Talanta 86 (2011) 208–213

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Flow–batch miniaturization

Severino S. Monte-Filho[a], Marcelo B. Lima[a], Stéfani I.E. Andrade[a], David P. Harding[a], Yebá N.M. Fagundes[a], Sergio R.B. Santos[b], Sherlan G. Lemos[a], Mario C.U. Araújo[a,*]

[a] Universidade Federal da Paraíba, CCEN, Departamento de Química, Caixa Postal 5093, 58051-970, João Pessoa, Paraíba, Brazil
[b] Instituto Federal de Educação Ciência e Tecnologia da Paraíba – IFPB, Avenida 1° de maio, 720, Jaguaribe, 58015-430, João Pessoa, Paraíba, Brazil

ARTICLE INFO

Article history:
Received 21 June 2011
Received in revised form 29 August 2011
Accepted 30 August 2011
Available online 3 September 2011

Keywords:
Flow–batch analyzer
Urethane–acrylate photo-resist
Microfabrication
Deep ultraviolet-lithography
UV–vis spectrophotometry
Determination of Fe(II)

ABSTRACT

This study introduces the first micro-flow–batch analyzer (μFBA). A simple, low-cost, deep urethane–acrylate photo-resist ultraviolet-lithographic technique was used in its development. Details of the microfabrication process are presented including; the use of two superimposed photo-masks to improve the micro-channel and stop chamber border definition, as well as integration of an LED/phototransistor photometric pair, while using an open nylon-thread (fishing line) micro-mixing system for solutions homogenization. The system was used for photometric determination of Fe(II) in oral solution iron supplements employing the well-known 1,10-phenanthroline method, with instantaneously prepared micro-chamber calibration solutions. All analytical processes were accomplished by simply changing the timing parameters in the control software. It must be emphasized here that there was no outside preparation of the standard calibration solutions; the mixing was all done in-chamber/in-line, with all solutions maintained flowing while being proportioned for the measurement processes. The μFBA results were acceptable when compared to the reference method, and comparable to normal flow-batch systems. It was possible both to project and build a low-cost probe with high sample throughput (about 120 h^{-1}), low relative standard deviations (about 1.1%), and reduced reagent consumption (30 times less than the reference method). The μFBA system based on urethane–acrylate presented satisfactory physical and chemical properties while keeping the flexibility, versatility, robustness, and multi-task characteristics of normal flow–batch analyzers. The μFBA system contributes to the advance of micro-analytical instrumentation, while realizing the basic principles of "Green Chemistry".

1. Introduction

Fonseca et al. [1,2] have recently, developed micro-devices for flow injection analysis (FIA) using deep UV-lithography and urethane–acrylate photo-resist. Cured urethane–acrylate photoresin presents good chemical solvent resistance, and has high resistance to acids (37% HCl) and concentrated alkalis (40% KOH, 80 °C) [3].

Given the large number of papers exploring FIA miniaturization, its success has been proven [4]. However, for each particular method, FIA systems require a specific apparatus assembly, which limits their widespread acceptance for routine laboratory analyses. The washing times, flow rates, reactors, tubing lengths and diameters are all critical. Moreover, the sample is injected by carrier that transports it to the detector, the flow reaching the detector has concentration gradients, which exclude discrete measurements (i.e. there is a sample zone in the flow), and decreases the analytical signal. To circumvent these drawbacks, flow–batch analyzers (FBAs) are a good alternative and have now been miniaturized.

During the last ten years versatile and flexible flow-batch analyzers have been explored to automate various analytical processes including screening [5–7]; titration [8–13]; analyte addition [13–16]; internal standard solution addition [17]; and in line matching of pH [18], salinity [19] or acidity [20] between standard solutions and samples. Flow–batch has been used for prior assays [9]; for developing titration concentration gradients [10], and concentration gradients for nonlinear calibrations [21]. Also included are enzymatic [22], chemiluminescence [23], nephelometric [24], and UV–vis photometric [25] analyses of pharmaceutical formulations, as well as, in-line coulometric generation of standards and titrants [11,13,14]. Liquid–liquid extractions [26] in-line univariant [23,24,27] and multivariate [22,28] calibrations have also been accomplished. Flow–batch systems have begun to be mentioned in flow analysis textbooks as well [4,29].

Flow–batch analyzers are instantaneous stop-chamber flow systems, which integrate batch and flow analysis methods into a single approach, through the use of programmed multi-commutation. The main component of the manifold is the mixing chamber (MC) into which different solutions can optionally be defined, added,

* Corresponding author. Tel.: +55 833 216 7438; fax: +55 833 216 7437.
E-mail address: laqa@quimica.ufpb.br (M.C.U. Araújo).

