Talanta 88 (2012) 717–723

Contents lists available at SciVerse ScienceDirect

Talanta

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

Turbidimetric and photometric determination of total tannins in tea using a micro-flow-batch analyzer

Marcelo B. Lima[a], Stéfani I.E. Andrade[a], David P. Harding[a], Marcelo F. Pistonesi[b], Beatriz S.F. Band[b], Mário C.U. Araújo[a,*]

[a] Universidade Federal da Paraíba, Departamento de Química, João Pessoa, PB, Brazil
[b] Universidad Nacional del Sur, Departamento de Química, Lab. FIA, Bahia Blanca, Argentina

ARTICLE INFO

Article history:
Received 1 November 2011
Received in revised form
24 November 2011
Accepted 25 November 2011
Available online 2 December 2011

Keywords:
Flow-batch system
Microfabrication
Urethane-acrylate
Tannins
Green and black tea
Photometric determination
Turbidimetric determination

ABSTRACT

Both turbidimetric and photometric determinations of total tannins in samples of green and black tea, using a micro-flow-batch analyzer (μFBA) were studied. The miniaturized system was formed using photocurable urethane-acrylate resin and ultraviolet lithography technique. The turbidimetric method was based on the precipitation reaction of Cu (II) with tannins in acetate medium at a pH of 4.5. The photometric method was based on the complexation reaction of tannins with ferrous tartrate. The turbidimetric μFBA was able to test 200 samples per hour. The photometric μFBA allowed 300 analyses per hour, generating 136 μL of residue per analysis. The paired *t* test, at a 95% confidence level, showed no statistically significant differences between results obtained by both methods and the reference method. The urethane-acrylate μFBA maintained satisfactory physical and chemical properties, and represents an improvement over conventional flow-batch analyzer.

1. Introduction

Tannins are a polyphenol group with high molecular weights. They are astringent and have the natural ability to complex with various macromolecules such as proteins, starch, cellulose, as well as minerals [1]. Tannins in general have antioxidant, anticarcinogenic, antimutagenic and antimicrobial activities and can assist in the prevention and treatment of diseases [2,3]. Tannins are found in common foods and drinks, but especially in green and black tea, which are consumed worldwide [4,5]. Green tea is derived from the infusion of twigs and dried leaves of *Camellia sinensis* [6], while black tea is obtained by oxidation of the same plant [7]. Quality control is of course important for the world's tea consumers, but the quantification of tannins has always been of particular interest to the industry, as well as governmental regulators [8,9].

Determination of tannins in tea samples is carried out regularly in food laboratories around the world [10]. The Folin–Ciocalteau spectrophotometric method is currently the official method for the determination of tannins in samples from plant tissues by the AOAC (Association of Official Analytical Chemists) [11]. However, this method is a relatively slow process, both in reagent preparation, and in product formation with tannins, it is also susceptible to interference from any reductive species present in the sample [10,12].

The official method in Japan (Official Chemical Analysis of Tea) uses the ferrous tartrate method as the reference method for determination of tannins in tea samples [13]. This method is based on the formation, in a buffered medium (pH 6.8) of a complex between ferrous tartrate and tannins in the tea, which absorbs light at around 560 nm. The ferrous tartrate method is more selective than the Folin–Ciocalteau method, and is unaffected by the coexistence of reducing agents, such as ascorbic acid [14]. It is being used by researchers for the determination of tannins in tea [15–18].

Another alternative for the determination of tannins in tea is the turbidimetric method [10]. This method consists in the precipitation reaction of Cu (II) with tannins in acetate medium at a pH of 4.5 monitored at around 470 nm [19]. This precipitation is due to the presence of functional groups in tannins, such as ortho-dihydroxyphenyl and carboxyl groups, that act as mono- or bidentate ligands [20].

Methods automation for analysis of tannins allows: faster controlled analysis, greater precision, less analyst interference, and a

* Corresponding author at: Department of Chemistry, CCEN, Federal University of Paraiba, Caixa Postal 5093, CEP 58051-970, Joao Pessoa, Brazil.
Tel.: +55 83 3216 7438; fax: +55 83 3216 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.2011.11.076

reduction of expenditures on samples and reagents. Several studies have been reported in the literature using automated flow systems for analysis of tannins in tea samples using both the turbidimetric [10,19] and ferrous tartrate [14–18] methods. But it would be interesting to have an automatic system capable of allowing the analyst to choose, depending on the reagents available in the laboratory, determine tannins using the same manifold and selecting one of these methods via software. This can be achieved by developing an automatized flow-batch analyzer.

