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Highly sensitive gas-diffusion sequential injection analysis based on flow manipulation

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ABSTRACT

The present paper describes approaches utilizing the powerful flow manipulation capabilities of sequential injection analysis (SIA) to substantially improve the efficiency of gas-diffusion separation compared to its traditional implementation in flow injection analysis (FIA). Ammonia, ethylamine, diethylamine and triethylamine were used as model analytes in this study. Eleven flow manipulation approaches involving continuous flow, stop-flow, oscillating flow, and the introduction of air bubbles to separate the sample zone from the donor solution were tested. Improvement in sensitivity compared to traditional gas-diffusion FIA exceeding one order of magnitude was achieved. It was observed that this improvement increased with the molecular size of the analyte.

1. Introduction

Samples with complex matrices often experience serious interference problems when measured by analytical flow techniques such as flow injection analysis (FIA) and sequential injection analysis (SIA). Online membrane-based separation (e.g., gas-diffusion (GD), dialysis and pervaporation) has shown considerable promise in minimizing such interferences [1].

GD FIA has been frequently used for the online determination of volatile (e.g., NH_3 [2]) or semi-volatile analytes (e.g., amines [3]), or non-volatile analytes (e.g., arsenite [4], Hg^{2+} [5], CN^- [6]) that can be converted online into volatile chemical species.

However, only a few GD SIA applications have been reported in the literature so far [7–13]. The majority of these applications involved the detection of ammonia [7–9]. Lukkari et al. [7] proposed a GD SIA system where detection was conducted in the acceptor channel of the GD cell using a bifurcated fibre optic cable. The analytical measurements were conducted under stop-flow conditions for both the donor and acceptor streams. Detection approaches based on Berthelot method and the use of acid–base indicators were compared and it was concluded that the latter approach offered higher reproducibility and wider calibration range. The same detection approach was utilized by Oms et al. [8] in a spectrophotometric GD SIA system where, prior to the analytical measurement, ammonia was collected in a static acceptor solution located in the sample loop of a six-port rotary injection valve. A similar GD SIA procedure with either spectrophotometric or conductometric detection was successfully applied by Rangel and co-workers [9] to the enzymatic determination of urea in milk. The same research group proposed GD SIA methods for the determination of sulphur dioxide in wine [10] and free chlorine [11]. Echols et al. [12] developed an amperometric GD SIA system for the determination of azides in environmental samples. Silva and Masini [13] applied the GD SIA approach to sulfide measurement in liquid samples.

It has been demonstrated that sensitivity in FIA systems employing electroanalytical stripping techniques can be improved substantially by oscillating the sample zone in the measuring cell. This has led to prolonged electrodeposition times and enhanced mass transport of the analyte towards the working electrode as a result of better mixing within the sample zone [14–16]. A similar approach for improving the sensitivity in GD SIA has been attempted in two of the studies outlined above [8,12]. In both of them the mass transfer of the analyte (i.e., ammonia [8] and azide [12]) was enhanced by stopping the acceptor stream while the sample zone was oscillated in the donor channel of the GD cell. However, due to the increased dispersion of the sample zone as a result of the oscillations the maximum improvement did not exceed 300%.

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In this paper, we report on several approaches, utilizing the powerful flow manipulation capabilities of SIA, for improving the efficiency of GD separation compared to its traditional implementation in FIA. Some of these approaches involved the introduction of air bubbles to minimize the dispersion problems associated with flow oscillations [8,12]. The effect of the analyte molecular mass on sensitivity was studied by using ammonia, ethylamine, diethylamine and triethylamine as model analytes.

2. Experimental

2.1. Reagents

The following reagents of analytical grade were used as received: sodium hydroxide (Lachema, Czech Republic), ammonium chloride (Balex, Czech Republic), ethylamine (EA) hydrochloride (Aldrich), diethylamine (DEA) hydrochloride (Aldrich), triethylamine (TEA) hydrochloride (Fluka), hydrochloric acid (Lachema, Czech Republic), and Bromocresol Green (Fluka).

