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A LED Based Photometer for Solid Phase Photometry: Zinc Determination in Pharmaceutical Preparation Employing a Multicommuted Flow Analysis Approach

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Neste trabalho é proposto um fotômetro baseado em LED (diodo emissor de luz) para fotometria em fase sólida. O fotômetro foi desenvolvido para permitir o acoplamento da fonte de radiação (LED) e do fotodetector direto na cela de fluxo, tendo um caminho óptico de 4 mm. A cela de fluxo foi preenchida com material sólido (C_{18}), o qual foi utilizado para imobilizar o reagente cromogênico 1-(2-tiazolilazo)-2-naftol (TAN). A exatidão foi avaliada empregando dados obtidos através da técnica ICP OES (espectrometria de emissão por plasma indutivamente acoplado). Aplicando-se o teste-*t* pareado não foi observada diferença significativa em nível de confiança de 95%. Outros parâmetros importantes encontrados foram faixa de resposta linear de 0,05 a 0,85 mg L^{-1} Zn, limite de detecção de 9 μg L^{-1} Zn (n = 3), desvio padrão de 1,4 % (n = 10), frequência de amostragem de 36 determinações por h, e uma geração de efluente e consumo de reagente de 1,7 mL e 0,03 μg por determinação, respectivamente.

In this work, a LED (light emitting diode) based photometer for solid phase photometry is described. The photometer was designed to permit direct coupling of a light source (LED) and a photodiode to a flow cell with an optical pathlength of 4 mm. The flow cell was filled with adsorbing solid phase material (C_{18}), which was used to immobilize the chromogenic reagent 1-(2-thiazolylazo)-2-naphthol (TAN). Aiming to allow accuracy assessment, samples were also analyzed employing ICP OES (inductively coupled plasma optical emission spectrometry) methodology. Applying the paired *t*-test at the 95% confidence level, no significant difference was observed. Other useful features were also achieved: linear response ranging from 0.05 to 0.85 mg L^{-1} Zn, limit of detection of 9 μg L^{-1} Zn (3σ criterion), standard deviation of 1.4% (n = 10), sampling throughput of 36 determinations *per* h, and a waste generation and reagent consumption of 1.7 mL and of 0.03 μg *per* determination, respectively.

Keywords: flow injection analysis, multicommuted flow analysis, solid phase spectrophotometry, LED based photometer, pharmaceutical formulation, green chemistry

Introduction

Nowadays, there is a demand for environmentally friendly analytical procedures in accordance with the Green Analytical Chemistry (GAC) guidelines.[1,2] The development of such procedures has been a challenge. Low reagent consumption and generating also low volume of waste are among the main requirements for achieving the environmental sustainability of the analytical new procedures. Solid phase spectrophotometry is among the methodologies that can afford facilities to attain the requirements.[3-5] Since the analyte is retained by the solid phase material, the pre-concentration step can be implemented without any setup modification.

Light emitting diode (LED) has become a reliable source for photometric purposes because of such useful features as durability and high light beam stability.[6-9] Its narrow emission band (λ = 25 nm) has enabled it to be used without a monochromator system, thus simplifying the photometer design.[10-12]

Multicommuted flow analysis process affords facilities to design active flow setup constituted by solenoid valves, which have been assembled to work as an independent commutator unit, thus allowing facilities to handle sample and reagent solutions controlled by microcomputer.[8-11,13,14]

Zinc is present in all living cells as a constituent of several molecules involved in proteins, lipids and

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carbohydrates metabolism, as well as several enzymes.[15,16] In this sense, zinc is considered as an essential element for all animals, including humans. The zinc deficiency leads to retarded growth and lower feeding efficiency, and inhibits the general well-being.[16] Because of its beneficial effects for humans, zinc has been included as a constituent of the pharmaceutical formulations available in the market.[17-19] Therefore, the determination of its presence in pharmaceutical products is a requirement for assuring quality. This has been done by employing spectrophotometry.[20,21] In this work, it is intended to design a LED based photometer integrated to a multicommuted flow analysis setup, which will be used to develop a solid phase photometric procedure. The facilities previously pointed out will be roused to obtain an efficient and not expensive equipment setup, to be used in the development of a solid phase photometric procedure for the zinc determination in pharmaceutical preparation.

