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Miniature flow-injection analysis manifold created by micromilling

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Abstract

The miniature flow injection analysis (μFIA) system is based on a mechanically engraved manifold in a PMMA-substrate. The channels have a trapezoidal cross-section with a depth of 150 μm and a width between 180 and 360 μm and the reactor volume is 3.75 μl. A light-emitting diode (LED) is used as radiation source for absorbance detection and optical fibres are used for transmitting light to and from the manifold. Computer controlled valves are used for directing the liquid flows maintained with syringe pumps. A sampling rate of approximately five determinations per minute was achieved with a reagent consumption of 10 μl min^{-1}. Four applications were implemented and tested; the performance was found to be comparable to that obtained with conventional FIA-set-ups despite the drastically reduced optical pathlength. Electrokinetic pumping was extensively evaluated but found to generally be very restricted in scope because the reagent solutions cannot usually be adapted to the requirements for generating the flow.

Keywords: Flow-injection analysis; Miniaturization; Light-emitting diodes; Syringe pumps

1. Introduction

Flow-injection analysis (FIA), intensively developed over the past 25 years, is a general approach to sample handling and most often represents an automated procedure for the common spectrophotometric procedures. In keeping with the current trend to miniaturization of analytical methods mainly concerned with electrophoretic separations on planar microfabricated systems (electrophoresis chips, micro-total analysis systems), also several approaches to micro-scale FIA-systems have been described. The advantages of miniaturization are compactness of the overall system, portability and, importantly, a significant reduction of reagent consumption which leads to cost savings and reduction of chemical wastes.

A forerunner of miniaturized systems were the integrated microconduits introduced by Ruzicka and Hansen in 1984 [1]. Flow channels, injection devices as well as detectors were integrated into a flat block of PVC. Semicircular channels with cross-sectional areas of 0.8 mm^2 were embossed or engraved with mechanical means and covered with a second plate glued onto the first one. The

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devices were, however, operated with a conventional peristaltic pump and the internal dimensions were not much reduced compared with conventional systems assembled with tubes, so that the gain in terms of overall miniaturization and reduction in reagent volumes was limited. Carlsson et al. reported a similar system employing a stepper motor driven piston pump which was used for the determination of phosphate [2]. Fettinger et al. also designed a miniature FIA-system fabricated with conventional machining techniques which was based on a number of stacked modules made from poly (methyl methacrylate) (PMMA) and having internal channels with diameters of 0.8 mm [3]. Van der Schoot et al. constructed miniature FIA manifolds in silicon which featured piezo-electric pumping and ion-selective field effect transistors (ISFET) for the determination of certain ions. Haswell and co-workers [4–7] described the fabrication of micro-scale manifolds in glass using photolithographic etching techniques which were used for the photometric determination of phosphate, nitrite and nitrate. As a pronounced electroosmotic flow (EOF) is obtained in glass when applying a voltage along a channel, these authors investigated the use of electrokinetic pumping for FIA in the micro-fabricated device.

The aim of the project reported on herein was to investigate the feasibility of creating a micro-scale manifold by conventional mechanical milling of PMMA and to explore the possibility of using electrokinetic pumping to implement common FIA-methods.

2. Experimental

2.1. Manifold

The channels were created in a plate of PMMA, (Irpen, Barcelona, Spain) of 50×110 mm by mechanical milling with a high frequency spindle (55 000 rpm) using a milling bit that has V-shaped tip (R30 from Ray, Nännikon, Switzerland). As can be seen in Fig. 1, the cross-section has a trapezoidal shape of approximately 150 μm depth and a width from 180 to 360 μm. The walls look