Deionized water (18 MΩ cm, Milli-Q RG, Millipore) was used for the preparation of all solutions.

Stock solutions of 2.0 M NaOH and 20 mM of ammonium chloride and the hydrochlorides of EA, DEA and TEA were used to prepare the corresponding standard solutions daily. The acceptor solution was prepared by dissolving 50 mg of Bromcresol Green in 0.72 mL 0.1 M NaOH and 20 mL 96% ethanol and the resultant mixture was diluted to 500 mL and acidified to pH 4 by drop-wise addition of 0.1 M HCl solution.

2.2. GD SIA system

A FIAlab-3500 sequential injection analyzer (FIAlab Instruments, USA) equipped with an optical fibre spectrophotometric detector (USB 2000, Ocean Optics, USA) set at 616.5 nm and a home-made GD cell was used in this study (Fig. 1).

The GD cell consisted of two rectangular Perspex blocks (9.5 cm length, 2.3 cm width, and 1.5 cm height) held together by stainless steel screws. The depth, width and length of the two serpentine channels of the GD cell were 0.5, 2.0 and 100 mm, respectively. A semi-permeable Teflon membrane (Pro-Tech, Australia, 0.076 mm thickness) was used to separate these two channels. The volume of each channel after assembling the GD cell with a membrane was 75 μL.

The volume of the mixing coil (Fig. 1) was 220 μL and the volume of the tubing connecting the mixing coil with the multiport selection valve and the GD cell was 50 μL. The flow rates of the donor and acceptor streams were 1.50 and 0.53 mL min^{-1}, respectively. The sample volume of 75 μL matched the volume of the donor channel of the GD cell.

2.3. Flow manipulation

2.3.1. Fluid structure of the donor stream

Two different fluid structures of the donor stream were used, i.e., unsegmented and segmented structures. In the former, the sample zone was directly enclosed by the donor solution. This structure corresponds to traditional unsegmented flow. In the segmented structure, the sample zone was separated from the donor solution by two air bubbles identical in size and aspirated before and after the aspiration of the sample.

2.3.2. Flow pattern of the donor and acceptor streams

The three flow patterns of the donor and acceptor streams utilized in this study were: traditional continuous flow, stop-flow and oscillating flow. The stop-flow pattern involved stopping the donor or acceptor stream for a predetermined period of time. The acceptor stream was stopped during the passage of the sample zone through the donor channel of the GD cell after which it was re-started. The donor stream was stopped when the sample zone had reached the GD cell to prolong its contact time with the membrane. In the case of air segmentation the original sample slug did not disperse and could be accommodated in the donor channel of the GD cell which had the same volume as that of the original sample (i.e., 75 μL). The oscillating flow pattern involved repetitive flow reversal of the direction of the donor or acceptor stream while the other stream was stopped. Another oscillating flow pattern was based on the simultaneous flow reversals of both streams. These flow reversals were in opposite directions. The donor stream was oscillated by the syringe pump by 40 μL in each direction while the acceptor stream was oscillated by the peristaltic pump by 10 μL. The donor stream oscillations started after the front of the sample slug was 20 μL downstream of the GD cell.

2.3.3. Flow configurations of the GD SIA manifold

The following flow configurations were compared in terms of sensitivity and sampling rate:

(a) *Continuous flow*. This traditional GD FIA/SIA configuration involved continuous flow of both the donor and acceptor streams without air segmentation.