The flow-batch analyzers are automated systems that use an instantaneous stop chamber and integrate batch and flow methods by using programmed multi-commutation [21]. The main component is the mixing chamber where the whole analytical process including; fluids addition, sample pretreatment, homogenization, precipitation, extraction, preparation of calibration solutions, and detection, takes place under the total control of the software [22–24]. The sample is processed seamlessly with less manipulation, less consumption of reagents and samples, less chemical waste, and less chance for human error. Classical methods (discrete) can be performed with precision, accuracy and speed similar to other flow analysis methods [25].

The flow-batch systems flexible and versatile have been developed for different applications such as: titration [26], screening analysis [27], standard addition [28] chemiluminescence [21], nephelometric [29] and liquid–liquid extractions [30], for example.

Recently, the flow-batch analyzer was miniaturized (μFBA) and applied analysis of Fe (II) ions in medicinal samples [31]. The microfabrication used deep ultraviolet lithography and photopolymerizable urethane-acrylate photoresist. It contained an integrated photometer (LED)/phototransistor for detection, and a nylon line homogenizing system. The same fabrication technique was applied to develop a new μFBA for total tannin determinations in green and black tea samples using the photometric (ferrous tartrate) and turbidimetric (copper (II) in acetate medium) methods.

2. Experimental

2.1. Reagents and standards

All reagents were of analytical grade and freshly distilled and deionized water (18 MΩ cm^{-1}) was used to prepare all solutions. A stock solution of 1000 mg L^{-1} of tannic acid was prepared with 0.1000 g of tannic acid (Labsynth) diluted to 100.0 mL with water.

For the photometric analysis a ferrous tartrate reagent solution was obtained by mixing 1.0000 g of heptahydrated ferrous sulfate (Vetec) with 2.0000 g of potassium sodium tartrate (Vetec) and 0.1000 g of sodium bisulfite (Reagen) diluted in water to 100.0 mL. The phosphate buffer pH 6.8 was prepared by mixing heptahydrated sodium phosphate 0.10 mol L^{-1} (Reagen) with 0.10 mol L^{-1} sodium hydroxide (Vetec).

For the turbidimetric analysis we used a solution of copper (II) at 0.1 mol L^{-1}, this solution was prepared by dissolving 0.6354 g of metallic copper (Merck) in nitric acid 50% (v/v) and diluted to 100.0 mL with a solution of 1% nitric acid, a solution of ammonium acetate 1.5 mol L^{-1} (Vetec), and a nitric acid solution 10% (v/v).

The commercial urethane-acrylate photoresist used for fabrication of the μFBA was acquired by Carimbos Medeiros Ltda, Brazil (MacDermid, flex-light trademark M050).

2.2. Preparation of samples

Samples were obtained from six emulsions of green and black tea purchased from different manufacturers and lots. These tea samples were purchased in local shops in Bahía Blanca, Argentina. For sample preparation, an amount of 0.5000 g of tea was heated for 10 min at 90 °C in about 50 mL of deionized water, the mixture was filtered, washing the residues and completing to 100.0 mL with deionized water.

2.3. Apparatus

To fabricate the micro-chamber (μCH) in urethane-acrylate resin, we used a commercial UV light source (Fotolight-MD2-A4, Carimbos Medeiros Ltda, Brazil), with two sets of mercury lamps (BLB-15W-T8, SCT black light).

For layout design of the μCH, the CorelDraw® X5 program was used. The layout printing was on polyester transparency films for laser printing using an HP LaserJet P2014. After UV exposure, channels on the substrate were revealed by removal of the non-exposed resin with an ultrasonic bath (model UltraCleaner800, Unique, Brazil).

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

2.4. Assembly of the μFBA system

A peristaltic pump (model 78001-12, 8 channel, Ismatec) for fluids propulsion was used which operated at 10 rpm. Minisolenoid valves (model LHDA 0531415H, Lee Company) were used for controlled fluid additions to the micro-mixing chamber. A 0.4 mm nylon wire was used for agitation within the micro-mixing chamber to ensure mix of added products. The nylon was adapted to a CD/DVD-ROM motor drive (model MDN3GT3CPAC, 2000 rpm, 5 Vdc). Teflon® tubes with 0.5 mm internal diameter were used for fluids transport.

The detection system integrated into the micro-mixing chamber was composed of an LED (light emitting diode) as source radiation, and a phototransistor for detection [32]. For the photometric detection we used a green LED with a maximum emission of 560 nm. For the turbidimetric detection we used a blue LED with a maximum emission of 470 nm. These devices, both LED and phototransistor having 5 mm of diameter, were mounted firmly outside the mixing chamber against the glass tube openings which have an internal diameter of 2 mm. The 2 mm diameter was chosen for the glass tubes to minimize the resulting chamber volume.