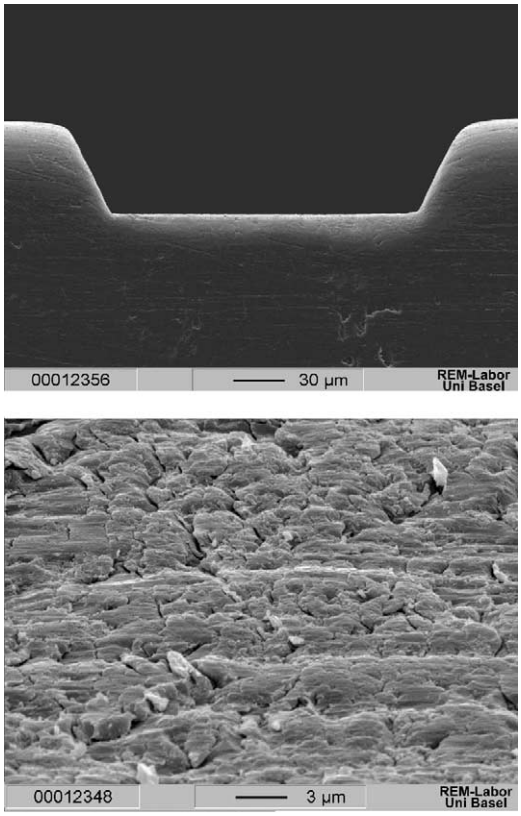

Fig. 1. Top, electron-microscope picture of the channel-geometry. Bottom, electron-microscope picture of the channel ground.

smooth under a light microscope, only the scanning electron-micrograph of Fig. 1, bottom, reveals some degree of roughness present. The channels are sealed with a second PMMA plate which is clamped tightly on top with the help of several screws. This arrangement has the advantage that it allows dismantling for cleaning if the channels should become blocked. A schematic drawing of the manifold is given in Fig. 2. Access to the channel system is provided by six 1/4 in.-UNF ports (A–E in Fig. 2) machined into the cover plate to accept standard fittings. A BAS Bee-Hive syringe pump system (BAS, West Lafayette, IN, USA) equipped with two 5 ml plastic syringes (Cosanum, Zürich, Switzerland) was used for conventional pumping of reagents (SP 1 and SP 2 in Fig. 2). Electrokinetic pumping was achieved by applying voltages from high voltage power

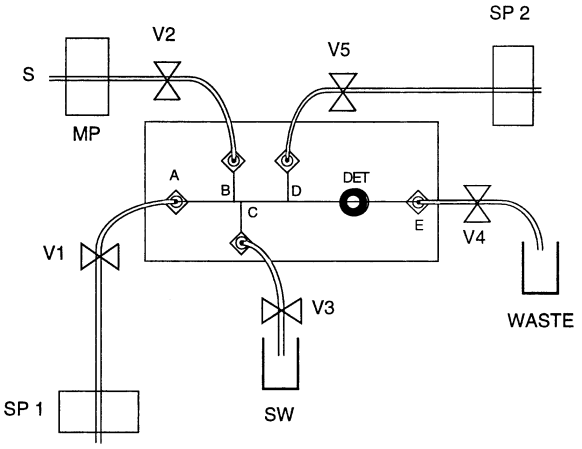

Fig. 2. Schematic overview of the PMMA microchannel device with optical detection. DET, optical cell for two SMA fiber connectors; SP 1, syringe pump for the main channel; SP 2, syringe pump for the side channel; V1–V5, valves; S, sample; SW, sample waste; MP, solenoid pump for sample injection. The distances between the marked points are: A–B, 2 cm; B–C, 0.3 cm; B–D, 2 cm; D–E, 5 cm; D-DET, 2.5 cm; A–E, 9.3 cm.

supplies (Model CZE1000R, Start-Spellman Ltd., Pulborough, England) to the ends of the channels with platinum wires. Five miniature solenoid valves (LFVA 1230113H, V1–V5) were used to control the flows and a solenoid pump (LPLA 1230350L, MP) (all from LEE, Westbrook, CT, USA) was used to fill the double-T section of the manifold (B–C in Fig. 2) for hydrodynamic sample injection [8]. This was effected by closing valves V1, V4 and V5 while opening V2 and V3 to pass the sample solution to the sample vial (SW) with pump MP. For the determination step proper, valves V2 and V3 were closed, V1 and V4 opened and syringe pump SP 1 started. The auxiliary flow through port D is optional. All valves and pumps were controlled via an interface with a computer program written in FORTH (UR/FORTH, Laboratory Microsystems Inc., Marina del Rey, CA) running on an IBM-compatible PC under DOS.