0039-9140/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2011.08.063

or removed with complete control [30]. This hybridization retains the reliability of classical batch mode methods, within a fully computer-controlled (in flow) system. A flow–batch system can be viewed as a multi-purpose analytical accessory, easily attached to conventional equipment for instrumental analyses. The main advantage of the flow–batch analyzer is that classical (discrete) methods can be utilized (in-flow) with precision and speed, and the analytical methods can be chosen and developed on software. FBA's present precision and accuracy equal to FIA systems with comparably high sampling frequencies, yet with discrete throughput, less consumption, less reagent/sample manipulation, less chemical waste, and less chance of human error which results in a lower cost per analysis.

Since the introduction of "lab-on-a-chip" devices in the early 1990s [31], miniaturization of analytical assays has become the reality for analytical instrumentation [32–36]. Besides portability, simpler operation, and time savings, miniaturization of analytical instrumentation brings dramatic volume reductions for reagents, samples, and waste. Miniaturization itself has become as important to analytical chemistry as the microchip is to electronics.

The first studies proposing micro-fabrication were based on the existing techniques of the microelectronics industry, or using the silicon substrate. These were adapted to quartz and glass, and although glass is the preferred material for use in micro-fluidic systems, the cost of producing systems in glass has driven the search for other materials.

The use of polymeric materials brings many benefits for simplified manufacturing. Microfluidic systems based on polymeric materials produced by photolithography [37], hot molding [38], laser ablation [39] and direct printing with polyester–toner systems [40] are already in general use. Fernandes and Ferreira [3] developed an alternative micro-fabrication method using photosensitive urethane–acrylate oligomer resin. The resin polymerizes when exposed to ultraviolet radiation at 330 nm, and results in the formation of channels from 100 to 1000 μm deep. Two sheets of urethane–acrylate photo-resist were fabricated using deep ultraviolet lithography and then joined and sealed with UV, to form a unique micro-fluidic device.

In this study, a micro-fabrication technique based on deep ultraviolet lithography and photo-polymerizable urethane–acrylate photo-resist was used to develop a micro-flow–batch analyzer (μFBA). It included an integrated photometer (light emitting diode (LED)/(PT) phototransistor pair) used for detection, and an inserted nylon thread micro-mixing/homogenizing system. The proposed μFBA was used to determine Fe(II) in iron-based supplements (oral solutions) using the 1,10-phenanthroline method [41–43], and the results were compared to a manual UV–vis photometric procedure (as reference) with the same oral samples. Details of the micro-fabrication process are presented.

2. Experimental

2.1. Reagents, solutions and samples

All the reagents were of analytical grade unless otherwise stated, and all dilutions were carried out using double distilled, de-ionized water (18 MΩ cm^{-1}).

A stock solution of 100.0 mg L^{-1} Fe(II) was prepared by dissolving 0.702 g of $(Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O)$, 1 g of ascorbic acid and 20 mL of concentrated H_2SO_4 in a 1 L volumetric flask. The acetate buffer pH 5.5 was prepared from 0.10 mol L^{-1} of acetic acid and 0.10 mol L^{-1} of sodium acetate. The reagent solution (0.10% (w/v) of 1,10-phenanthroline) was prepared by dissolving 0.100 g of 1,10-phenanthroline in 1 mL of concentrated HCl and 50 mL of water and then diluted quantitatively to the mark in a 100 mL volumetric flask.

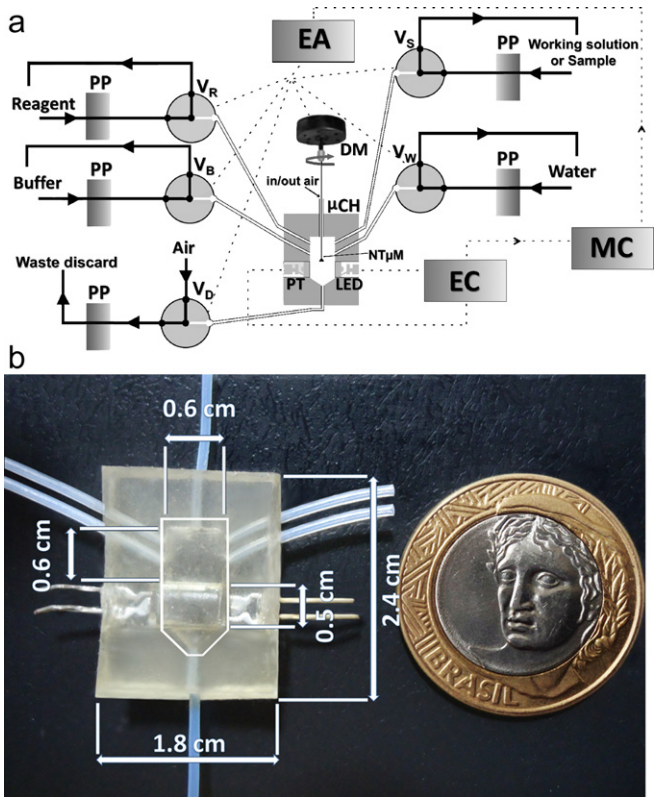

Fig. 1. (a) Schematic diagram of the μFBA and (b) photograph of the micro-chamber with its dimensions. Micro-chamber (μCH); peristaltic pump (PP); mini-solenoid valves for reagent (V_R), sodium acetate/acetic acid buffer pH 5.5 (V_B); standard working solution or samples (V_S); water (V_W) and waste discard (V_D); CD/DVD-ROM drive motor (DM); nylon thread micro-mixer (NTμM); electronic actuator (EA); microcomputer (MC); light emitting diode (LED); phototransistor (PT); electronic circuitry for the LED/PT photometric pair (EC).