All tasks, such as data acquisition, valve, and drive motor activation, were done using a USB interface (USB6009, National Instruments®), which activated a lab made controller module. The software was developed in LabVIEW® 7.1 (National Instruments®).

2.5. Lithographic micro-chamber fabrication process

As outlined in Fig. 1, the μFBA was fabricated based on the methodology described by Monte-Filho et al. [31].

Initially the required layouts or templates were developed and printed. Each one was set between a 2.0 mm acrylic plate, and a 3.4 mm rubber frame forming the mold, as shown in Fig. 1a. The urethane-acrylate resin was then deposited on the mold and immobilized by another acrylic plate, as sketched in Fig. 1b and c, respectively. The thickness of the rubber framing (in this case 3.4 mm), allows you to define the volume of the system.

The layouts were engraved in the resin by exposure to UV radiation in two steps. First the top of both layers were cured (polymerized) when exposed for 150 s, as shown in Fig. 1d. Next, the bottom was cured by exposure for 350 s, as illustrated in Fig. 1e. The uncured resin layers were removed with the aid of an ultrasonic bath in detergent solution 10% (v/v) for 15 min. These layers were then dried in a nitrogen flow.

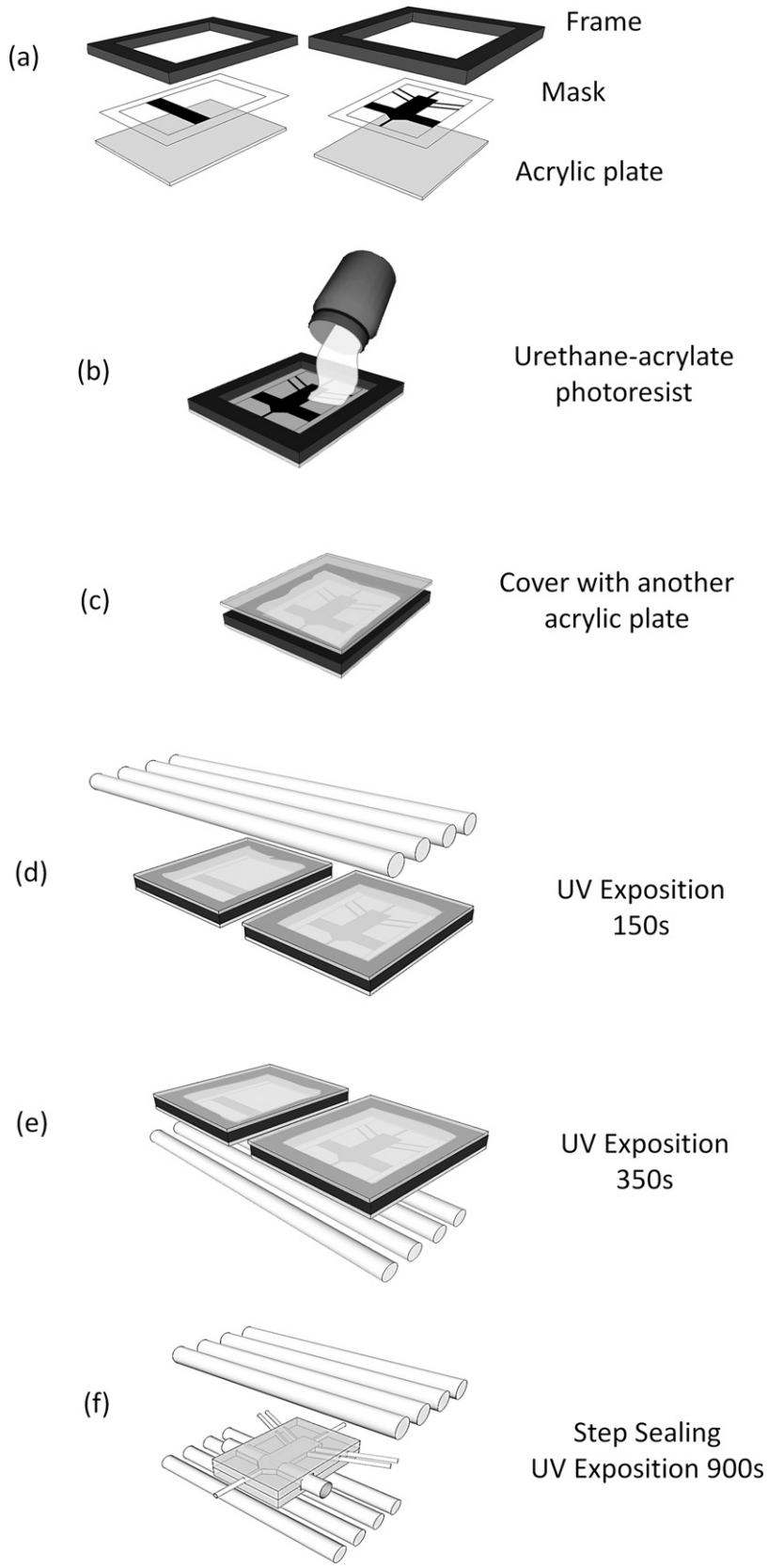

Fig. 1. Schematic diagram illustrating the procedure for construction of the μFBA. (a) Mold mounting with the required layout, (b) depositing the urethane acrylic resin over the mold, (c) immobilizing the resin with the second acrylic plate, (d) radiation exposure UV for 150 s, (e) radiation exposure UV for 350 s, (f) layer sealing with inserted teflon and glass tubes, radiation exposure UV for 900 s.

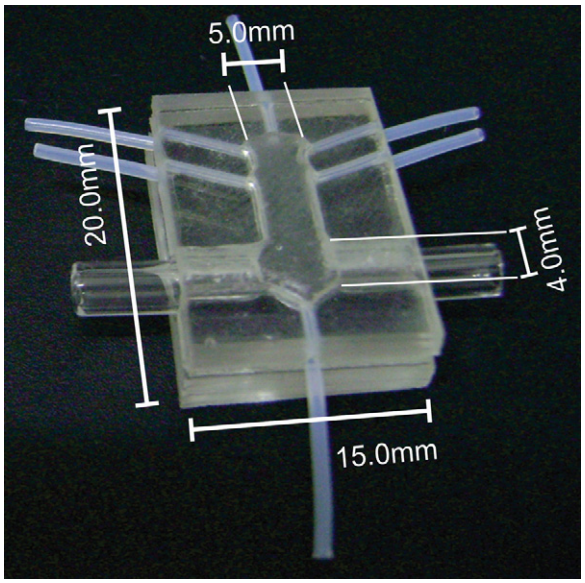

Fig. 2. Photograph of the microfabricated μFBA with its dimensions (mm).

In the sealing step, the layers were superimposed appropriately to form the required channels. Tubes of Teflon® and glass were carefully inserted into their respective channels. The system was again exposed to UV radiation in a single step of 900 s on both sides, as shown in Fig. 1f. This exposure time allows for an efficient and irreversible seal.