Experimental

Apparatus

The equipment setup included an IPC4 Ismatec (619 Oak Street, OakHarbor, WA, USA) peristaltic pump equipped with Tygon pumping tubes, flow lines of polyethylene tubes (0.8 mm internal diameter), a PC microcomputer equipped with a PCL711 Advantech (38 Tesla Street, 100 Irvine, CA, USA) electronic interface card, two normally closed and one normally open solenoid pinch valves (P/N P091-11 and P/N 225 NResearch Inc, 267 Fairfield Ave, West Caldwell, NJ, USA), a homemade digital interface to control the solenoid valves,[11] a regulated power supply (12 V, 1 A) to power the solenoid valves, a flow cell machined in acrylic for solid phase packing, a photodetector DAL 10530 Thales (Dorchester, Dorset, DT13SY, UK), a high-brightness LED (λ_{max} = 490 nm) of narrow emission beam (< 20 ºC?), a regulated power supply (−12 V, +12 V) to feed the photometer, two printed circuit boards made of fiber glass to assemble the interfaces and resistors, as described in Figure 1.

Reagents and solution

All chemicals used were of analytical grade. Purified water presenting electric conductivity less than 0.1 μS cm^{-1} was used throughout. A 1000 mg L^{-1} Zn(II) stock solution was prepared by dissolving 1.000 g of metallic zinc in 10 mL of concentrated nitric acid. After dissolution, the volume was made up to 1000 mL with water. Intermediate stock solution (100 mg L^{-1}) was used to prepare zinc reference solutions with concentrations ranging from 0.05 to 1.20 mg L^{-1} (prepared by diluting with water). A Triton X-100 solution of 5% (m/v) concentration was prepared by dissolving 5 g of solid in water. A 10 μg mL^{-1} TAN solution was prepared by dissolving 1 mg of 1-(2-thiazolylazo)-2-naphthol (TAN, Merck) in 1 mL of ethanol. After dissolution, the volume was increased to 100 mL with Triton X-100 solution. TAN was immobilized on C_{18}-bonded silica (60-100 μm) obtained from a Sep-Pak cartridge (Waters), which was packed into the flow cell channel. The hexamethylenetetramine (hexamine) (0.8 mol L^{-1} buffer solution) (pH = 6.4) was prepared by dissolving 84.1 g of solid in 900 mL of water. After