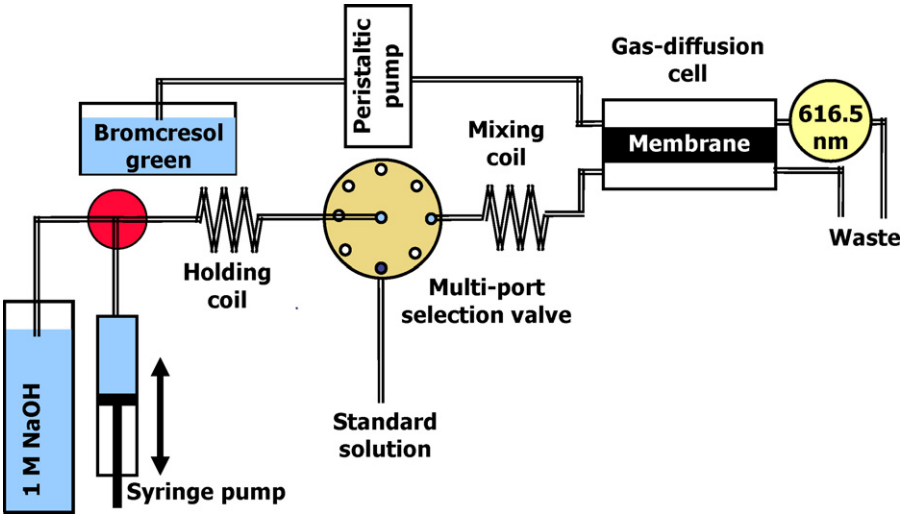

Fig. 1. Schematic of the GD SIA system.

(b) *Continuous flow with sample segmentation*. The sample zone was separated from the donor solution by air bubbles.
(c) *Stop-flow*. This traditional GD FIA stop-flow configuration involved stopping the acceptor stream until the unsegmented sample zone had passed through the GD cell.
(d) *Stop-flow with sample segmentation*. This flow configuration was similar to the previous one. However, in this case the sample zone was separated from the carrier solution by air bubbles.
(e) *Double stop-flow with sample segmentation*. Both the acceptor and donor streams were stopped for a predetermined period of time after the air-segmented sample zone had reached the GD cell.
(f) *Oscillating donor stream*. The acceptor stream was static during the oscillations of the unsegmented donor stream.
(g) *Oscillating acceptor stream*. The unsegmented donor stream with the central section of the sample zone located in the donor channel of the GD cell was static during the oscillations of the acceptor stream.
(h) *Oscillating donor stream with sample segmentation*. The acceptor stream was static during the oscillations of the donor stream with an air-segmented sample zone.
(i) *Oscillating acceptor stream with sample segmentation*. The segmented donor stream with the sample zone located in the donor channel of the GD cell was static during the oscillations of the acceptor stream.
(j) *Oscillating donor and acceptor streams*. Both unsegmented streams were oscillated simultaneously after the sample zone had reached the donor channel of the GD cell.
(k) *Oscillating donor and acceptor streams with sample segmentation*. Both streams were oscillated simultaneously after the air-segmented sample zone had reached the donor channel of the GD cell.

The remaining possible flow configurations which involved stopping or oscillating the donor stream while flowing continuously the acceptor stream were expected to produce lower sensitivity due to the extensive spreading of the protonated analyte in the acceptor stream.

2.4. Influence of the system parameters

Since the aim of this study was to investigate the effect of different flow manipulation techniques on sensitivity in GD SIA, full optimization of the flow system was not conducted. Only parameters which were relevant to the complete conversion of the ammonium and amine cations into the corresponding volatile molecular species in the acceptor stream were studied. These were the concentration of NaOH in the carrier stream and the volume of the air bubbles used to separate the sample zone from the donor solution.

The concentration range of NaOH in the donor solution studied was between 0 and 2.0 mol L^{-1}. The sensitivity was compared by measuring the maximum absorbance for 0.6 mM NH_4Cl standards. The flow configuration was *stop-flow with sample segmentation* employing 5 μL air bubbles.

The volume of the air bubbles was varied from 0, corresponding to unsegmented flow, to 20 μL. The concentration of NaOH in the carrier was 1.0 mol L^{-1}. The remaining GD SIA parameters were identical to those in the NaOH experiments outlined above.