urethane–acrylate photo-resist (Macdermid, trademark Flexlight M050) was acquired from Carimbos Medeiros Ltda, São Paulo – Brazil.

Before analysis with the proposed μFBA and the reference method, each iron-based supplement (oral solution) sample was diluted 1:2500 with 2% (v/v) of H_2SO_4.

2.2. Apparatus

For construction of the μFBA, a commercial photo-exposure machine (Fotolight-MD2-A4, Carimbos Medeiros Ltda, Brazil), with two sets of mercury lamps (F15 W T12 Sylvania black light) were used to expose the urethane–acrylate photo-resist to UV radiation. Overhead transparencies used as photo-masks were printed with a laser printer (HP LaserJet P2014). The micro-device was designed using CorelDRAW® X3. The revelation of the polymeric substrate after UV exposition was done by removing the non-exposed resin with an ultrasonic bath (model Ultracleaner 800A, Unique, Brazil).

A model 8453 Hewlett-Packard (HP) diode array UV–vis spectrophotometer, equipped with cuvette (with an inner volume of about 4 mL and an optical path of 1.0 cm) was used for the absorbance measurements employing the reference method.

2.3. The μFBA

Fig. 1a presents the schematic diagram of the μFBA. To propel the solutions, an 8-channel peristaltic pump (Ismatec®, Switzerland) operating at 10 rpm was used. Minisolenoid valves (model LHDA 0531415H, Lee Company, USA) were employed to

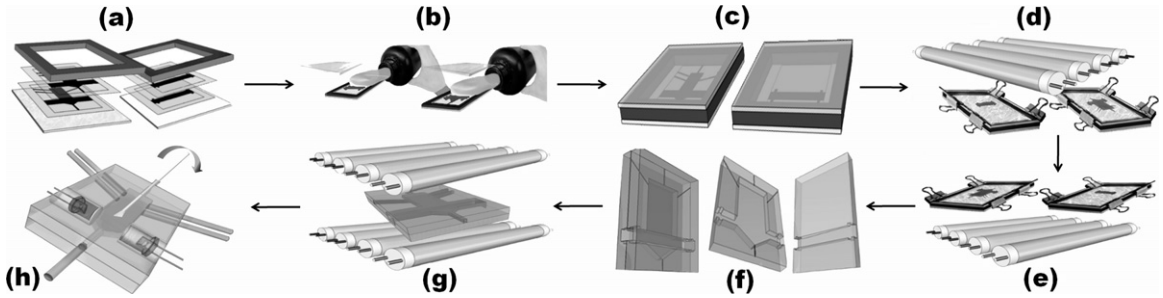

Fig. 2. Manufacturing process diagram of the μFBA.

control flow directions and volumes. Teflon® tubes with 0.5 mm internal diameters were used to transport fluids.

A nylon 0.4 mm fishing line was used to ensure adequate mixing of the solutions inside the micro-chamber. The nylon thread was adapted to a CD/DVD-ROM drive motor (model MDN3GT3CPAC, 2000 rpm, 5 V d.c.), which controlled the mixing. Of course, the nylon thread entrance into the micro-chamber is left unsealed. So that, when solutions are introduced or removed from the micro-chamber, the inner air escapes by this air passage (in/out).

A simple laboratory-made photometer [44] using a 5.0 mm diameter LED (light emitting diode) as light source, and a 5.0 mm diameter phototransistor as detector was built in to the μFBA and employed for absorbance measurements. Its performance was evaluated for photometric determinations of Fe(II) in iron-based supplements (oral solutions) with 1,10-phenanthroline (Phen) as a complexing agent [41–43]. Due to the broad absorption spectrum of the Fe(II)–Phen complex (about 510 nm), a green LED with maximum emission at 522 nm was used as the light source.

All related μFBA tasks, such as data acquisition, valve, and drive motor activation, were performed through a USB interface (USB6009, National Instruments®, USA), which activates a lab made controller module. The controlling software was developed in LabVIEW® 5.1 (National Instruments®, USA).

Fig. 1b presents an image of the μFBA ready for use, which is inserted in a closed plastic black box with dimensions 8.5 cm × 10.0 cm × 4.0 cm, to prevent effects of spurious radiation when the μFBA is in operation.