Detection was carried out by measuring the optical absorption through the depth of the channel. A high intensity green light-emitting diode (LED) (Type NSPG500S; λ_{max}: 525 nm, half width: 30 nm) purchased from Nichia Chemical Industries (Tokushima, Japan) was used as light source. The dome of the LED was cut and the resulting rough flat surface was polished to obtain close access (about 0.2 mm) to the active site. The LED was mounted on a 3-stage-micropositioner (Stock No. A38528 from Edmund Scientific, Barrington, NJ) for optimizing coupling of the emitted light directly into two optical fibres of 100 μm active diameter (FG-100-GLA from Thorlabs, Newton, NJ). Note, that no focussing elements were used. The other ends of the fibres were fitted with optical SMA-connectors (Thorlabs), one of which was used to guide light to the detection cell, while the other serves as reference channel. The design of the detector cell on the manifold is illustrated in Fig. 3. Two male SMA-sockets made in-house from aluminium were glued onto round PMMA disks of 10 mm diameter and 0.9 mm thickness which are press-fit into two holes machined at the appropriate places into the manifold. A third optical fibre of 200 μm core diameter (FT-200-URT, Thorlabs) was used to guide the trans-

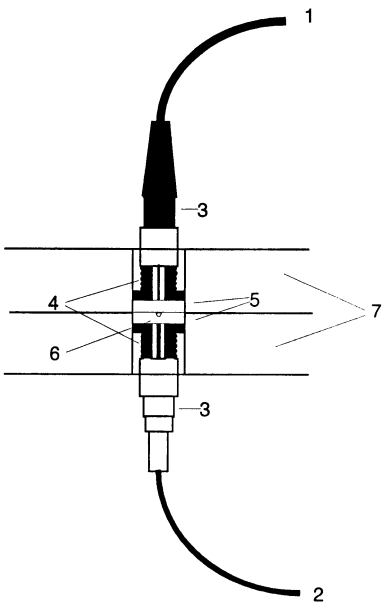

Fig. 3. Cross-sectional drawing of the optical cell. (1) light delivering optical fibre of 100 μm diameter; (2) light collecting fibre, 200 μm; (3) SMA connectors; (4) inserts made from aluminium with an outside thread for the SMA connectors; (5) PMMA inserts of 0.9 mm thickness; (6) channel; (7) PMMA plates.

mitted light to the detector circuitry. The latter consists of two photodiodes fitted with SMA sockets (Stock No. 633-363 from RS Components, Corby, Northants, UK) to accept the optical fibre from the cell as well as the reference fibre, which are connected directly to a log ratio amplifier as described in detail elsewhere [9–12]. Data acquisition was performed with a MacLab System (ADInstruments, Hastings, UK) on a 7300/166 Power Macintosh computer (Apple, Cupertino, CA). Photocurrents from the photodiodes were determined by connecting the diodes to the input of an operational amplifier in the current-to-voltage convertor configuration.