2.5. Calibration

Four different calibration procedures were tested with standard solutions of NH_4Cl in the concentration range from 0.1 to 1.2 mmol L^{-1}. These were: (a) *continuous flow*; (b) *continuous flow with sample segmentation*; (c) *stop-flow*; and (d) *stop-flow with sample segmentation*. The remaining SIA parameters were identical to those in the optimization experiments outlined above.

EA, DEA and TEA hydrochlorides were calibrated in the same concentration range but under flow configurations (c) and (d) only.

2.6. Influence of the stop-flow time in the absence of flow oscillations

The maximum absorbance for 0.1 mM of each one of the four analytes studied (NH_4Cl and the hydrochlorides of EA, DEA and TEA) was measured in the case of stop-flow times varying between 0 (i.e., *continuous flow with sample segmentation*) and 120 s. For non-zero stop-flow times the flow configuration was *double stop-flow with sample segmentation*. The remaining experimental conditions were identical to those in the calibration experiments.

2.7. Influence of the flow oscillations

The influence of the flow oscillations on the sensitivity of determination of NH_4Cl was studied for the following six flow configurations: *oscillating donor stream*, *oscillating acceptor stream*, *oscillating donor stream with sample segmentation*, *oscillating acceptor stream with sample segmentation*, *oscillating donor and acceptor streams*, and *oscillating donor and acceptor streams with sample segmentation*. Similar experiments were conducted with the hydrochlorides of the three amines studied. However, in these experiments the two configurations which produced the highest sensitivity in the determination of NH_4Cl were only employed, i.e., *oscillating donor stream with sample segmentation* and *oscillating donor and acceptor streams with sample segmentation*. The remaining experimental conditions were identical to those in the calibration experiments.

3. Results and discussion

3.1. Influence of the NaOH concentration and the size of the air bubbles

Varying the concentration of NaOH in the donor stream between 0.1 and 2.0 mol L^{-1} had negligible effect on the maximum absorbance (0.63 ± 0.02). A significantly lower maximum absorbance value (i.e., 0.07) was obtained when the donor stream did not contain any NaOH. The non-zero signal in this case was due to the partial hydrolysis of NH_4Cl.

It was found that varying the volume of the air bubbles separating the sample zone from the carrier stream (0–20 μL) did not influence the maximum absorbance (0.62 ± 0.01). This result suggested that the air bubbles only restricted the spreading of the sample zone thus minimizing sample dispersion without preventing mixing between the reagent (NaOH) and the analyte (NH_4Cl). This effect, which is responsible for sample carry-over in air-segmented continuous flow analyzers [17], can be explained with the formation of a thin film of the donor solution between the leading air bubble and the tube walls, which mixes with the sample zone. This continuous mass exchange process introduced NaOH into the sample zone in quantities sufficient for the complete conversion of the ammonium cation into ammonia.

3.2. Calibration

The NH_4Cl calibration results for stop- and continuous flow with or without sample segmentation, presented in Fig. 2, indicated that while air segmentation of the sample did not affect the system performance, stopping the acceptor stream reduced substantially its sensitivity. While the former finding was in line with earlier observations regarding the lack of influence of the air bubble size on

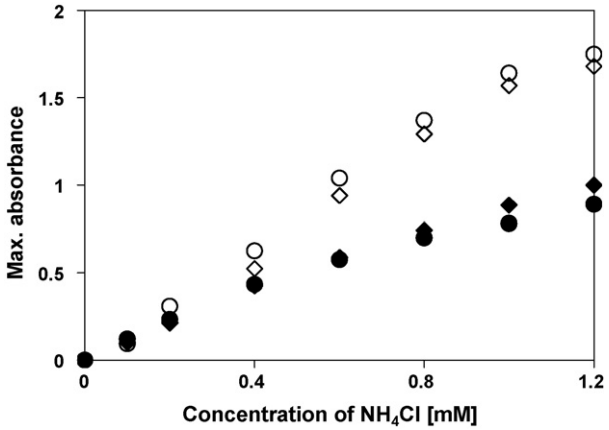

Fig. 2. Calibration data for NH_4Cl in the cases of: *continuous flow* (◊), *continuous flow with sample segmentation* (○), *stop-flow* (♦), and *stop-flow with sample segmentation* (●) (experimental conditions: sample: 75 μL of 0.6 mM NH_4Cl; donor stream: 1.0 M NaOH).

