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SEQUENTIAL INJECTION ANALYSIS IN CAPILLARY FORMAT WITH AN ELECTROOSMOTIC PUMP

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Summary—A system of sequential injection analysis (SIA) in capillary format based on an electroosmotic pump is introduced. The system exhibits excellent reliability and reproducibility (relative standard deviation 0.6–0.8%) for both simple and complex chemical reaction systems. The simplicity and flexibility of the field-decoupled electroosmotic pump has been found to be ideally suited for SIA. There is significant potential for miniaturizing the necessary instrumentation.

Flow injection analysis (FIA) has been very successful as a laboratory technique as well as a versatile tool for the processing, manipulation and automation of fast liquid phase analysis of samples. Although the applications of FIA to process analysis are growing, wide acceptance is deterred by the need for complex flow manifolds, multi-channel pumps and involved plumbing schemes. While such arrangements function well in the laboratory, they are often unsuitable for use in the industrial environment. This was the major reason for the pursuit of a simple and universal single-line flow analysis scheme, embodied in the Sequential Injection Analysis (SIA) system, first introduced by Ruzicka and Marshall.[1]

Tijssen[2] was the first to explore flow analysis in capillary dimensions. In this work, tubes as small as 28 μm in diameter were used. However, the overall volume of the detector and connecting tubes were relatively large ($\sim$25 μl), requiring a large dilution flow through a T-piece to reduce the residence time in the detector. This study was primarily aimed at determining the superiority of helically coiled conduits over straight tubes in reducing dispersion. Small diameter tubes were investigated to reduce volumetric dispersion with an ultimate view to increase sample throughout, to levels as high as several thousand/hr. No chemistry was carried out, toluene was directly injected in isooctane and optically monitored. A typical operational regime would have involved both high pressures and relatively high flow rates.

Since Tijssen's original work, other efforts have been made towards high throughput flow analyzers.[3] Despite early predictions to the contrary, no great need for process analyzers that can handle hundreds of samples/hr, much less thousands, has emerged. The demand has rather focused on mechanically simple analyzers that can perform complex chemistry with a minimum of downtime and operator training. Additionally, overall system miniaturization and the reduction of consumables and waste generated continue to be desirable in any analytical instrument. If short (3–30 cm) capillary conduits (25–150 μm bore) are used with low flow rates (0.5–5 μl/min), it can be readily computed that such systems can be used at very low operating pressures and offer residence times in the same range as those used in conventional FIA. Greater residence times are preferably achieved by flow cessation or reversal methods rather than increasing the length of the conduit; in this way the necessary pressure and the amount of liquids consumed are minimized. Therefore, it would be beneficial to carry out SIA in a capillary format.

Some general advantages of capillary format flow analysis have been described elsewhere.[4,5] A field-decoupled electroosmotic pump[6] is an ideal pumping system for SIA, the flow direction is readily and reproducibly reversed and the flow rate can be maintained with a high degree of reproducibility. In SIA, the sample and reagent(s) are sequentially injected. Mixing is

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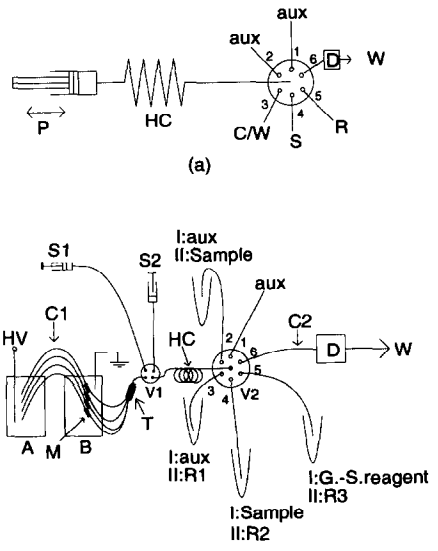