2.2. Solutions and procedures

The dispersion factor was determined by using a solution of Nuclear Fast Red approximately 0.1 mM in concentration. The EOF was evaluated by injection of Safranin O and Sulforhodamin B in buffers at pH values of 3.9 (sodium formiate), 4.7 (sodium acetate), 7.0 (potassium dihydrogen phosphate), 8.0 (tris(hydroxymethyl)aminomethane) and 9.0 (borate) all at 2.5 mM. The procedure for the iron determination was adapted from [13]. The reagent solution contained 12.3 mM 1,10-phenanthroline monohydrate and 0.1 M ammonium acetate in deionized water, and its pH value was adjusted with acetic acid to 4.5. The iron stock solution was made up by dissolving 1.404 g of ammonium iron (II) sulphate in a mixture of 50 ml water and 20 ml of conc. sulphuric acid and diluted to 1 l. This stock solution contains 200 ppm of iron (II). Standard solutions of different iron concentrations were prepared by diluting appropriate amounts of the stock solution with water. Heavy metal determinations were carried out according to Regan et al. and Engström et al. [14,15]. A solution of 2 mM 4-(2-pyridylazo)resorcinol (PAR) and 10 mM *N*-{tris(hydroxy-metyl)metyl}-3-aminopropanesulfonic acid (TAPS) in ultrapure water at pH 8.5 (adjusted with NaOH) was employed as reagent. Metal stock solutions consisted of 1 mM $ZnSO_4$ or 1 mM $CuSO_4$. Chloride determination was carried out according to Cheregi and Danet [16]. A mercury thiocyanate solution as reagent was prepared by dissolving 3.033 g $Fe(NO_3)_3$, 62.2 mg of $Hg(SCN)_2$ and 0.3 ml 65% HNO_3 in 100 ml ultrapure water. A chloride stock solution was prepared by dissolving 1.648 g of NaCl in 1 l of deionized water. Nitrite determinations were carried out according to Anderson [17]. As reaction medium a solution containing 0.4 M ammonium chloride and 0.3 M sodium chloride was prepared. The sulphanilamide reagent was prepared by dissolving 1 g of sulphanilamide in 5 ml of conc. hydrochloric acid in 100 ml of reaction medium. As coupling reagent a solution of 0.1 M *N*-(1-naphthyl)ethylendiamine dihydrochloride and 4 g of sodium chloride in 100 ml of the reaction medium was used. The standard stock solution of sodium nitrite was 1 mM in 0.7 M sodium chloride. For performing the calibration plot the stock nitrite solution was diluted with a solution of 0.7 M sodium chloride. All solutions were degassed for 15 min by ultrasonic agitation and filtered through a 0.45 μm membrane filter (BGB Analytik, Anwil, Switzerland). Chemicals were purchased from two different suppliers (Fluka, Buchs, Switzerland and Merck, Darmstadt, Germany) and were all of analytical grade. Purified water (Millipore, Bedford, MA, USA) with a conductivity of 18.2 nS cm^{-1} was used for all aqueous solutions. To avoid trapping of air bubbles in the channel system a small amount of Triton X100 was added to the reagent solutions.

3. Results and discussion

3.1. Characterization of the detector cell

In a first step it was ascertained that the current from the photodiode is within the dynamic range of the detector circuitry. The photocurrent was measured as typically 10 nA, and this is indeed well within the useful range. In a second experiment, the contribution of ambient light to the detector signal was determined. It was found that any current obtained from the photodiode when the LED was turned off is indistinguishable from its dark-current (obtained by placing the assembly in a light tight box) of approximately 25 pA. It is, therefore, possible to operate the device in ambient light and shielding is not necessary. Thirdly, the

amount of stray light from the LED-source which is by-passing the measuring channel and falling onto the collection fibre was determined by filling the channel with black ink. Again, the resulting photocurrent was in the order of the dark current of the photodiode (30 pA), and therefore, only a negligible level of stray light from the source was reaching the detector.

3.2. Characterization of the manifold

The dispersion coefficient D, as introduced by Ruzicka and Hansen [18], was determined for the manifold by comparing the absorbance of a dye injected with the absorbance obtained when filling the manifold completely with the dye solution. D is a measure of the dilution of the injected sample and defined as the ratio C^0/C^{max}, where C^0 is the analyte concentration in the original solution and the C^{max} corresponds to the maximum of peak on injection. A value of 1.5 was obtained. This means that the dispersion of the manifold is limited and characteristic of a system where mixing with a reagent is not needed, such as when a selective detector (e.g. an ion-selective electrode) is employed rather then spectrophotometry. However, as evident from the results reported below, this is not a limitation as the auxiliary side channel (point D on Fig. 2) can be used for adding reagent.