2.4. The manufacturing process of the μFBA

The manufacturing process of the μFBA was based on that described by Fernandes and Ferreira [3]. As shown in Fig. 2, identical photolithographic masks were printed on overhead transparencies using a laser printer (HP LaserJet P2014), and afterwards were superimposed and visually aligned employing a stereomicroscope (Carl Zeiss, model Stemi 2000C). They were then placed between a 2 mm acrylic plate, and a 3.4 mm rubber frame to form a mold (Fig. 2a), that defines the thickness of one layer of the micro-chamber. The urethane–acrylate photo-resist was then spread (Fig. 2b) into the mold, which was closed using another acrylic plate as presented in Fig. 2c. The mold was subjected to exposition under UV radiation for 50 s using the upper lamps of the photo-exposure unit (Fig. 2d), and then for 100 s using the lower lamps (Fig. 2e). After exposure, the unexposed resin was removed in an ultrasonic bath with a 2% (v/v) detergent solution for 10 min, and dried under a nitrogen flow.

Two different layers were prepared so that, when joined, they would form the channels, micro-chamber, and cavities to support the LED and the PT (Fig. 2f). Then, both layers were joined and sealed by exposure to UV radiation in a nitrogen atmosphere for 15 min (Fig. 2g). The process causes irreversible plate adhesion. Finally, a thin layer of liquid resin sealer was applied to the exterior surfaces of the Teflon® tubes, LED and PT, adapted to μFBA and exposed to UV for 15 min (Fig. 2h). The nylon thread micro-mixer (fishing line) is inserted after the resin sealer is applied. The optical path of the μFBA photometer is about 0.6 cm.

2.5. Analytical procedure for the μFBA

Before starting the procedure, the working solutions of each channel are pumped and recycled towards their flasks (Fig. 1a). Afterwards, valves V_W V_S, V_R and V_B are simultaneously switched ON for a time interval of 3.00 s, and the working solutions are pumped towards the micro-chamber (μCH) to fill the channels between the valves and the μCH. Then, immediately, valve V_D is switched ON for 5.00 s, and the excess solution in the μCH is discarded by peristaltic pump (PP) aspiration. This channel filling and μCH evacuation process should be performed whenever changing fluids or reservoirs to avoid superfluous air in the channels.

The μFBA analytical procedure for Fe(II) determination in iron-based supplements (oral solutions) using the 1,10-phenanthroline method is briefly described using the μFBA diagrams and control timelines presented in Fig. 3. This procedure carries out in-line preparations in the micro-chamber (μCH) of the blank (Fig. 3a), the calibration solutions (Fig. 3b), and the sample (Fig. 3c). The time intervals employed for each in-line preparation are for the driver motor (DM), valve switchings (V_W, V_S, V_R, V_B and V_D), and absorbance measurements (A). Each preparation Fig. 3a–c includes the required programmed mixing/homogenization of the μCH contents, absorbance measurements, emptying, and cleaning of the chamber. In Fig. 3b, t_W and t_S represent water and working solution addition times. These times vary in proportion to the standard solution concentration being prepared. Homogenization is carried out by switching ON the drive motor, and after absorbance measurements, the μCH is emptied by switching ON valve V_D, It is then cleaned by simultaneously switching ON valve V_W and DM, to add water to the μCH and agitate. Soon afterwards, valve V_D is switched ON to discard the contents of the μCH. This cleaning/evacuation procedure should be performed at least three times in order to guarantee efficient cleaning of the μCH. All preparations are done using a peristaltic pump flow-rate of $32.0 \pm 0.2\ \mu L\ s^{-1}$ ($n = 10$) for all channels.

For in-line blank preparation, valves V_W, V_R and V_B are simultaneously switched ON at the times indicated. Water, reagent and buffer are brought into the μCH, mixed and the blank absorbance is measured.

The in-line preparations of calibration solutions with 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 mg L^{-1} of Fe^{2+} were completed using a standard working solution of 20.0 mg L^{-1} Fe(II), which itself was prepared by adequate dilution (1:5) of a standard stock solution 100.0 mg L^{-1} Fe(II). In these preparations, valves V_W, V_S, V_R and V_B are simultaneously switched ON at the indicated times. Water, standard working solution, reagent and buffer are sent into the

