 Reactor length values for the different values of F_D. Time of residence of NH_3 bolus in thermostatted bath, 50 s. [EB]=4.5×10^{-5}M, pH=3.6; [NaOH]=0.012 M; Vi=408 μL; T=50 ºC

F_D, mlmin^{-1}	R, cm.
0.10	42
0.20	84
0.30	126
0.40	168
0.50	210
0.60	252
0.70	294
0.80	336
0.90	378
1.00	420
1.20	504
1.40	588
1.50	630
1.70	714
2.00	840

pressure exerted on the membrane on the side of the donor solution is much greater than on the other side (acceptor solution). This would alter the dialysis process and would modify the fluorescence value of the baseline because the dye would be very slightly diluted owing to the passage of droplets of donor solution around the sides of the membrane. This process also alters the analytical signal, which is why the maximum working temperature should not surpass 70 ºC in order to minimize this phenomenon as much as possible.

The above two experiments, with two flow-rate ratio conditions of F_D/F_A=2.0 and F_D/F_A=3.9, were repeated for the same experimental conditions, but in continuous sampling mode, varying the corresponding reactor-thermostatting temperature between 25 and 90 ºC.

The heating time of the NH_4^+ solution under a continuous flow regime was identical for each length of thermostatted reactor; the difference lay in that when working with this mode the dispersion of the acceptor bolus was eliminated, which is why the analytical signal, once it had reach the maximum value, thereafter remained constant. The fact of eliminating the process of dispersion of the acceptor bolus means that the analytical signal, ΔI_F, will be higher than when the same solution is injected.

The values of ΔI_F obtained at 522 nm are plotted for both experiments in Fig. 7.

The qualitative result obtained was similar to that observed in the experiments in which 408 μl of a standard solution of NH_4^+ (1.0 mg l^{-1} of NH_4^+) was injected; the higher the thermostatting temperature of the reactors, the higher the analytical signal. This indicates that when the difference in temperature between the acceptor and donor solutions increased, the process of NH_3 diffusion through the membrane also increased.

From the qualitative point of view, the values of ΔI_F obtained on passing the solution of NH_4^+ in continuous sampling mode are higher than those obtained upon injecting the same solution. Additionally, the ΔI_F values, which indicate the yield of the diffusion of the NH_3 generated, were higher in the experiment in which the flow rate ratio was greatest: F_D/F_A=3.9 as compared with F_D/F_A=2.0.

In both experiments against temperature, the values ΔI_F up to 65 ºC fitted a linear relationship, the equations of the straight lines being:

$$F_D/F_A = 2.0: \quad \Delta I_F = (4 \pm 8) + (4.6 \pm 0.1)T\,^{\circ}C \quad R^2 = 1.0$$

$$F_D/F_A = 3.9: \quad \Delta I_F = (17 \pm 7) + (8.5 \pm 0.1)T\,^{\circ}C \; R^2 = 0.9969$$

Influence of the flow rates of the acceptor and donor solutions

The flow rates of the donor and acceptor solutions, F_D and F_A, are two variables to be taken into account when studying yield in the diffusion of a gaseous species through a Teflon membrane. From scrutiny of the pertinent literature, it may be seen that in order to achieve the best results most authors propose that work should be carried out under conditions in which F_D=F_A.

With a view to checking the effect of both flow rates, in the scheme shown in Fig. 1 the following experimental