sensitivity, the latter one was unexpected on first glance. It suggested that at higher ammonium concentrations in the standards, the boundary layer of the static acceptor solution at the membrane/solution interface became saturated with the volatile species. As a result of this effect, the ammonia concentration gradient across the membrane decreased thus lowering the ammonia mass transfer rate across the membrane. Flowing the acceptor solution in the continuous configuration prevented this effect from taking place and consequently this resulted in higher sensitivity. This observation suggested that flow oscillations should be expected to increase sensitivity. Similar results were obtained for the calibration of EA, DEA and TEA hydrochlorides in the cases of stop- and continuous flow with sample segmentation.

3.3. Influence of the stop-flow time in the absence of flow oscillations

The maximum absorbance for all four model analytes increased initially with the stop-flow time and reached a limiting value at around 60 s (Fig. 3).

3.4. Influence of the flow oscillations

The results for oscillating donor stream, oscillating acceptor stream, oscillating donor stream with sample segmentation, oscillating acceptor stream with sample segmentation, oscillating donor and acceptor streams, and oscillating donor and acceptor streams with sample segmentation for 0.1 M NH_4Cl are shown in Fig. 4a. These results indicated substantially higher sensitivity in the case of sample segmentation which, as mentioned earlier, prevented sample dispersion. Flow oscillations enhanced mixing and decreased the thickness of the stagnant diffusion layer at the membrane/solution interface. This resulted in faster mass transfer of the analyte in the donor stream towards the membrane and in the acceptor stream away from the membrane. Both effects contributed to a more efficient overall mass transfer of the analyte across the membrane. This also explained the further improvement in sensitivity when both the donor and acceptor streams were oscillated in comparison to the situation when only one of them was oscillated. This improvement was very pronounced at low number of oscillations, in particular at one oscillation, when only a fraction of the analyte was transported across the membrane. At higher number of oscillations almost complete transfer of the analyte into the acceptor stream took place and oscillating the acceptor stream in addition to the donor stream became less important for improving sensitivity.

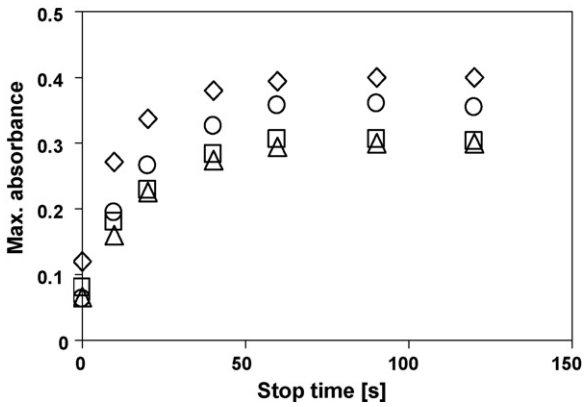

Fig. 3. Influence of the stop time on the maximum absorbance for ammonia (◊), EA (○), DEA (△) and TEA (□) in the case of *double stop-flow with sample segmentation* (experimental conditions as in Fig. 1).