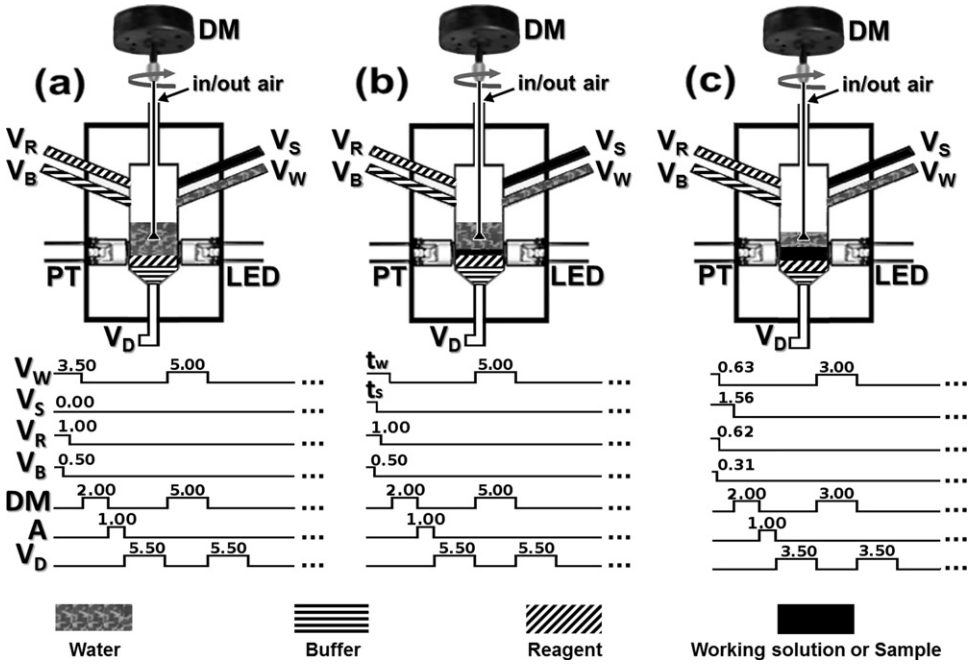

Fig. 3. The μFBA diagrams and control timelines for in-line preparations in the micro-chamber of (a) blank, (b) calibration solutions with 1.0–10.0 mg L^{-1} Fe^{2+}, and (c) sample. In (b), t_W and t_S represent water and working solution addition times. These times vary in proportion to the standard solution concentration being prepared. The time intervals (in seconds) are for the driver motor (DM), switching valves (V_W, V_S, V_R, V_B and V_D), and absorbance measurements (*A*).

μCH, homogenized, and the absorbencies of the calibration solutions are measured.

For in-line preparation of the samples, previously diluted (1:2500) iron-based supplement (oral solutions) samples were used. The procedure is similar to the in-line preparation of calibration solutions. The difference is that the samples are used instead of the standard working solution.

2.6. Analytycal procedure of the reference method

For comparison, the proposed μFBA performance was evaluated against a manual reference UV–vis spectrophotometric method analyzing the same oral supplement samples. In the manual procedure calibration solutions with 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 mg L^{-1} Fe^{2+} were prepared by taking 20 mL of reagent (1,10-phenanthroline), 10 mL of buffer (acetate buffer pH 5.5) and 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 mL of 100.0 mg L^{-1} Fe(II) standard stock solution and completed with water to the mark of a 100 mL volumetric flask. Afterwards, 50 mL of each previously diluted (1:2500) iron-based supplement (oral solution) sample, 20 mL of reagent and 10 mL of buffer were transferred to a 100 mL volumetric flask and completed with water to the mark. Finally, about 3 mL of each calibration solution and each of the sample Fe–Phen complexes produced were transferred to the cuvette and their absorbencies measured at 510 nm.

3. Results and discussion

3.1. Characterization of the μFBA

Deep UV-lithography is easily reproducible and utilizes adhesive sealing in which two layers, partially cured by exposure to UV, are put in contact and polymerized. Due to the risk of clogging, the technique requires caution and optimization of the exposure times [2]. The resin was first submitted to an infrared analysis to verify the minimum time required for polymerization of a 3.0 mm thick layer with constant UV exposure. The absorbance at 8840 cm^{-1} was measured from 20 s to 600 s of exposure time. The chosen wavelength is dependent on the vibrations of acrylate groups CH and CH_2 at the second overtone. The absorbance decreases as the polymer is produced, and acrylate groups located at the end of the oligomer chain are consumed [45]. After about 400 s absorbance reaches a minimum value, which does not change significantly during the exposure. A minimum time of 15 min is stipulated to ensure effective sealing of the layers. After curing the photo-resist, a colorless and transparent material is obtained that does not absorb radiation in the visible range, allowing the use of optical detectors [3].

Fonseca et al. [1] demonstrated the necessity of using photo-masks with lines wider than 200 μm to produce channels, which will not clog during the sealing process (due to irregularities in toner deposition). They also observed that the depth of the channels depends on the relative position of the photo-mask (a parallel exposure to the lamps leads to deeper channels) and the channel width is always narrower than the photo-mask line, which was attributed to the light scattering and the use of a non-collimated radiation beam.