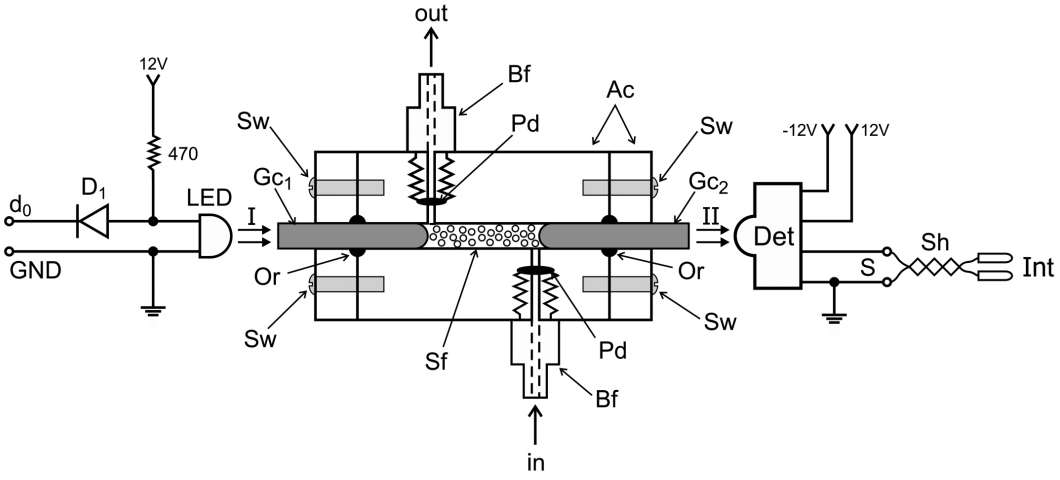

Figure 1. Pictorial view of the solid phase photometer: d_0 = input control line, GND = ground coupling; LED = light emitting diode, λ_{max} = 490 nm; I and II = radiation beams; Gc_1 and Gc_2 = glass cylinders, 20 mm long and 2.0 diameter; Sw = screw; Or = rubber O`ring; Ac = cutaway of the acrylic plates that comprise the body of the flow cell; Fs = solid phase material (C_{18}); Bf = barb-fitting; Pd = polyester cloth disk, 400 mesh aperture; in and out = solution input and output, respectively; and Det = photodetector DAL 10530 IPL; S = signal in mV; Sh = shielded cable; Int = coupling to the analog input of the PCL711 interface card.

dissolution, pH was adjusted with hydrochloric acid. In order to bring the volume level to 1000 mL, water was added. A 5% (m/v) sodium thiosulfate solution was prepared by dissolving 5.0 g of solid in 100 mL of water. A 5% (m/v) ascorbic acid solution was prepared by dissolving 5.0 g of solid in 100 mL of water.

Sample preparation

Samples of pharmaceutical formulations were acquired at the local market. Sample amounts of 0.500 g were weighed and carefully transferred to porcelain crucibles, which were placed into an electric stove and heated at 430 °C for 12 h. After cooling at the laboratory temperature, the crucibles were washed with 5 mL of concentrated hydrochloric acid to dissolve the solid residue. After dissolution, the volume was made up to 500 mL with water. Prior to analysis, a sample aliquot of 13.0 mL plus 1 mL of ascorbic acid and 1 mL thiosulfate solutions were transferred to a 25 mL volumetric flask. The volume was completed with hexamine buffer solution.

Chromogenic reagent immobilization

Approximately 100 mg of solid phase (C_{18}) were placed on a filter (cellulose acetate membrane 0.45 μm) supported on a glass funnel in order to allow the washing with methanol. The washing was carried out by slowly pouring a volume of 2 mL of methanol over the solid phase. Afterwards, the solid phase was transferred to a 2 mL vial and a 1.5 mL volume of TAN solution was added. The vial was maintained in a freezer (4 °C) while the immobilization proceeded. After immobilization, the solid phase was filtered and transferred to the cell flow. The remaining material was stored in the freezer to be used to refill the flow cell. The immobilization assays were carried out using time intervals of 1, 2, 4 and 6 h.

Experimental setup

The experimental setup comprised a LED based photometer and a flow system module, as described below. As shown in Figure 1, the photometer is constituted by a radiation source (LED) and a photodetector (Det), which were attached to the flow cell to form a compact unit of downsized dimension.

The glass cylinder (Gc_1) acted as a waveguide, by conducting the radiation beam (I) along the analytical path of the flow cell, which was filled with the solid phase material (C_{18}). After transmission through the solid phase, the radiation beam (II) was conducted by the other glass cylinder (Gc_2) towards the photodetector (Det), generating a potential difference signal (S) that presents a linear relationship with the intensity of the radiation beam (II). The solid phase material causes a strong attenuation of the radiation beam due to scattering effects. Thus, the intensity of radiation beam (II) was lower than that of radiation beam (I). Rubber O-rings were installed in order to prevent leakage of fluid and also to allow a correct alignment between the radiation source (LED) and the photodetector (Det). Polyester disks (Pd) placed in the threaded hole of the barb fitting prevented the solid phase material from being displaced from the flow cell by sample and eluent streams.

The packing of the flow cell with solid phase material was carried out by maintaining the central body of the flow cell tightly attached to the left part, while an amount of bonded silica (C_{18}), containing the immobilized chromogenic reagent (TAN), was inserted into the flow channel by using a small spatula. Afterwards, the second glass cylinder (Gc_2) was inserted into the flow cell channel and then it was slowly moved down in order to compact the solid phase material. The distance between the glass cylinders (Gc_1 and Gc_2) defined the optical path length of the flow cell. This distance can be varied by changing the amount of solid material inserted into the flow cell channel. The control line (d_0) was coupled to the digital interface output port[11] in order to allow the LED shining to be controlled by a software.