Teflon® tubes (0.8 mm diameter) fixed in the channels allow μCH communication with the external environment, making possible the insertions of fluids as well as the agitator shaft. The glass tubes used have one end inside the closed μCH, an external diameter of 4.0 mm and a 2.0 mm internal diameter. These tubes allow flexibly, the integrated detection in the μCH.

Fig. 2 shows a photo of the microsystem and its dimensions, as manufactured by the procedure described above. The detail of the attachment and sealing of the tubes can be seen in this photography. This microsystem is mounted onto a suitable support in a black (darkroom) box (10.0 cm × 8.0 cm × 4.0 cm), to preserve the system from the effects of spurious environmental radiation while in operation.

2.6. Analytical procedure

Before starting the analytical procedure, working solutions for each channel are pumped and re-circulated to their respective reservoirs (Fig. 3a). Then the mini-valves V_S, V_{R1}, V_{R2} and V_C are opened for 3.0 s and the working solutions (S, R1, R2, and C) are pumped to the micro-chamber to fill the channels between the valves and the chamber. Then immediately, the discard valve V_W is opened for 5.0 s and then any solution inside the μCH is emptied using the peristaltic pump for aspiration. This channels filling procedure is very important and must be carried out whenever there is a change of the reservoir liquids.

Fig. 3a rpresent the schematic diagram of the μFBA used for determination of tannins by the Cu (II) in acetate medium and the ferrous tartrate methods. They have in common every preparation step for mixing/homogenization in the μCH, measurement of absorbance, disposal and cleaning. Homogenization is performed by the drive motor (DM), the absorbance is measured and the μCH is emptied. Afterwards the μCH is cleaned by simultaneous activation of V_C and the drive motor, adding cleaning solution (C) while activated. Then, V_W is opened to discard the contents of the μCH. This cleaning and disposal procedure must be done twice to effectively clean the μCH.