Fig. 1. (a) Standard sequential injection system: P, piston pump; HC, holding coil; C/W, carrier/wash solution; S, sample; R, reagent; D, detector; W, waste reservoir. (b) Schematic diagram of our experimental arrangement: HV, high voltage power supply; A, B, pumping electrolyte solution containers; M, membrane joint; C1, pumping capillary; T, 4 × 1 union; HC, holding coil; V1, four-way valve; S1 and S2, syringes; V2, 6 × 1 selector valve; R1, R2, R3, reagents; aux, unused auxiliary solution port, typically dipped in the carrier electrolyte to prevent siphoning. The sample/reagent arrangements for the nitrite determination system is denoted by I and that for the ammonia determination system is denoted by II. See text for details.

achieved by hydrodynamic dispersion and diffusion. Referring to Fig. 1a, a typical measuring cycle[7] comprises of the sequential aspiration of (a) the carrier/wash solution (with selector valve in position 3), (b) sample solution (with the valve in position 4) and (c) necessary reagent solution(s) (with the valve in position 5, and other available positions, such as 1, 2 *etc.*) into the holding coil (HC), and then propelling the entire contents of HC out to waste via the detector D with the valve in position 6. The analytical reaction product is thus monitored by the detector. If required, better mixing can be obtained by moving the piston forward and backward a few times with the valve in position 6, before the mixture is driven out.

The experimental arrangement for the capillary format SIA system described here is similar to the standard SIA format except that all conduits are capillaries and the fluid driving system is an electroosmotic pump. In this communication, we show two illustrative applications: the determination of nitrite–nitrogen by the Griess–Saltzman reactions,[8,9] and the determination of ammonia–nitrogen by the nitroprusside-catalyzed Berthelot reaction.[10] In the first case, all the necessary reagents can be mixed together in a single solution, so the determination procedure is simple. The second reaction involves the sequential addition of three separate reagents to the sample and constitutes a more challenging task. The effect of (a) varying the reagent introduction sequence, (b) the amount of each reagent and (c) the sample–reagent mixing style are readily studied by the present system and are discussed.

EXPERIMENTAL

Reagents

Nitrite–nitrogen. The Griess–Saltzman (G.–S.) reagent was prepared by dissolving 0.1 g *N*-1-naphthylethylenediamine dihydrochloride and 0.503 g sulfanilic acid in ~50 ml distilled water, adding 10 ml concentrated HCl, and finally diluting to 100 ml. Nitrite stock solution (1 m*M*) was prepared by dissolving sodium nitrite in distilled water; working solutions of different concentrations were made by dilution.

Ammonia–nitrogen. Phenol/sodium nitroprusside solution (R1) was prepared by dissolving 1.0 g phenol and 0.5 g sodium nitroprusside in 50 ml distilled water. Alkaline EDTA reagent solution (R2) was made by dissolving 5.0 g disodium ethylenediaminetetraacetate dihydrate and 2.0 g sodium hydroxide in 100 ml distilled water. Sodium hypochlorite reagent solution (R3) was obtained by diluting 8 ml commercial bleach (Clorox, 5% w/v NaOCl) to 100 ml.

Pumping electrolyte solution: 2 m*M* sodium tetraborate, diluted from a 0.5*M* stock solution in water, was used.

Instrumentation

The experimental arrangement is shown schematically in Fig. 1b. The high voltage source was a CZE1000 power supply (Spellman High Voltage, Plainview, NY). The pumping capillaries consisted of four individual segments (C1, 40 cm × 75 μm i.d. × 375 μm o.d.). Plastic bottles, A and B, respectively 100 ml and 25 ml, contained the pump electrolyte solution. The joint M was composed of a tubular Nafion membrane (Perma-Pure Products, Toms River, NJ, U.S.A.), T was a 4x1 union constructed from PVC tubing and silicone adhesive. A four-way valve (V1, Dionex Inert Valve, Dionex Co., Sunnyvale, CA) was incorporated between the pump and the analytical system. In the