The flow system module was designed based on the multicommutation process.[9-11] A diagram of this module is shown in Figure 2. In this configuration, all valves are switched OFF and the reconditioning solution (Bf) is

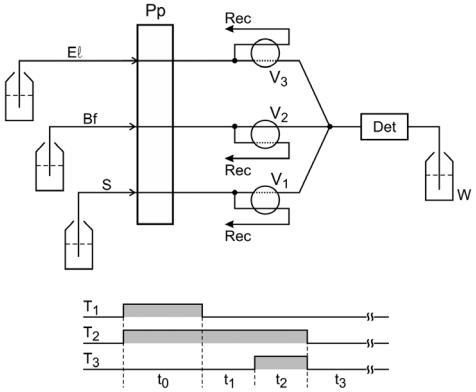

Figure 2. Diagram of the flow system manifold: V_1, V_2 and V_3 = solenoid pinch valves; S, Bf and El = sample, buffer and eluting solutions, respectively, flow rate of 1.0 mL^{-1}; Pp = peristaltic pump; Rec = circulation of solutions; Det = photodetector; W = waste vessel. Continuous and dotted lines into the valves symbols indicate the solutions pathway while valves were switched OFF or ON, respectively. T_1, T_2 and T_3 = timing course to switch ON/OFF the solenoid valves V_1, V_2 and V_3, respectively. Shadow surfaces beneath of the timing lines indicate that the respective valve is switched ON; t_0, t_1, t_2 and t_3 = sample inserting, signal reading, analyte eluting, and solid phase reconditioning steps, respectively.

flowing through the flow cell (Figure 1) towards the waste (W), while streams of sample (S) and eluting solution (El) are conducted towards their storing recipients.

Procedure

When the control software was run, the microcomputer read the dark signal (Sd) generated by the photodetector (Det), which was done by maintaining the LED switched OFF. Afterwards, the microcomputer enabled the LED to shine by sending a control signal through line d_0 (Figure 1). The signal read under this condition (Sf) and the dark measurement (Sd) were both saved, so that they can be later used to calculate the absorbance. Afterwards, the analytical run comprising sample loading, signal reading, the eluting and reconditioning steps were carried out following the pattern depicted in the valves timing course (Figure 2). Under this condition, while the sample solution (step t_0) was pumped through the flow cell towards the waste vessel (W), eluting and reconditioning solutions were pumped back to their storing vessels. The signal reading step (t_1) was carried out while maintaining all valves switched OFF. In this way, the reconditioning solution flowed again through the flow cell to allow signal measurement to be carried out, thereby maintaining a similar condition to that used to read the reference measurement (Sf), as described above. While signal reading was proceeding, the sample and eluting solutions were directed back to their storing vessels. Afterwards, steps t_2 and t_3 were performed following a similar pattern in order to complete the analytical run. Other runs were then performed by cycling through the same four steps. The variables studied included flow rate, time interval for sample loading and reagent concentration in order to establish the best operational conditions. These assays were done by following the working pattern depicted in Figure 2.

Results and Discussion

General comments

While the sample solution flowed through the flow cell, the Zn(II) ions reacted with the immobilized chromogenic reagent (TAN) on the solid phase (C_{18}) that was packed into the flow cell channel, forming a colored compound that absorbed light with a maximum of around 580 nm, thus allowing that a LED with an emission beam (whose maximum was around 590 nm) was used as a radiation source (Figure 2).

The parameters that can affect the overall performance of the proposed setup including sensitivity, linear response range, reagent concentration and flow rate were investigated and the results are presented in the following sections.

Effect of the reconditioning solution

Since the solid phase reconditioning condition can affect the reaction between Zn(II) ions and the immobilized chromogenic reagent (TAN) on the C_{18}, as well as the lifetime of the immobilized reagent, this parameter was the first one studied. The assays were performed using Zn(II) standard solutions with concentrations ranging from 0.05 to 1.20 mg L^{-1}. Flow rate and time interval for insertion of the sample solution were maintained at 1.0 mL min^{-1} and 40 s, respectively. Water and hexamine solution were used to perform the solid phase reconditioning. For both cases, the results presented similar behavior when zinc concentrations were higher than 0.15 mg L^{-1}. On the other hand, when zinc concentrations were lower than this value, the solid phase material presented a different behavior using hexamine and water as reconditioning fluids, with use of water resulting in signals identical to the blank measurement. For this reason, hexamine buffer solution was selected for the solid phase reconditioning.