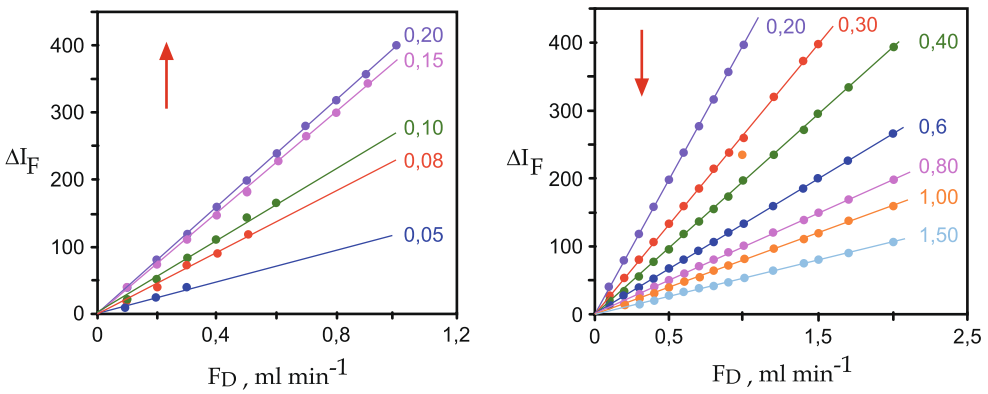

Fig. 8 Influence of flow rates of the donor (F_D) and acceptor(F_A) solutions. [EB]=4.5×10^{-5}M, [NaOH]=0.012 M; V_i=408 μl; [NH_4^+]=1.0 mg l^{-1}; T=50 ºC: The values of F_A (ml min^{-1}) are indicated in the corresponding lines

Table 3 Equations of the straight lines obtained on plotting the values of ΔI_F against the flow rate of the donor solution (F_D) for all the values of F_A assayed for both techniques

F_A mlmin^{-1}	Classic technique	Continuous technique
0.05	ΔI_F=(-1±3) + (126±13) F_D; R^2=0.9890	ΔI_F=(-2±3) + (195±15) F_D; R^2=0.9873
0.08	ΔI_F=(-6±5) + (245±15) F_D; R^2=0.9894	ΔI_F=(-4±5) + (365±20) F_D; R^2=0.9882
0.10	ΔI_F=(-2±3) + (291±8) F_D; R^2=0.9971	ΔI_F=(-1±4) + (468±19) F_D; R^2=0.9912
0.15	ΔI_F=(-4±6) + (359±11) F_D; R^2=0.9934	ΔI_F=(2±6) + (623±8) F_D; R^2=0.9994
0.20	ΔI_F=(-1±4) + (400±8) F_D; R^2=0.9994	ΔI_F=(-1±1) + (663±2) F_D; R^2=0.9999
0.30	ΔI_F=(-2±5) + (271±9) F_D; R^2=0.9890	ΔI_F=(0±1) + (442±1) F_D; R^2=0.9992
0.40	ΔI_F=(-1±3) + (199±7) F_D; R^2=1	ΔI_F=(0.3±0.4) + (330.7±0.4) F_D; R^2=1
0.60	ΔI_F=(-1±4) + (139±4) F_D; R^2=1	ΔI_F=(0.2±0.5) + (220.8±0.5) F_D; R^2=0.9998
0.80	ΔI_F=(-1±5) + (99±3) F_D; R^2=0.9999	ΔI_F=(0.5±0.8) + (166.5±0.6) F_D; R^2=0.9999
1.00	ΔI_F=(-0.3±0.2) + (79.8±0.2) F_D; R^2=0.9999	ΔI_F=(0.8±0.6) + (132.6±0.9) F_D; R^2=0.9998
1.50	ΔI_F=(-0.6±0.4) + (52.8±0.3) F_D; R^2=1	ΔI_F=(0.9±0.7) + (88.5±0.6) F_D; R^2=0.9999

conditions were fixed: [EB]=4.5×10^{-5}M, pH=3.6; [NaOH]= 0.012 M; [NH_4^+]=1.0 mg l^{-1}, V_i=408 µl, thermostatting temperature=50ºC, and the values of F_D were modified from 0.1 mL.min^{-1} to 2.0 ml min^{-1}, while F_A was varied from 0.05 mL.min^{-1} to 1.50 ml min^{-1}.

In order to ensure that the time taken by the NH_3 bolus to cross the thermostatted reactor would be constant -50 s- so that the bolus temperature could be maintained constant and independent of the value of F_D, the reactor length was varied for each flow rate of the donor solution (Table 2).