The timing diagrams that illustrate the analytical procedures for both the turbidimetric and photometric methods are described in Fig. 3b and c, respectively. The time intervals chosen for each in-line preparation are for the drive motor (T_{DM}), valve switchings (T_S, T_{R1}, T_{R2}, T_C and T_W), and absorbance measurements (T_A). All preparations used a peristaltic pump (PP) with a flow of $34.0 \pm 0.3\ \mu L\ s^{-1}$ ($n = 10$) for all channels.

2.6.1. Procedure for Cu (II) in acetate medium

For in-line blank preparation, valves, V_{R1} and V_{R2} are simultaneously activated for 1.0 s, the mixing for 2.0 s and after that the absorbance signal is measured.

The in-line preparation of calibration solutions from 20 to 100 mg L^{-1} were performed using the working solution of 200 mg L^{-1} of tannic acid prepared from the appropriate dilution of stock solution of 1000 mg L^{-1}. In these preparations valves V_S, V_{R1}, and V_{R2} are activated simultaneously. Working solution (S), acetate reagent (R1) and Cu^{2+} (R2) are added to the μCH, homogenized and the absorbance is monitored at 470 nm. V_{R2} is opened for 0.5 s and the on times for V_S and V_{R1} vary proportionately with the concentration of the standard solution being prepared.

The procedure for in-line preparation of the sample is similar to the preparation of calibration solutions. The prepared samples (see Section 2.2) were used without further dilution. Samples are used instead of the working standard solution. The time intervals used for this analysis are shown in Fig. 3b. This method used a 10% nitric acid solution for μCH cleaning.

2.6.2. Procedure for ferrous tartrate

The procedure for blank preparation and the calibration solutions is similar to that described in Section 2.6.1. For the calibration solutions, the working solution (S), phosphate buffer pH 6.8 (R_1) and ferrous tartrate reagent (R_2) are added to the μCH, homogenized and the absorbance is measured at 560 nm. V_{R2} is opened for 0.5 s and the on times V_S and V_{R1} vary proportionately with the concentration of the standard solution being prepared.

For in-line preparation of the sample, the in-line procedure is similar to the preparation of calibration solutions. The prepared samples as described in Section 2.2 were used without further dilution. The difference here is also that the samples are used instead of the standard working solution. The time intervals used for this analysis are shown in Fig. 3c. This method used deionized water for cleaning the μCH.

2.7. Reference method

Results obtained with the proposed mini-system were compared with the official Japanese ferrous tartrate method [13,14]. Calibration solutions from 20 to 100 mg L^{-1} of tannic acid were prepared by adding appropriate volumes of 1000 mg L^{-1} stock solution, 10.0 mL of ferrous tartrate solution, 50.0 mL of phosphate buffer (pH 6.8) and water to complete to a final volume of 100.0 mL in a volumetric flask. For determination of tannins in the tea samples we added 10.0 mL aliquots of the sample, 10.0 mL of reagent and 50.0 mL of buffer using the same conditions as were used in determining the calibration solutions. After 10 min of reaction, the absorbance of all solutions, both calibration and sample were measured at 550 nm.