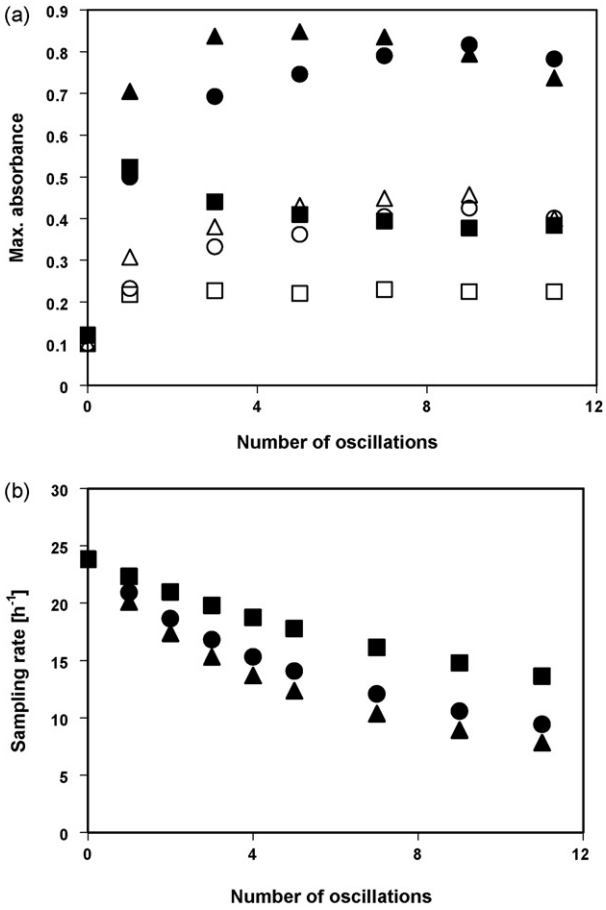

Fig. 4. Influence of the number of oscillations on the maximum absorbance (a) and sampling rate (b) in the case of: *oscillating donor stream* (○), *oscillating acceptor stream* (□), *oscillating donor stream with sample segmentation* (●), *oscillating acceptor stream with sample segmentation* (■), *oscillating donor and acceptor streams* (△), and *oscillating donor and acceptor streams with sample segmentation* (▲).

It was also observed that at higher number of oscillations of the acceptor stream sensitivity started to decrease. This effect was clearly visible when only the acceptor stream was oscillated and could be explained by the lack of air segmentation in this stream which resulted in higher dispersion.

In the case when both streams were oscillated, the enhanced analyte mass transfer towards the membrane in the donor stream

compensated for the dispersion effects in the acceptor stream. As a result, the decrease in sensitivity became only visible after five oscillations. The introduction of air bubbles into the acceptor stream to suppress dispersion was not possible because of interferences with the spectrophotometric detection.

Fig. 4b presents the influence of the flow configuration and the number of oscillations on sampling rate in the case of NH_4Cl. These results indicated that at higher number of oscillations there was a substantial decrease in sampling rate. The structure of the donor stream (i.e., presence or absence of air segmentation) did not affect the sampling rate and therefore only the results with air-segmented donor stream are shown in Fig. 4b.

On the basis of the NH_4Cl results presented in Fig. 4a and b it was concluded that one oscillation in the case of *oscillating donor and acceptor streams with sample segmentation* offered an acceptable compromise between the requirements for high sensitivity and high sampling rate. Under these conditions there was a 6-fold increase in sensitivity compared to the corresponding flow configuration without oscillations (*stop-flow with sample segmentation*). The maximum 7-fold improvement was achieved after five oscillations.

Since the sensitivity in the case of sample segmentation was considerably higher compared to the configuration with continuous donor stream, oscillations experiments for EA„ DEA and TEA hydrochlorides were conducted with air segmentation only. The corresponding results followed the same trend as the results for NH_4Cl (Fig. 4a). However, because of the larger molecular size of the three amines compared to that of ammonia, the mass transfer across the membrane was slower. Maximum sensitivity for all three amines was obtained after five oscillations. This sensitivity (i.e., 10 for TEA, 11.5 for DEA and 12.5 for EA) was between 10 and 12.5 times higher than that in the case when oscillations were not used.

4. Conclusions

On the basis of the result obtained in this study it can be concluded that flow manipulation in GD SIA utilizing a second pump for the acceptor stream can improve substantially sensitivity compared to conventional GD FIA. This improvement was more pronounced for higher molecular size analytes. Best results were obtained when both the donor and acceptor streams were oscillated and the sample zone was separated by air bubbles from the donor solution. The sensitivity improvement due to this flow manipulation approach was between 7- and 12.5-fold.

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