ΔI_F values were obtained by injecting 408 µl of a standard solution of NH_4^+ (1.0 mg l^{-1}) in the cases in which the F_D/F_A ratio was ≤ 6.0, since in these cases the baseline remained constant and without alterations, while in the cases in which $F_D/F_A \geq 6$ the baseline was significantly altered because the pressure exerted on the lower part of the membrane (when $F_D >> F_A$) was so high that it permitted the passage of droplets of the donor solution to the acceptor solution, thus modifying the pH of the latter and eliciting a modification of the signal due to a process other than the gaseous diffusion of NH_3. The same but inverse phenomenon was observed when $F_A >> F_D$.

In the experiments in which $F_D \neq F_A$, it was clearly possible to observe, even better so when the difference was greater, that the Teflon membrane is subject to pressure from the side with the greatest flow rate, constantly moving up or down or vice-versa, but without there being contact between the donor and acceptor solutions. When $F_D \approx F_A$, this process was not observed.

The same phenomena occurred when the standard solution of NH_4^+ was passed in continuous sampling mode through channel C_3. Plotted against the flow rates of the donor solution, F_D, the ΔI_F obtained with this flow mode fitted straight lines for all the F_A values assayed. As an example, Fig. 8 shows the experiments when 408 µl of the standard solution of NH_4^+ was injected. The trend of the results in the mode in which the standard solution of NH_4^+ was passed in continuous sampling mode was similar, but with lower ΔI_F values in all cases (Table 3).

It may be seen that the slope of the straight lines initially increased when the value of F_A was kept between 0.05 ml min^{-1} and 0.20 ml min^{-1}, thereafter decreasing as the values of F_A were varied between 0.20 ml min^{-1} and 1.50 ml min^{-1}. This value splits the results into two different blocks: for values of F_A between 0.05 ml min^{-1} and

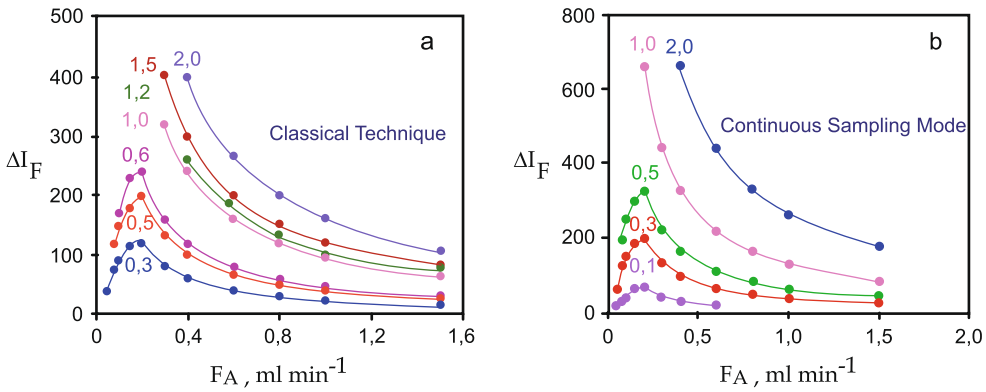

Fig. 9 Influence of flow rates of donor and acceptor solutions on the analytical signal. Variation of ΔI_F with F_A for different values of F_D. a) Classic technique; b) Continuous technique. [EB]=4.5×10^{-5}M, pH=3.6, [NaOH]=0.012 M; V_i=408 µl; [NH_4^+]=1.0 mg l^{-1}

0.2 ml min^{-1}, the proportionality constant increased with the F_A value, while for F_A values higher than 0.20 ml min^{-1}, the proportionality constant (slope) decreased with the increase in F_A.

It thus appears that the final NH_3 concentration in the acceptor bolus, as a function of the value of F_D, is differentially dependent on the value of F_A if this latter value is greater or lesser than 0.20 ml min^{-1}. The physical processes accounting for this kind of behavior are different in these two zones of F_A.