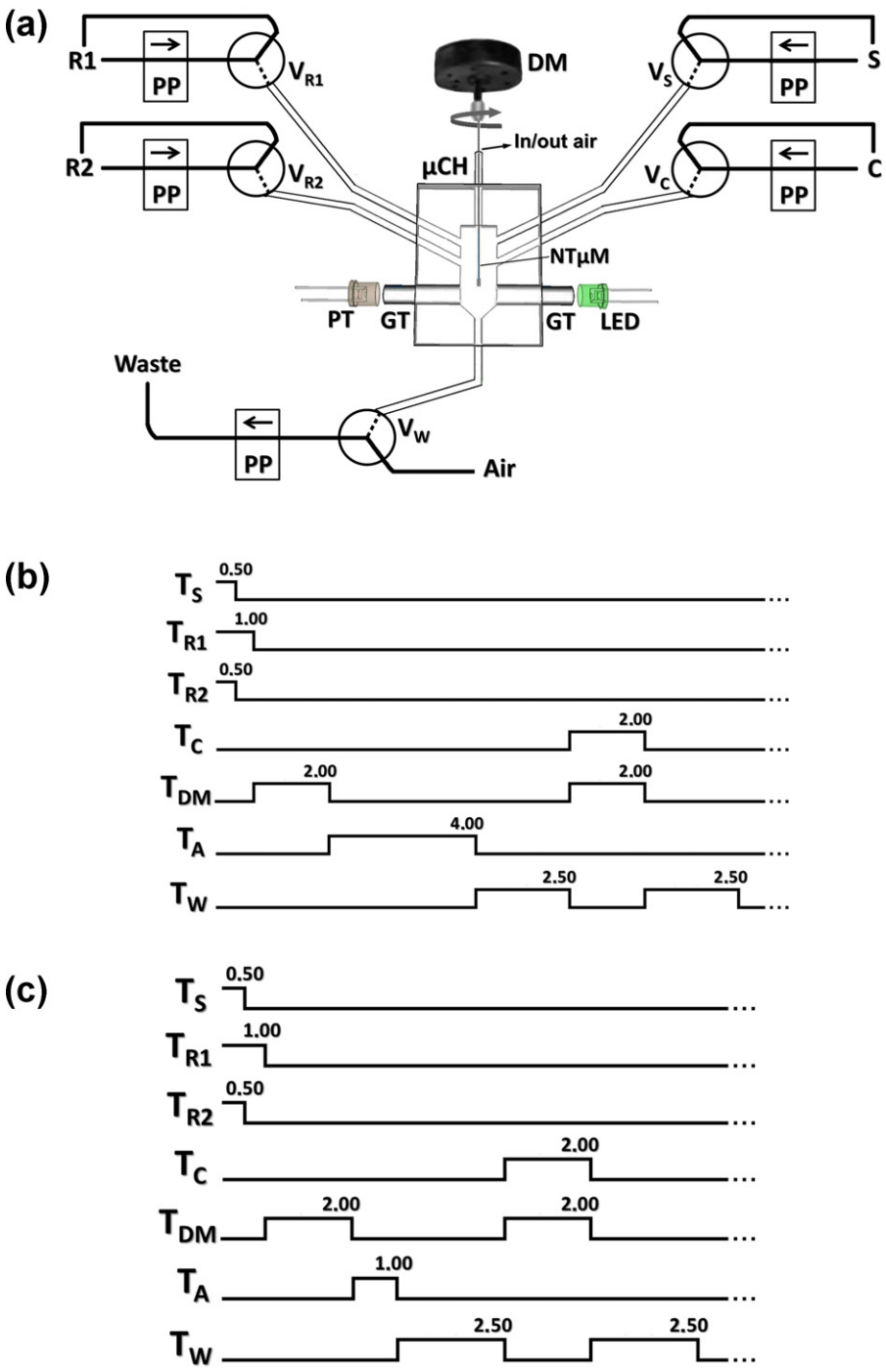

Fig. 3. (a) The µFBA diagram, (b) photometric and (c) turbidimetric time diagrams for samples. Micro-chamber (µCH), peristaltic pump (PP), drive motor (DM), nylon line mixer (NTµM), glass tube (GT), light emitting diode (LED), phototransistor (PT), minivalves solenoids (V_S, V_{R1}, V_{R2}, V_C and V_W), working solution or sample (S), discharge (W). For turbidimetric determination: R1, ammonium acetate 1.5 mol L^{-1}; R2, Cu (II) 0.1 mol L^{-1}; C, HNO_3 10% (v/v). For photometric determination: R1, phosphate buffer pH 6.8; R2, ferrous tartrate; C, water. The time intervals (in s) T_S, T_{R1}, T_{R2}, T_C and T_W, correspond to the minivalves (V_S, V_{R1}, V_{R2}, V_C and V_W); T_{DM}, drive motor; T_A, absorbance measurements.

3. Results and discussion

3.1. Characterization of the microsystem

Lithographic microfabrication with ultraviolet urethane-acrylate resin is easily reproducible. The sealing of the two partially cured layers with UV exposure was performed with both the Teflon and glass tubes already properly positioned in their respective channels. Due to the risk of clogging, the technique requires caution, and proper control of the exposure time. A time of 900 s is needed to ensure effective sealing of the layers. After curing the photoresist, a colorless and transparent material is obtained which does not absorb radiation in the visible region, allowing the use of optical detectors [33,34].

Monte-Filho et al. [31] describe the µFBA using detection devices permanently inserted into the resin and polymerized. For a more flexible system, glass tubes were attached to µCH. Thus, we employed the same system for both determinations,

measuring turbidity with a blue LED and for photometric absorbance, a green LED for. The use of glass tubes allows the micro flow-batch to be used with different LED/phototransistor couplings for various applications.

In conventional FBA systems the mixing chamber contains a magnetic strip that allows fluids agitation. In the μFBA system agitation is performed using nylon line with a shovel tip. Complete mixing of the solutions in the micro-chamber was obtained in less than 2 s, due to the high speed of (2000 rpm), CD/DVD-ROM motor.