Table 1. Protocol for nitrite–nitrogen determination

Valve position	Time spent (*sec*)*	HV (*kV*)	Comments
6 to 1	0.5	+15	0.5 *sec* required for valve action†
1	0.5	+15	Ports 1 to 3 are not being
1 to 2	0.5	+15	used. However, there is no
2	0.5	+15	particular advantage in using
2 to 3	0.5	+15	three ports adjacent since the
3	0.5	+15	valve does not rotate
3 to 4	0.5	+15	bidirectionally.
4‡	145	+15	Washing the sampling capillary
4‡	15	0	Replacing the sample solution
4	150§	−15	Aspirating sample, *ca.* 3.7 *µl.*
4 to 5	0.5	−15	
5	30	−15	Aspirating G.-S. reagent, ~740 *nl*
5 to 6	0.5	0	
6	270	+15	Propelling the product through the detector to waste reservoir
Total cycle time	614.5		

*The times were not optimized.

†This can be reduced by higher pneumatic pressure or by using helium as the pressurizing gas. The switching time is, however, fast enough to not hae toturn off the pump.

‡This step was omitted for single sample experiments, such as reproducibility experiments, system-optimization experiments, *etc.*

§This time was reduced to 30 *sec* for single sample experiments.

operating position the pump and the analytical system were connected. In the other position, syringes S1 and S2, respectively, filled the pumping conduit with pumping electrolyte and the holding coil with carrier solution. In both present cases, the pumping electrolyte solution was the same as the carrier solution since the chemical reaction systems were compatible with the pumping electrolyte. The capillary holding coil HC (40 cm × 250 µm i.d. × 350 µm o.d.) had a volume of ~20 µl. The electropneumatically operated 6 × 1 selector valve V2 (the common port may be connected to any of six selectable ports, type 5012P, Rheodyne Inc., Cotati, CA, U.S.A. modified in-house as described below) was automatically controlled through a programmable microcontroller (LS-100, Minarik Electric, Los Angeles, CA). Capillaries, 375 µm in o.d. and 150 µm in i.d., were directly put in the stator ports of the valve and fixed in place with silicone adhesive. The original rotor with a slot volume of ~10 µl was replaced with a Kel-F rotor with a machined scratch mark. A special tool was machined for making the scratch, with a wedge-shaped tip, measuring 1.5 mm at the tip with a wedge width of 30 µm at the bottom and a wedge angle of 15.5°. The scratch was ~125 µm (0.005 in.) deep, at this height from the bottom. The wedge width is 80 µm. The notch was 5.5 mm long and the computed volume of the notch of trapezoidal cross section is ≤50 nl. For the detection capillary C2, the detection window was located at ~15 cm from the valve. The length of the sample introduction capillary was ~7 cm, and all others were ~20–25 cm. The optical detector D was a model 206 PHD UV/Vis instrument (Linear Instruments/Spectra-Physics). The waste reservoir is represented by W. The construction of the field-decoupled electrolyte pump has been previously described in detail.[6]

Procedure

Determination of nitrite–nitrogen. Ports 1–3 were not used and capillaries connected to these ports were dipped in 2 m*M* $Na_2B_4O_7$ auxiliary solutions. Port 4 was connected to the nitrite sample solution, port 5 to the G.-S. reagent solution and port 6 to the detector (Fig. 1b). Although the optimum detection wavelength has been reported to be 542 nm,[11] the absorption band is broad, the absorptivity at 555 nm is >95% that of the maximum. We used a detection wavelength of 555 nm for future adaptation to an on-column light emitting dioxide based detector usable with capillary systems,[12] high brightness GaP emitters have an emission centered at this wavelength.[13] The protocol is described in Table 1.

Determination of ammonia–nitrogen. Ports 1–6 were, respectively, connected to 2 m*M* $Na_2B_4O_7$, sample, R1, R2, R3 and detector. The detection wavelength was set at 630 nm. The operational parameters for a measurement cycle are listed in Table 2.