Plotting of the values of ΔI_F against those of F_A, Fig. 9, confirms the above results. It may be seen that for all F_D values the dependence of ΔI_F on F_A is different when the value of F_A is smaller or larger than 0.20 ml min^{-1}. Plotting the results of the experiments in which F_D is greater than 1.0 ml min^{-1} is misleading, since maximum fluorescence values are not obtained for F_A=0.20 ml min^{-1} because the values at which $F_D/F_A \geq 6$ are affected by the passing of solution droplets around the membrane.

From a practical point of view, it may be deduced that the maximum value of ΔI_F is obtained by neglecting the anomalous values for F_A=0.20 ml min^{-1} and F_D= 1.00 ml min^{-1} (or F_A=0.30 ml min^{-1} and F_D=1.5 ml min^{-1}; F_A=0.40 ml min^{-1} and F_D=2.0 ml min^{-1}).

From scrutiny of the dependence of the value of ΔI_F on the flow rates of the donor and acceptor solutions it may be inferred that a dual relationship exists, not only a dependence on one of the variables, and that this relationship also seems to be different, whatever the values of F_D, for two zones of F_A values separated by the value of 0.20 ml min^{-1}.

To attempt to elucidate this dual dependence, the values of ΔI_F were plotted against the F_D/F_A ratio (Fig. 10).

It is again seen that the F_A value of 0.20 ml min^{-1} splits two different zones of behavior of the value of ΔI_F *versus* the values of F_D/F_A.

For F_A values $\leq$ 0.20 ml min^{-1}, Fig. 10 a, the higher the value of F_D/F_A, the higher the analytical signal, although in turn depending on the value of F_A. The relationship between ΔI_F and the value of F_D/F_A in all cases fitted equations with straight lines, whose slope increases with the increase in the value of F_A:

$$F_A = 0.05: \quad \Delta I_F = (6 \pm 8) + (6 \pm 2)F_D/F_A \qquad R^2 = 0.9889$$

$$F_A = 0.08: \quad \Delta I_F = (3 \pm 3) + (17 \pm 3)F_D/F_A \qquad R^2 = 0.9934$$

$$F_A = 0.10: \quad \Delta I_F = (2 \pm 2) + (29 \pm 2)F_D/F_A \qquad R^2 = 0.9979$$

$$F_A = 0.15: \quad \Delta I_F = (0.8 \pm 0.2) + (57 \pm 2)F_D/F_A \qquad R^2 = 09994$$

The values of ΔI_F *vs.* the F_D/F_A ratio for values of $F_A \geq$ 0.20 ml min^{-1} (Fig. 10 b) fitted equations with straight lines that did not differ significantly for a 95% confidence level:

$$\Delta I_F = (0.6 \pm 0.1) + (82 \pm 3)F_D/F_A; \quad R^2 = 0.9998$$

For F_A values $\geq$ 0.20 ml min^{-1}, the analytical signal increased with the increase in the F_D/F_A ratio, a practical limit being $F_D/F_A \approx 5$, after which the pressure that one of the sides of the membrane was subjected to became so high that a transfer of fluid occurred from one side to the other, altering the baseline and hence the analytical signal.

A similar study performed in continuous sampling mode and under the same experimental conditions afforded similar results in qualitative terms, although the value of F_A after which ΔI_F was dependent upon the F_D/F_A ratio was in this case 0.15 ml min^{-1}, a linear relationship with an equation of

$$\Delta I_F = (0.8 \pm 0.2) + (138 \pm 4)F_D/F_A; \quad R^2 = 0.9996$$

being observed.

From the practical point of view, the highest ΔI_F values were obtained for F_A=0.15 ml min^{-1} and F_D=0.90 ml min^{-1}.