3.2. μFBA application

The copper (II) in acetate medium method obtained a satisfactory analytical curve for the determination of tannins in tea samples with the regression equation $Y = -0.0025 + 0.0002C$; where Y is the absorbance and C is the analyte concentration in mg L^{-1} of tannic acid. The linear correlation coefficient, r^2, was 0.997 in the range between 20 and 100 mg L^{-1}. Its calibration curve was statistically validated by variance analysis showing no lack of fit at a 95% confidence level. The LOD and LOQ were 6.89 and 10.06 mg L^{-1} respectively.

For the reference method applied to the μFBA the regression equation was $Y = -0.0043 + 0.0010C$; where Y is the absorbance and C is the analytic concentration in mg L^{-1} of tannic acid. The linear correlation coefficient, r^2 was 0.9994 in the range between 20 and 100 mg L^{-1}. The calibration curve was statistically validated by variance analysis showing no significant lack of fit in the proposed model at a 95% confidence level. The LOD and LOQ were 4.74 and 6.97 mg L^{-1} respectively.

The LOD and LOQ for both methods were calculated based on the criteria established by IUPAC, the LOD was evaluated as three times the standard deviation of the blank measurement, and the LOQ was evaluated as being 10 times the standard deviation of the blank measure [35]. The LOD and LOQ values, although relatively high may be acceptable for the analyte under study, considering the high concentrations of tannin in green and black tea [10].

Table 1 presents the results obtained for the proposed microsystem for turbidimetric and photometric analysis, and those obtained for the reference spectrophotometric method. These results were statistically significant at a 95% confidence level when using the paired t test.

The μFBA presented an analytical frequency of 300 samples per hour for the ferrous tartrate method, with a waste generation of 136 μL per analysis. The turbidimetric method, copper (II) in acetate medium displayed an analytical frequency of 200 samples per hour, with a waste generation of204 μL per analysis.

The turbidimetric analysis needed more signal monitoring (4.0 s), due to the need to maintain a reproducible precipitate formation. The turbidimetric method's analytical frequency was lower than that of the photometric method.

The analytical curves obtained using the μFBA system had less slope or sensitivity, because absorbance measurements were made using an LED/phototransistor pair in a shorter optical path, about 0.5 cm. In the reference method, absorbance measurements were performed at 560 nm with a cuvette with 1.0 cm optical path.

Table 1
Results for tannin determinations in samples of green and black tea (mg L^{-1}) using both turbidimetric (μFBA–Turb.), photometric (μFBA–Phot.) methods and the spectrophotometric method with UV–vis as reference.

Samples	μFBA–Turb.	μFBA–Phot.	Reference
Green tea	41.48 ± 0.05	41.50 ± 0.03	41.74 ± 0.01
Green tea	49.21 ± 0.03	48.01 ± 0.05	47.92 ± 0.02
Green tea	61.62 ± 0.05	63.76 ± 0.03	64.35 ± 0.03
Black tea	46.47 ± 0.04	47.21 ± 0.01	47.40 ± 0.03
Black tea	54.57 ± 0.05	54.97 ± 0.01	55.19 ± 0.02
Black tea	44.14 ± 0.06	45.01 ± 0.03	44.87 ± 0.00
RSD% (n = 5)	0.05	0.03	0.02

4. Conclusion

The μFBA system proposed, successfully carried out the analysis of tannins in samples of green and black teas. The glass tubes attached to the μCH allowed changing the source radiations, a blue LED was used for turbidimetric monitoring and a green LED for the photometric determination. The urethane-acrylate resin used as substrate, proved chemically stable throughout the study and the fabrication methodology proved to be fast, and efficient while producing a robust or sturdy assemblage.

We obtained high speed microanalysis, thanks to optimizations in the injection times and volumes, in a system that allowed both homogenization (2 s) and discrete measurements. Essentially equal to a traditional flow-batch analyzer [22,24], the miniaturized or μFBA proposed can be used in many analytical applications; determinations for pharmaceuticals, foods, and clinical laboratory analysis of biological fluids, while guaranteeing significant reductions in the consumption of reagents and samples, and generating much less chemical waste.

Acknowledgements

The authors would like to thank the Brazilian agencies (CNPq and CAPES) for research fellowships and scholarships.