Considering the above, we proposed the use of two superimposed photo-masks to obtain the deeper and wider channels required for the micro-chamber of the μFBA (Fig. 4). This figure shows a simplified illustration of the effect of two photo-masks overlapping in relation to seal perfection (unfilled portion of an ideal printed line). Fonseca et al. [1] demonstrated that a toner printed line has imperfections in the printed limit of this line, which can be considered as random (noise). Consequently, one can assume that with two superimposed transparencies the definition of the borders of the channels and the micro-chamber is improved (Fig. 4a). This overlapping acts on the non-collimated beam (Fig. 4b) by minimizing radiation with a smaller angle of incidence (Θ1) on the resin at the expense of radiation with a higher angle of incidence (Θ2). This phenomenon combined with parallel positioning of the design to the lamp overlapping allows the formation of deeper channels with better defined edges. By eliminating the use of printed photo-masks and phototypesetter technology the construction process becomes simpler and cheaper.

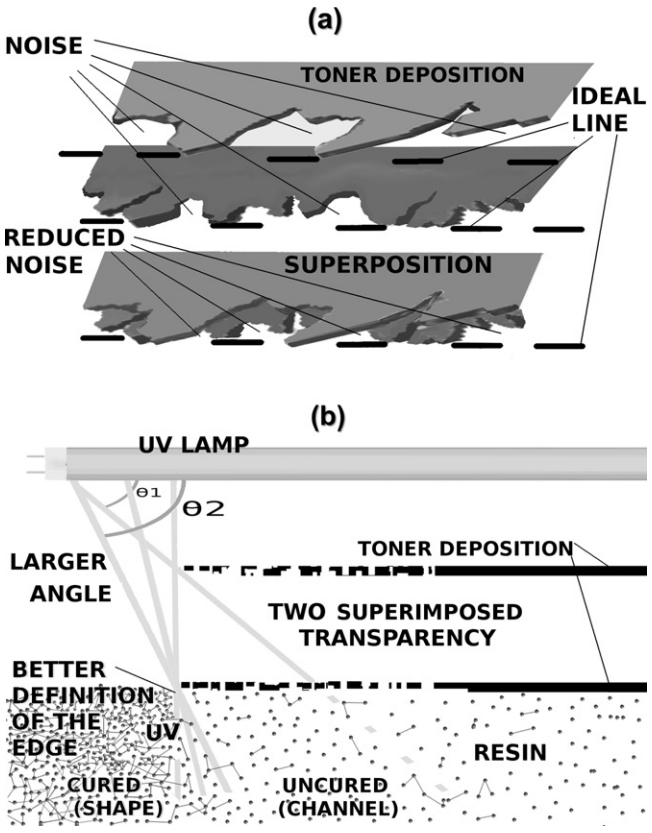

Fig. 4. (a) Simplified illustration of the effect of two photo-masks overlapping in relation to seal perfection (unfilled portion of an ideal printed line), with two superimposed transparencies the definition of the borders of the channels and the micro-chamber is improved. (b) Simplified illustration of the effect of two superimposed photo-masks on the non-collimated UV beam with a smaller (Θ1) and larger (Θ2) angle of incidence.

The micro-chamber depth is twice the supporting layer depth of the LED and the PT. Fitting of the photometric components depends on the elastomeric properties of the material. However, both the LED and the PT had to be cut and polished to provide the correct optical path, and to prevent any dead space between the components and the micro-chamber wall.

In the macroscopic FBA, the dilution/mixing/reaction chamber contains a magnetic stirring bar to allow all the chemicals to be thoroughly mixed similarly to classical batch analysis methods. Mixing and dilution of reagents, samples, and other solutions can be easily controlled through software. For the μFBA, reduced dimensions made it impossible to use a magnet bar inside the micro-chamber. To overcome this difficulty, a stirring rod with a flat end (shovel-shaped) was formed from a nylon thread. The complete mixing of solutions within the micro-chamber was obtained in under 2 s, due to the high speed of the CD/DVD-ROM drive motor. Fig. 2h shows, in perspective, the three-dimensional model developed accommodating the stirring rod, tubing, LED, and PT.

3.2. The μFBA evaluation

The equations for the analytical curves obtained using the μFBA and the reference method are respectively: $A = 0.018C_{Fe(II)} + 0.023$ ($r^2 = 0.998$) and $A = 0.14C_{Fe(II)} + 0.14$ ($r^2 = 0.9993$). Both curves are linear in the 1.0–10.0 mg L^{-1} Fe(II) range. The analytical curve of the μFBA presents less slope or sensitivity, because the absorbance measurements are carried out using a LED/phototransistor pair, which has a shorter optical path (about 0.6 cm) and uses a green LED with a maximum emission of 522 nm as its light source. In the reference method, absorbance measurements were performed using 510 nm, and a cuvette with an optical path of 1.0 cm. The spectrum of the Fe(II)–Phen complex has a maximum absorption at 510 nm. We believe that using a 510 nm LED, and increasing the optical path to 1.0 cm will give similar results to our reference method by augmenting sensitivity.