Influence of the NH_4^+ concentration. Analytical calibrations

In order to determine the relationship between the concentration of NH_4^+ (in mg l^{-1}) and the analytical signal, and to check the range of applicability of the proposed method, solutions of NH_4^+ were prepared at concentrations ranging between 0.02 and 1.90 mg l^{-1}. These were injected into the flow system or passed in continuous sampling mode, setting the variables at the values indicated in Fig. 1, and

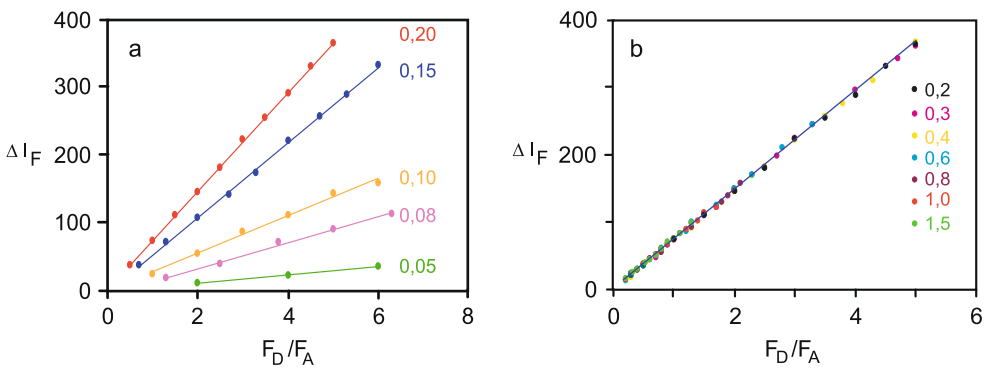

Fig. 10 Influence of flow rates of donor and acceptor solutions on the analytical signal using the classic technique. Values of ΔI_F versus the F_D/F_A ratio for different values of F_A, indicated in the corresponding lines. A) $F_A \leq$ 0.20 ml min^{-1}; B) $F_A \geq$ 0.20 ml min^{-1}. [EB]=4.5×10^{-5} M (pH=3.6), [NaOH]= 0.012 M; V_i=408 μl; [NH_4^+]= 1.0 mg l^{-1}

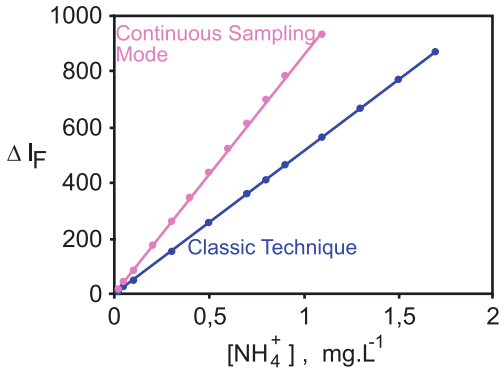

Fig. 11 Determination of ammonium by gas diffusion in continuous mode using Eosin Bluish as fluorescent indicator. Analytical calibrations. Experimental conditions. λ_{exc}=536 nm, λ_{em}=552 nm, R_{exc}= 5 nm, R_{em}=5 nm, pH=3.60, [EA]=4,5×10^{-5}M

setting the pH of the acceptor solution at 3.60 and the concentration of [EB] at 4.5×10^{-5}M. The values of ΔI_F, which were the means of three experiments, are shown in Fig. 11.

It may be seen that, in continuous sampling mode, for the same concentration of NH_4^+ higher signals are obtained, such that the calibration obtained with this technique is more sensitive than that provided by the classic technique.

For the classic technique, a linear relationship was observed between the concentration of NH_4^+ and the analytical signal up to [NH_4^+]=1.5 mg l^{-1}. The straight line fits the equation:

$$\Delta I_F = (0.3 \pm 1.0) + (514 \pm 1)[NH_4^+], \text{mg l}^{-1} \quad R^2 = 1.00,$$

while for the inverse technique, the linearity range reached 1.1 mg l^{-1}, the straight line fitting an equation of:

$$\Delta I_F = (4 \pm 10) + (859 \pm 17)[NH_4^+], \text{mg l}^{-1} \; R^2 = 0.9994,$$

both equations having a 95% confidence interval.

The departure from linearity is due to the quenching effect, mentioned previously, observed for high concentrations of the basic form of EB.