Table 2. Protocol for ammonia–nitrogen determination

Valve position	Time spent (*sec*)	HV (*kV*)	Comments
6 to 1	0.5	+15	
1	0.5	+15	
1 to 2	0.5	+15	
2	60*	+15	Washing the sampling capillary
2	15	0	15 *sec* for sample replacement
2	4	−15	
2	120†	−15	Aspirating sample, *ca.* 3 *µl*
2 to 3	0.5	−15	
3	3	−15	Aspirating R1, *ca.* 74 *nl*
3 to 4	0.5	−15	
4	5	−15	Aspirating R2, *ca.* 120 *nl*
4 to 5	0.5	−15	
5	20	−15	Aspirating R3, *ca.* 490 *nl*
5	0	0	
5 to 6	0.5	0	
6	180‡	+15	Propelling the product through the detector to waste reservoir
Total cycle time	410.5		

*Sixty seconds were allowed for all of the previous sample in the sampling capillary to be washed out.

†A relatively long withdrawal period was used to make sure that the undispersed sample was introduced into the principal conduit.

‡This period allows complete retainment of the detector baseline.

RESULTS AND DISCUSSION

Hardware modification for use in capillary systems

As obtained, the multiport selector valve had a rotor slot volume of ~10 µl. This volume is large relative to capillary dimensions. When the valve was used without modification in the SIA system, the product zone suffered from very large dispersion. The baseline peak width of the analyte signal (at 5% of the peak height) was ~25 min. Replacement with a rotor of a slot volume of ~50 nl, machined in-house, reduced this peak width to ~2 min, under the same experimental conditions.

General attributes of the system

It should be mentioned at the outset that the present experiments were conducted to demonstrate the feasibility of electroosmotically pumped capillary format SIA. The long time of each measurement cycle are not intrinsic limits of the system, they can be dramatically reduced by using greater pumping rates (by incorporating more pump capillaries in parallel).

When the sample capillary was switched from one sample to another, the sampling capillary was first washed with the carrier solution to avoid sample carry-over. Obviously, the sampling capillary should be as short as possible, to increase the sample throughput and decrease the sample consumption. The length of the sampling capillary we used was ~7 cm because of the physical limitations of the hardware. It would have been possible to reduce the wash period by using a narrower bore capillary. Another means to reduce sample carry-over with minimum time penalty is to aspirate air after each sample, considerably reducing the necessary wash time. There did not appear to be any special problems in aspirating air bubbles between liquid segments in the present system and on-column detectors did not suffer from bubble entrapment problems.

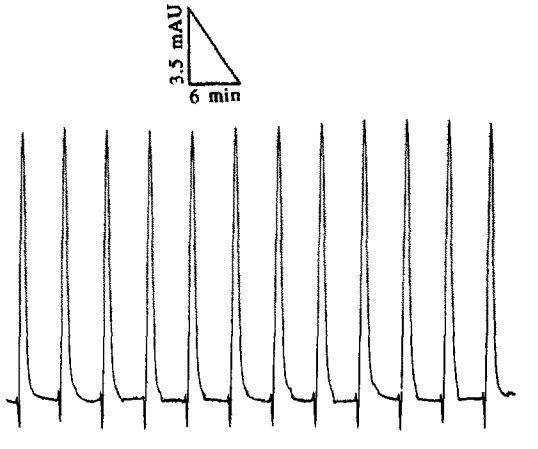

Fig. 2. Reproducibility for nitrite measurement. HV: 15 kV; sample aspiration: 30 sec; reagent aspiration: 30 sec; product propelling: 270 sec; detection window: 150 µm i.d. capillary; detection wavelength: 555 nm; sample: 160 µ*M* sodium nitrite.

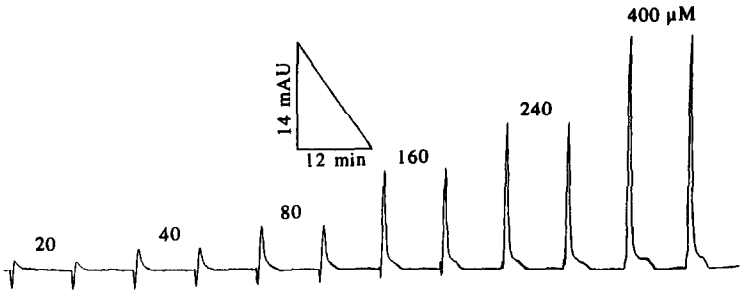

Fig. 3. Calibration experiment for nitrite measurement. Sampling capillary wash: 2.2 min; sample aspiration: 2.2 min; sample aspiration: 2.5 min; sample concentration: 20–400 μM. Other conditions as in Fig. 2.