Table 1
Results for Fe(II) determinations (mg L^{-1}) in iron-based supplements (oral solutions) by using the μFBA and the manual UV–vis spectrophotometric method.

Samples	μFBA	Manual
1	3.9	3.9
2	4.4	4.3
3	4.1	4.1
4	4.3	4.3
5	4.1	4.1
R.S.D. ($n = 3$)	1.1%	0.2%

Table 1 shows the results obtained in the determinations of Fe(II) for the iron-based supplements (oral solutions) using the μFBA, and the reference method. No statistically significant differences at a confidence level of 95% were observed between the results when applying the paired *t*-test.

The μFBA presented a high analytical frequency of 120 samples h^{-1}, low relative standard deviations (about 1.1%), and reduced reagent consumption (30 times less than the reference method).

4. Conclusions

This study proposed the fabrication of a micro-flow–batch analyzer, which was accomplished by employing deep UV-lithography with photo-polymerizable urethane–acrylate photo-resist. The micro-fabrication technique proved to be simple and reproducible. The use of two superimposed overhead transparencies enables deep micro-channel and micro-chamber creation and avoids clogging. The system was accurate when applied to the determination of Fe(II) in iron-based supplements. With few manufacturing steps, it was possible to both project and build a low-cost microprobe, displaying high frequency discrete sampling throughput comparable with normal flow-batch systems [5–28].

Acknowledgements

The authors would like to thank Dr. Alexandre Fonseca and Dr. I. M. Raimundo Jr. for their important suggestions, and the Brazilian agencies CNPq (for research fellowships) and CAPES (for scholarships).

References

[1] A. Fonseca, I.M. Raimundo Jr., J.J.R. Rohwedder, L.O.S. Ferreira, Anal. Chim. Acta 603 (2007) 159–166.
[2] A. Fonseca, I.M. Raimundo Jr., J.J.R. Rohwedder, R.S. Lima, M.C.U. Araújo, Anal. Bioanal. Chem. 396 (2009) 715–723.
[3] J.C.B. Fernandes, L.O.S. Ferreira, J. Braz. Chem. Soc. 17 (2006) 643–647.
[4] F.A. Iñón, M.B. Tudino, in: M. Trojanowicz (Ed.), Advances in Flow Analysis, Wiley-VCH, Weinheim, 2008, pp. 3–42.
[5] R.A.C. Lima, S.R.B. Santos, R.S. Costa, G.P.S. Marcone, R.S. Honorato, V.B. Nascimento, M.C.U. Araújo, Anal. Chim. Acta 518 (2004) 25–30.
[6] J.A. Nascimento, A.G.G. Dionísio, E.C.L. Nascimento, S.K.B. Freitas, M.C.U. Araújo, Quim Nova 33 (2010) 351–357.
[7] E.C.L. Nascimento, M.C.U. Araújo, R.K.H. Galvão, J. Braz. Chem. Soc. 22 (2011) 1061–1067.
[8] R.S. Honorato, M.C.U. Araújo, R.A.C. Lima, E.A.G. Zagatto, R.A.S. Lapa, J.L.F.C. Lima, Anal. Chim. Acta 396 (1999) 91–97.
[9] R.S. Honorato, E.A.G. Zagatto, R.A.C. Lima, M.C.U. Araújo, Anal. Chim. Acta 416 (2000) 231–237.
[10] E.P. Medeiros, E.C.L. Nascimento, A.C.D. Medeiros, J.G. Veras Neto, E.C. Silva, M.C.U. Araújo, Anal. Chim. Acta 511 (2004) 113–118.
[11] C. Pasquini, E.V. Aquino, M.V. Rebouças, F.B. Gonzaga, Anal. Chim. Acta 600 (2007) 84–89.