Under these conditions, the concentrations of the limits of detection calculated from the IUPAC expression, bearing in mind that X_B^{max}=0.8 for both techniques, were: C_L= 10 μg l^{-1} (classic technique) and C_L=5 μg l^{-1} (continuous sampling mode).

Precision of the methods

The precision of the methods was studied by preparing standard solutions of NH_4^+ with contents of 50 and 0.50 μg l^{-1}; n=12 for each concentration.

After the samples (408 μL) had been injected or passed in continuous sampling mode, the ΔI_F values were obtained at λ_{em}=552 nm, and after the corresponding statistical treatments, values of the relative standard deviation: S_R=3.4% ([NH_4^+]=50 μg l^{-1}) and S_R=3.0% ([NH_4^+]=0.05 μg l^{-1}) for the classic technique and S_R= 3.6% ([NH_4^+]=50 μg l^{-1}) and S_R=3.2% ([NH_4^+]= 0.05 μg l^{-1}) for the continuous sampling mode were obtained. The determination rate was 15 samples per hour for the classic technique, while this ratio was considerably reduced in the case of the continuous sampling technique.

Validation of the methods

The two methods of determination were validated, starting out from a certified standard of NH_4^+ (CRM 409), from which a solution with a known concentration of the analyte was obtained. The standard solution was prepared by weighing 21.0000 g of liquid from the vial, bringing the final volume up to 25.0 mL. This solution proved to have a concentration of 1.60 mg l^{-1} (with a reliability of 0.04 mg l^{-1} for 95% probability). This solution was used to prepare other more dilute ones, taking aliquots and bringing volume up to the desired level with bidistilled water.

To check the validity of the method, solutions with a concentration of NH_4^+ between 0.64 and 0.064 mg l^{-1} were injected (408 μl) or passed in continuous sampling mode, depending on the technique employed, and the mean value of ΔI_F was measured, performing three determinations for each concentration.

The concentration values, in mg l^{-1}, found (Tables 3 and 4) for a 95% confidence level did not differ significantly.

Conclusions

In the processes of gas diffusion through solid membranes inserted into flow systems, the final yield in the process of collecting the volatile species in an acceptor solution

Table 4 Determination of ammonium by gas diffusion in continuous mode employing Eosin Bluish as fluorescent dye. Validation of the method. λ_{exc}=536 nm, λ_{em}=552 nm, R_{exc}=5 nm, R_{em}=5 nm, [EB]= 4.5×10^{-5}M, pH=3.60

	[NH_4^+], mgl^{-1} *	
Added	Found	
	Classic T.	Continuous T.
0.64±0.02	0.62±0.04	0.66±0.04
0.032±0.01	0.33±0.05	0.30±0.05
0.19±0.01	0.21±0.05	0.18±0.05
0.13±0.01	0.10±0.06	0.14 0.06

*Mean values of three determinations. 95% confidence

depends on the variables affecting the diffusion equilibrium, such as differences in temperature between the acceptor and donor phases, and the difference between the flow rates of both phases; the best results are obtained when $F_D > F_A$ (with the limitation of $F_D/F_A \leq 6$) because the pressure difference on both sides of the membrane favors analyte transfer, and because a "preconcentration" process occurs from the donor to the acceptor phase. The pressure increases with the increase in the F_D/F_A ratio. The conjunction of the variables affecting the final yield of analyte transfer is such that for $F_A \geq 0.20$ ml min^{-1} the relationship between the final analytical signal and the volume injected, the temperature of the donor solution and the F_D/F_A flow rate ratio, is linear (with the limitations explained above) over a broad range of V_i values. The same linear relationship is seen when the analyte solution is made to flow continuously, although with a higher proportionality constant owing to the disappearance of the dilution processes due to dispersion of the donor and acceptor boluses. It may be inferred that the theory proposed in the literature attributing the best yields when $F_D = F_A$ does not hold.

The use of a fluorescent indicator for monitoring the amount of NH_3 collected in the acceptor solution improves the sensitivity of the procedure. NH_4^+ can be determined at values clearly below the parametric values set by current legislation for drinking water.

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