Determination of nitrite–nitrogen

The peak height reproducibility (Fig. 2) for a sample containing 160 μM nitrite was 0.6% ($n = 12$) relative standard deviation. Figure 3 shows a calibration series. The negative absorbance in front of the signal peak was due to the negative absorbance of the G.-S. reagent relative to the carrier. These results (peak height *vs.* concentration) exhibited a good linear relationship ($r = 0.9995$) over the range of 20–400 μM nitrite.

Determination of ammonia–nitrogen

Reagent introduction sequence. The mechanism of the Berthelot reaction has been described in the literature.[14–16] Ammonia first reacts with hypochlorite in alkaline medium forming monochloramine. The monochloramine slowly couples with aquopentacyanoferrate (an equilibrium product of nitroprusside in aqueous solution). The aquopentacyanoferrate-coupled monochloramine then reacts with two molecules of phenol, producing the deep blue-colored indophenol. On the basis of the reaction mechanism, the following procedure was recommended:[15] (1) adjust sample to a pH of 10.5, (2) add hypochlorite reagent, (3) add nitroprusside and (4) add phenol reagent. On the other hand, the EPA approved standard automatic method[17] is based on the following introduction sequence: (1) sample, (2) phenolate, (3) hypochlorite and (4) nitroprusside. In our experiments, following a previously published study from this laboratory on this reaction,[18] the phenolate and nitroprusside were mixed as R1, alkaline EDTA as R2, and hypochlorite as R3. Results of different sequences of reagent-introduction are shown in Fig. 4. Under conditions 3 and 4, where hypochlorite reagent was added before phenolate/nitroprusside and alkaline EDTA, the peak height signals were higher than in other cases, as reported in the literature.[15] Because the hypochlorite reagent is in more than a thousand-fold excess, a great difference in sensitivity with a different order of reagent addition should not be expected. However, as Fig. 4 also shows, the reproducibilities were the worst under these conditions. If alkaline EDTA was introduced immediately after the sample, followed by hypochlorite and the phenolate/nitroprusside reagent (condition 6, similar to the recommended procedure[15]), the highest signal was not obtained. This is probably due to the fact that one is not dealing with a homogeneous solution in SIA. Alkaline EDTA served the desired function of not only adjusting the sample pH, it also unavoidably separated the sample spatially form the other reagents. This illustrates that one needs to be cautious in directly adapting methods from homogeneous reaction chemistry to SIA systems. In principle, it would be possible to aspirate repeatedly very small segments of different fluids in turn (sample–R1–R2–R3, Sample–R1–R2–R3, *etc.*) to achieve better mixing, as shown in a recent

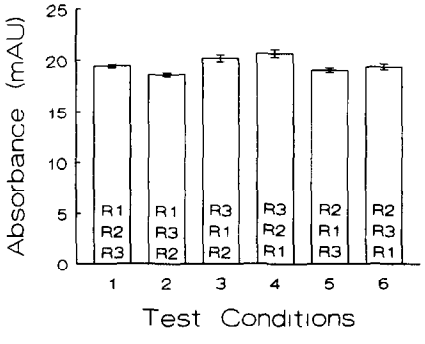

Fig. 4. Results of different reagent-introduction sequences. Sample: 500 μM ammonium chloride; estimated reaction time: 150 sec; Aspiration times: Sample 20 secs, R1 3 sec, R2 5 sec, R3 20 sec. The reagent introduction order is indicated in each bar, from top to bottom. Product propelling time: 180 sec.

publication on the use of a novel computer controlled micropump in FIA.[19] However, this can be a very time-consuming process if finite reaction periods must be allowed after each reagent addition. In any case, for the presence case, since the different sequences did not affect the product absorbance in a large fashion, condition 1 (similar to the EPA approved automatic procedure),[17] was selected for further experiments because it produced the best reproducibility.