[12] M.B. Silva, C.C. Crispino, B.F. Reis, J. Braz. Chem. Soc. 21 (2010) 1854–1860.
[13] S.C.B. Oliveira, E.C.S. Coelho, T.M.G. Selva, F.P. Santos, M.C.U. Araújo, F.C. Abreu, V.B. Nascimento, Microchem. J. 82 (2006) 220–225.
[14] V.B. Nascimento, T.M.G. Selva, E.C.S. Coelho, F.P. Santos, J.L.S. Antônio, J.R. Silva, E.N. Gaião, M.C.U. Araújo, Talanta 81 (2010) 609–613.
[15] L.F. Almeida, V.L. Martins, E.C. Silva, P.N.T. Moreira, M.C.U. Araújo, Anal. Chim. Acta 486 (2003) 143–148.
[16] L.F. Almeida, V.L. Martins, E.C. Silva, P.N.T. Moreira, M.C.U. Araújo, J. Braz. Chem. Soc. 14 (2003) 249–253.
[17] J.E. da Silva, F.A. da Silva, M.F. Pimentel, R.S. Honorato, V.L. da Silva, M.C.B.S.M. Montenegro, A.N. Araújo, Talanta 70 (2006) 522–526.
[18] R.S. Honorato, J.M.T. Carneiro, E.A.G. Zagatto, Anal. Chim. Acta 441 (2001) 309–315.
[19] J.M.T. Carneiro, A.C.B. Dias, E.A.G. Zagatto, R.S. Honorato, Anal. Chim. Acta 455 (2001) 327–333.
[20] J.M.T. Carneiro, R.S. Honorato, E.A.G. Zagatto, Fresenius J. Anal. Chem. 368 (2000) 496–500.
[21] M.C. Souza, V.L. Martins, L.F. Almeida, O.D. Pessoa Neto, E.N. Gaião, M.C.U. Araujo, Talanta 82 (2010) 1027–1032.
[22] M. Grünhut, M.E. Centurión, W.D. Fragoso, L.F. Almeida, M.C.U. Araújo, B.S.F. Band, Talanta 75 (2008) 950–958.
[23] M. Grünhut, V.L. Martins, M.E. Centurión, M.C.U. Araújo, B.S.F. Band, Anal. Lett. 44 (2010) 67–81.
[24] C.C. Acebal, M. Insausti, M.F. Pistonesi, A.G. Lista, B.S.F. Band, Talanta 81 (2010) 116–119.
[25] S.K.B. Freitas, V.L. Silva, A.N. Araújo, M.C.B.S.M. Montenegro, B.F. Reis, A.P.S. Paim, J. Braz. Chem. Soc. 22 (2011) 279–285.
[26] J.M. Silva, E.V. Anjos, R.S. Honorato, M.F. Pimentel, A.P.S. Paim, Anal. Chim. Acta 629 (2008) 98–103.
[27] L.F. Almeida, M.G.R. Vale, M.B. Dessuy, M.M. Silva, R.S. Lima, V.B. Santos, P.H.D. Diniz, M.C.U. Araújo, Talanta 73 (2007) 906–912.
[28] V. Visani, S.R.R.C. Barros, H.A. Dantas Filho, L.F. Almeida, R.A.C. Lima, W.D. Fragoso, T.C.B. Saldanha, M.C.U. Araújo, Ecl. Quím. 34 (2009) 37–47.
[29] C. Pasquini, M.V. Rebouças, in: S.D. Kolev, I.D. Mckelvie (Eds.), Comprehensive Analytical Chemistry Advances in Flow Injection Analysis and Related Techniques, Elsevier, Amsterdam, 2008, pp. 617–638.
[30] E.A.G. Zagatto, J.M.T. Carneiro, S. Vicente, P.R. Fortes, J.L.M. Santos, J.L.F.C. Lima, J. Anal. Chem. 64 (2009) 524–532.
[31] A. Manz, D.J. Harrison, E.M.J. Verpoorte, J.C. Fettinger, A. Paulus, H. Ludi, H.M. Widmer, J. Chromatogr. 593 (1992) 253–258.
[32] H. Becker, C. Gärtner, Electrophoresis 21 (2000) 12–26.
[33] M.A. Schwarz, P.C. Hauser, Lab Chip 1 (2001) 1–6.
[34] Q. Xue, F. Foret, Y.M. Dunayevskiy, P.M. Zavracky, N.E. McGruer, B.L. Karger, Anal. Chem. 69 (1997) 426–430.
[35] M.H. Sorouraddin, M. Amjadi, M. Safi-Shalamzari, Anal. Chim. Acta 589 (2007) 84–88.
[36] S.I. Ohira, K. Toda, Anal. Chim. Acta 619 (2008) 143–156.
[37] M.J. Madou, Fundamentals of Microfabrication, second ed., CRC Press, New York, 2002.
[38] L. Martynova, L.E. Locascio, M. Gaitan, G.W. Kramer, R.G. Christensen, W.A. Maccreham, Anal. Chem. 69 (1997) 4783–4789.
[39] M.A. Roberts, J.S. Rossier, P. Bercier, H. Girault, Anal. Chem. 69 (1997) 2035–2042.
[40] A.M. Tan, K. Rodgers, J.P. Murrihy, C. O'mathuna, J.D. Glennon, Lab Chip 1 (2001) 7–9.
[41] D.A. Skoog, D.M. West, F.J. Holler, Fundamentals of Analytical Chemistry, sixth ed., Saunders College Publishing, New York, 1992.
[42] Method 3500-Fe B, in: Standard Methods for the Examination of Water and Wastewater, twentieth ed., American Public Health Association, Baltimore, 1998, pp. 3–76.
[43] ASTM E394-00, in: Standard Test Method for Iron in Trace Quantities Using the 1,10-phenanthroline method, 2000.
[44] E.N. Gaião, R.S. Honorato, S.R.B. Santos, M.C.U. Araújo, Analyst 124 (1999) 1727–1730.
[45] S.C. Araujo, Y. Kawano, Polím Cienc. Tecnol. 4 (2001) 213–221.