3.3. Evaluation of pumping systems

In principle, the most elegant method for propelling solutions is the use of electrokinetic pumping as then no mechanically moving parts have to be employed. For this reason, first of all, the EOF in the PMMA manifold was determined by injection of a cationic and of an anionic dye. From the intermediate value of the time delay time to the detector, the extent of the EOF could be estimated as a constant 4.3 10^{-6} cm^2 V^{-1} s^{-1} for the pH range from 5 to 9. This EOF is slightly lower then the corresponding value in fused silica [19], and clearly indicates that pumping, even of neutral species, by applying a voltage across channels is feasible in PMMA. Below pH 5 the EOF is, however, significantly reduced, presumably because of protonation of acidic groups present on the surface of the polymer. It was found possible to determine iron(II) with the phenanthroline method using electrokinetic pumping as illustrated by the peaks of Fig. 4. A linear calibration curve for the concentration range from 20 to 1300 μM was obtained and the detection limit was determined as 5 μM. However, attempts to adapt other methods to electrokinetic pumping (such as heavy metal determination with pyridylazoresorcinol, the determination of cyanide with chloramin T, of nitrite with the Griess method and others) were found to be fraught with difficulties. It was generally not possible to find operating conditions which would satisfy the needs for electrokinetic pumping (pH value > 5, low ionic strength for limited electrical conductivity of the solution and high EOF) as well as those of the spectrophotometric method (reagent concentrations, flow rates, pH value).

Also considered was the use of miniature piezoelectric pumps. Such devices of outer dimensions of approximately 2×2 cm^2 and 5 mm thickness were purchased from an institute active in micromachining which made them available for experimental purposes. It was found that these devices were not adequate because only low pressures could be obtained and the flow rates were not

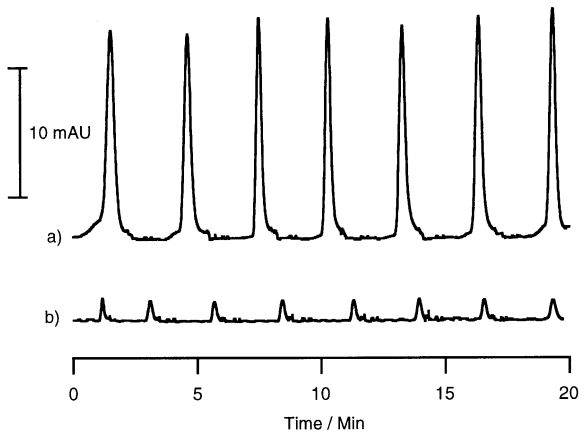

Fig. 4. Iron determination with electrokinetic pumping. Repeated injections of (a) 358 μM and (b) 18 μM iron solutions with manual injection and EOF as pump. Reagent solution: 12.3 mM phenanthroline in 1 mM NaOAc buffer at pH 5. Potential between the two vials: 2 kV.

stable. At least in our hands these devices were not sufficiently robust. It must also be considered that a special electronic driver circuitry, which can deliver a voltage of several hundred volts is required for piezoelectric devices, which is not trivial and requires considerable space much exceeding that of the pumps themselves, thus negating some of the reduction in size achieved.

Syringe pumps provide a further means of creating small flow rates. Relatively high pressures can be sustained and the flow rate is independent of back pressure and other parameters. Critical might be the creation of pulses as syringe pumps are driven by stepper motors. However, for the models used for our purposes, the length which the syringe piston travels is divided into 829 925 steps over the 60 mm calibration length, which was found to result in a more then adequately smooth flow. Syringe pumps were, therefore, adapted as the means of choice, although this also limits the degree of miniaturization of the overall system.