Sample–reagent aspiration time. For multireagent SIA experiments, the aspirated length (amount) of each solution, especially the solutions added inbetween the others, is an important experimental parameter. Figure 5 shows how the product signal peak height changes with the aspiration time of each solution. Reagents R1 and R2 were introduced between the sample and R3. Their aspiration times should be as short as possible to minimize the spatial isolation effect. Note that one can use increased reagent concentrations and reduced volumes to introduce the same total amount; this decreases spatial isolation. We used here twice as concentrated a hypochlorite reagent than previously used in batch experiments. Based on the results shown in Fig. 5a and b, respective aspiration times of 3 and 5 sec (1 sec aspiration $\approx$1.4 mm length of a plug of solution in a 150 μm i.d. capillary) appeared to be the optimum choices for R1 and R2. These values were then used for all further experiments. In the liquid introduction sequence R3 was aspirated last and no spatial isolation effect on other reagents was involved. A relatively long aspiration time for R3 was beneficial as it helped the sample–reagent mixing. Based on the results from Fig. 5c, 20 sec aspiration for R3 was selected. Aspiration times >20 sec decreased the peak height due to sample zone dispersion, although the peak area remained approximately the same. Considerations for the amount of the sample aspirated were different from that for the reagents because the analyte was the limiting reagent. The concentrations of the reagent solutions aspirated were orders of magnitude more concentrated than that of the analyte. Preliminary experiments with dyes were made to ensure that each reagent dispersed sufficiently into sample zone. Therefore, the sample zone dispersion would be the major factor that affects the absorbance peak height signal of the product. Figure 5d shows the relationship between the peak height and the sample aspiration time. The peak height increased exponentially with the sample aspiration time up to 20 sec. At longer aspiration times, a plateau absorbance level was eventually reached, suggesting that at this point the sample volume is large enough to approach a dispersion factor of unity. When the amount of the product as measured by peak area was plotted against the sample aspiration time (Fig. 6), the observed relationship was linear up to an aspiration time of 10 sec. This indicates that all of the sample was sufficiently penetrated by the reagents up to this point. At longer sample aspiration times, reagent

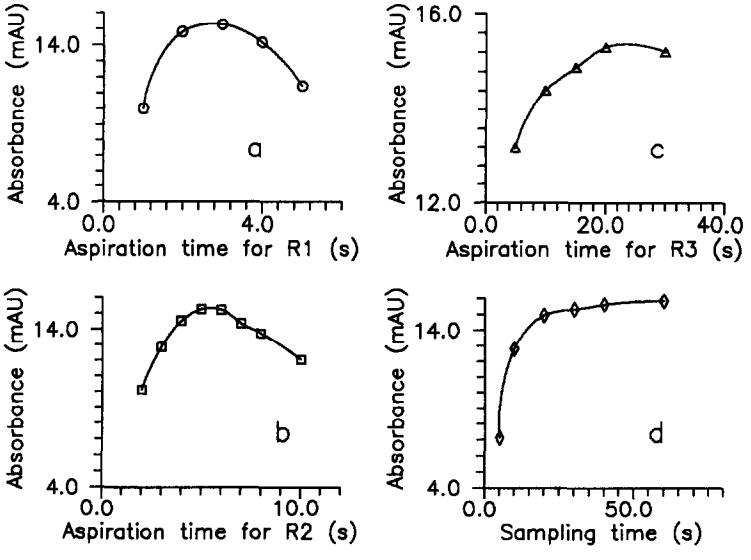

Fig. 5. Effects of different aspiration times on the absorbance signals. (a)–(d) Varying aspiration times for R1, R2, R3 and sample, respectively. Other conditions are the same as condition 1 in Fig. 4.