References

[1] A.A. Bele, V.M. Jadhav, V.J. Kadam, Asian J. Plant Sci. 9 (2010) 209–214.
[2] K. Khanbabaee, T. Ree, Nat. Prod. Rep. 18 (2001) 641–649.
[3] C.S. Yang, X. Wang, G. Lu, S.C. Picinich, Nat. Rev. 9 (2009) 429–439.
[4] C. Cabrera, R. Artacho, R. Giménez, J. Am. Coll. Nutr. 25 (2006) 79–99.
[5] X. Xi, X. Wei, Y. Wang, Q. Chu, J. Xiao, Arch. Biol. Sci. 62 (2010) 669–676.
[6] H. McKinley, M. Jamieson, Handbook of Green Tea and Health Research, Nova Science Publishers, New York, 2009.
[7] W. Luczaj, E. Skrzydlewska, Prev. Med. 40 (2005) 910–918.
[8] A.B. Sharangi, Food Res. Int. 42 (2009) 529–535.
[9] Y. Clement, Prev. Med. 49 (2009) 83–87.
[10] E. Piccin, H.J. Vieira, O. Fatibello-Filho, Anal. Lett. 38 (2005) 511–522.
[11] Official Methods of Analysis of AOAC International, Official Method 952.03, In Section 26.1.37, 8th, AOAC International, Gaithersburg, 2005.
[12] M.C. Yebra, M. Gallego, M. Valcárcel, Anal. Chim. Acta 308 (1995) 357–363.
[13] Official Methods of Analysis of Tea, Jap. Tea Res. Station Bull. 6 (1970) 167–172.
[14] Y.T. Hung, P.C. Chen, R.L.C. Chen, T.J. Cheng, Food Chem. 118 (2010) 876–881.
[15] R.L.C. Chen, C.H. Lin, C.Y. Chung, T.J. Cheng, J. Agric. Food Chem. 53 (2005) 8443–8446.
[16] Y.T. Hung, P.C. Chen, R.L.C. Chen, T.J. Cheng, Sens. Actuators B 130 (2008) 135–140.
[17] T.J. Cheng, H.Y. Hsiao, C.Y. Chung, Microchim. Acta 169 (2010) 117–122.
[18] M. Nakayama, N. Shigemune, T. Tsugukuni, H. Tokuda, T. Miyamoto, Int. J. Food Sci. Technol. 45 (2010) 2071–2079.
[19] M. McDonald, I. Mila, A. Scalbert, J. Agric. Food Chem. 44 (1996) 599–606.
[20] N. Slabbert, in: R.W. Hemingway, P.E. Laks (Eds.), Plant Polyphenols, Plenum Press, New York, 1991, pp. 421–436.
[21] M. Grünhut, V.L. Martins, M.E. Centurión, M.C.U. Araújo, B.S.F. Band, Anal. Lett. 44 (2011) 67–81.
[22] R.S. Honorato, M.C.U. Araújo, R.A.C. Lima, E.A.C. Zagatto, R.A.S. Lapa, J.L.F.C. Lima, Anal. Chim. Acta 396 (1999) 91–97.
[23] S.D. Kolev, I.D. Mckelvie, Advances in Flow Injection Analysis and Related Techniques, 5th ed., Elsevier, Hungary, 2008.
[24] 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.
[25] J.M.T. Carneiro, A.C.B. Dias, E.A.G. Zagatto, R.S. Honorato, Anal. Chim. Acta 455 (2001) 327–333.
[26] 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.
[27] 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.
[28] L.F. Almeida, V.L. Martins, E.C. Silva, P.N.T. Moreira, M.C.U. Araújo, Anal. Chim. Acta 486 (2003) 143–148.
[29] C.C. Acebal, M. Insausti, M.F. Pistonesi, A.G. Lista, B.S.F. Band, Talanta 81 (2010) 116–119.

[30] M.B. Lima, M. Insausti, C.E. Domini, M.F. Pistonesi, M.C.U. Araújo, B.S.F. Band, Talanta (2011), doi:10.1016/j.talanta.2011.10.055.
[31] S.S. Monte-Filho, M.B. Lima, S.I.E. Andrade, D.P. Harding, Y.N.M. Fagundes, S.R.B. Santos, S.G. Lemos, M.C.U. Araújo, Talanta 86 (2011) 208–213.
[32] E.N. Gaião, R.S. Honorato, S.R.B. Santos, M.C.U. Araújo, Analyst 124 (1999) 1727–1730.
[33] J.C.B. Fernandes, L.O.S. Ferreira, J. Braz. Chem. Soc. 17 (2006) 643–647.
[34] A. Fonseca, I.M. Raimundo Jr., J.J.R. Rohwedder, L.O.S. Ferreira, Anal. Chim. Acta 603 (2007) 159–166.
[35] A.D. McNaught, W. Andrew, IUPAC Compendium of Chemical Terminology, 2nd ed., Royal Society of Chemistry, Cambridge, 1997.