3.4. Applications

The determination of iron(II) was then implemented using syringe pumps. The phenanthroline reagent was pumped through the main as well as the auxiliary channel. As can be seen from the peaks in Fig. 5, the relative reproducibility is good (R.S.D = 1.1%, $n = 6$) and a sampling rate of up to about five determinations per minute could be achieved (Fig. 5). All experiments for the iron determinations were performed with a flow rate of 10 μl min^{-1} for each of the syringe pumps. Since the reagent consumption per analysis is only 10 μl, the system can handle 1000 samples without refilling. By using two 5 ml syringes this means a refill is only need after 4 h. In Fig. 5, a small peak is visible ahead of the analyte peak. It is thought that this is due to a pressure pulse introduced by the action of the valves. For the measurements of small concentrations this artefact can disturb, but it is possible to eliminate this interference by creating a short delay (5 s) after the injection and before the start of the main flows. The results are summarized in Table 1.

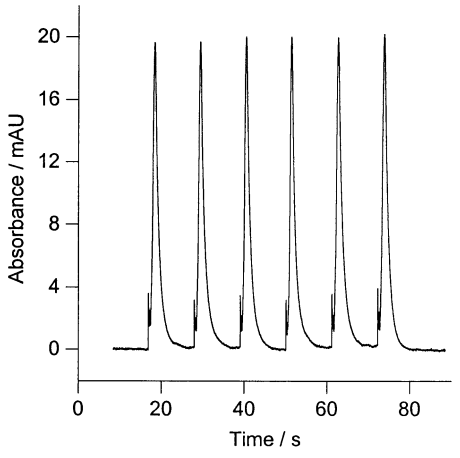

Fig. 5. Iron determination using syringe pumps. Repeated injections of a solution containing 1.79 μM of Fe^{2+}. Reagent solution: 12.3 mM phenanthroline in 0.1 M ammonium acetate buffer at pH 4.5. Flow rates: main channel 10 μl min^{-1}, side channel 10 μl min^{-1}.

The second application investigated was the determination of total heavy metal concentration with PAR as reagent. PAR is a highly sensitive and non-selective reagent applicable for screening of heavy metals useful for example in industrial waste water analysis. The FIA setup used was identical to that of the iron determination, only the reagents and the flow rates are different. Similar results as for the iron determination were obtained as again summarized in Table 1.

The determination of chloride was also found possible (see Table 1). This method is based on the reaction of Cl^- with $Hg(SCN)_2$ to liberate SCN^- which in turn reacts with Fe^{3+} to produce the red $Fe(SCN)^{2+}$ species. The analysis for nitrite determination is more challenging because it is based on a two step reaction, in which nitrite first reacts with sulphanilamide and the product must then react with diamine as a coupling reagent to form the diazo dye detected. In this case the second syringe (SP 2 in Fig. 2) was, therefore, filled with a different reagent. The results summarized again in Table 1 indicate that also this mode is possible. Note, that in this case the flow rates for both syringes had to be kept comparatively low in order to allow sufficient time for the reaction to take place.

Table 1
Summary of experimental parameters and results

	Flow rate (μl min^{-1})	Sensitivity (mAU μM^{-1})	Correlation coefficient (R^2) (linear regression)	Detection limit ($3 \times S/N$) (μM)	Linear range (μM)
Iron	SP 1 = 4, SP 2 = 4	0.18139	0.995	33	35–1800
Total heavy metals (PAR)	SP 1 = 8, SP 2 = 8	0.13216	0.9945	7	10–200
Chloride	SP 1 = 5, SP 2 = 20	0.0386	0.988	158	1000–4200
Nitrite	SP 1 = 1, SP 2 = 1	0.11262	0.9995	4	5–150

4. Conclusions

It was found possible to construct a versatile miniature FIA manifold using conventional machining methods. This results in a significant reduction of reagent consumption, and therefore, also of analysis costs. The move to reduce reagent consumption is not only economical but also environmentally friendly as there is also a proportional decrease in waste products. Using the mechanical machining method for the fabrication of the manifold omits the expensive and complicated etching processes which require the use of special equipment in a clean room. With few alterations it would be possible to operate the system from a battery as power source and a compact portable instrument is feasible. An important finding is also the fact that electrokinetic pumping was found to be highly limited in scope, as it is usually not possible to find suitable conditions that match both the requirements for pumping and photometric detection.

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