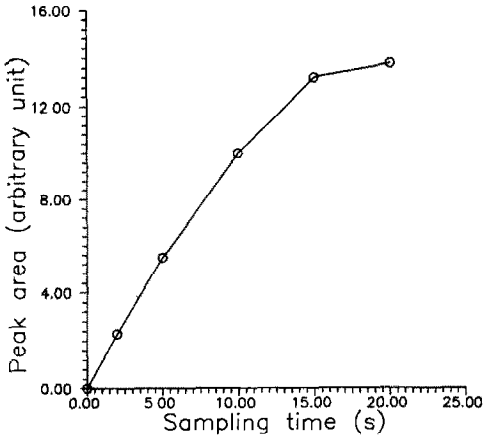

Fig. 6. Product signal (peak area) *vs.* aspiration time of the sample. Conditions as in Fig. 5d.

penetration into some portion of the sample zone clearly became insufficient.

Control of mixing by dispersion. As in FIA, any modification of experimental parameters in SIA is often centered on the control and the attainment of the desired degree of dispersive mixing of the sample and the reagent solutions. While mixing also occurs by diffusion, little can be done to directly control diffusion. However, hydrodynamic dispersion can be controlled in a number of ways. As has been previously shown,[20] one efficient method is to use sequential backward and forward flow, repeated as many times as necessary. By controlling the length of each flow step and the number of repetitions, the dispersion can be precisely controlled. In the present experimental system with an introduction sequence of sample (20 sec), R1 (3 sec), R2 (5 sec), and R3 (5 sec), after the R3 aspiration step was finished and the valve was switched to the detection capillary (port 6), the solution was propelled forward for 5 sec and then withdrawn backward for 5 sec and finally propelled out through the detector. This greatly helped the sample–reagent mixing. The peak height increased about 50% and the peak area increased about 80%, compared with the results without this flow oscillation. However, as the aspiration time of R3 was increased, the peak area enhancement upon flow oscillation became less significant. The enhancement disappeared altogether when the aspiration time of R3 was $\geqslant$20 sec. The effect of increasing the R3 aspiration time on how flow oscillation affected the peak height was even more noticeable. If the R3 aspiration time was $\geqslant$10 sec, the peak height decreased upon using flow oscillation. It appears that after 20 sec aspiration of R3, all necessary reagents were dispersed sufficiently into the sample zone. Further dispersion of these reagents could no longer increase the product yield (peak area), while further sample zone dispersion, if induced by flow oscillation, decreased the peak height. These results suggest that, at least under these circumstances, increasing the aspiration time of the last introduced reagent solution may be a better way to achieve the desired dispersive mixing compared to flow oscillation.

Reproducibility. The Berthelot reaction is not a fast reaction. In test tube experiments, 2.5 min was required to reach 90% of the reaction. In the standard procedure,[17] 10 min are used for the reaction to reach a stable absorbance. In our experiments, the time interval between the end of R3 aspiration and the passage of product peak through the detector was $\sim$2.5 min, this could be considered the reaction time. This means $\leqslant$90% completion of the reaction was achieved. In spite of the fact that the reaction was not complete, the calibration curve showed excellent linearity ($r = 1.0000$) over a range of 50–600 μM ammonia. The reproducibility was also very good: measurements of 200 μM ammonia solution exhibited a peak height RSD of 0.81% ($n = 9$).

CONCLUSIONS

As argued by Gubeli *et al.*,[20] the sequential injection analyzer is much less complex than any present chemical analyzer, including traditional flow injection schemes. This is especially true from a process analysis viewpoint. Capillary SIA schemes reduce the consumption of reagents and sample, minimize the physical size of the conduit and the instrument, reduce maintenance, lower instrument cost and improve the measurement precision. There is often the impression that the concentration sensitivities for capillary systems are limited due to the limited optical pathlength. First, great sensitivity is frequently not necessary in process analysis (although this can be a major factor in environmental analysis). In capillary SIA, the sample volume is small but is nevertheless much larger than in capillary electrophoresis, since analyte resolution is not of concern. The product zone is long enough to permit the use of a long pathlength U-cell or Z-cell to improve the sensitivity.[21–23] Other techniques, such as using a larger capillary[25] or a bubble blown into a

capillary[24] as the detection window, can also be used to increase the detection sensitivity.

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