Effect of the sample flow rate

The signal generated by the photometer presented a direct relationship with the amount of the analyte (Zn) that reacted with the immobilized chromogenic reagent (TAN) on the solid phase. This shows that the flow rate of sample solution can affect the signal generated. Aiming to find the best operational condition, a set of assays was implemented by varying the flow rate of sample solution from 0.8 up to 1.6 mL min^{-1}. In the last case, a leakage of sample solution in the flow line connections was observed. Therefore, the essay with that concentration was aborted. The results obtained using flow rates with values up to 1.4 mL min^{-1} yielded the results shown in Table 1.

These assays were carried out using Zn(II) standard solutions with concentrations ranging from 0.05 to

Table1. Effect of the sample solution flow rate

Flow rate / (mL min^{-1})	Sample volume / µL	Intersect / (mg L^{-1})	Slope	Linear coefficient (r)
0.8	532	0.1289	0.7050	0.996
1.0	664	0.1460	0.7324	0.993
1.2	800	0.1543	0.7052	0.990
1.4	932	0.1470	0.6233	0.990

Results are average of three consecutive measurements.

1.20 mg L^{-1}, with the time interval for sample solution insertion (t_0, Figure 2) maintained at 40 s. The decrease of slope shows that for flow rates higher than 1.0 mL min^{-1}, the analyte retention by the solid phase becomes less efficient. It is proposed the following explanation for this phenomenon: the speed of Zn ions through the flow cell channel increased with the flow rate, so that the time for contact with the solid phase decreased, thus impairing the compound formation. Taking into consideration the facts that the slope is the parameter related to the sensitivity and that linearity coefficients (r) were similar in the first two cases, it was selected the flow rate of 1.0 mL min^{-1} for the further assays. When the results related to Zn(II) concentrations higher than 0.85 mg L^{-1} were included, the linearity of the analytical curve was lessened. Thus, these standard solutions were not used for the subsequent assays.

Effect of the sample volume

Since in solid phase spectrophotometry the generated signal depends on the amount of analyte that forms a complex with the immobilized chromogenic reagent, a set of experiments was carried out in order to evaluate the effect of varying the sample volume. The assays were done using standard solutions with concentrations of 0, 0.05, 0.15, 0.30, 0.55 and 0.85 mg L^{-1} Zn(II), yielding the results shown in Table 2.

Table 2. Effect of sample volume

Time interval / s	Sample volume / µL	Intersect / (mg L^{-1})	Slope	Linear coefficient (r)
30	500	0.0972	0.3593	0.998
40	666	0.1087	0.4618	0.996
60	1000	0.1184	0.5379	0.990
80	1333	0.1231	0.5786	0.989

Results are average of three consecutive measurements.

Taking the volume of 500 µL as a reference, the increase in volume was 33, 100 and 166%, while slopes increased by 28, 48 and 57%, respectively. These volumes are directly related with the time intervals elapsed while the sample inserting step proceeded (see step t_0, Figure 2). These results indicated that the efficiency with which the compound was formed decreased as the sample volume pumped through the phase solid increased. Nevertheless, since the signal increased throughout the range of volumes assayed, this effect could be exploited as a resource for improving the sensitivity. Additionally, it was observed that as sample volume increased, the lifetime of the immobilized chromogenic reagent on the solid phase underwent diminution. In order to assure a complete elution of the analyte, the time interval for the eluting step was increased, which might have also caused a partial elution of the chromogenic reagent. Taking into consideration this effect, as well as sample consumption, the sample loading time of 40 s was selected.

Acidity effect

Several mineral acids can be used for Zn(II) elution from the solid line, but the analyte elution can be performed without removing the immobilized chromogenic reagent on the solid phase material, when hydrochloric acid was used.[4] For this reason, hydrochloric acid solution was selected to implement the current work. A set of assays was performed in order to find the appropriate concentration and aliquot volume of hydrochloric acid to be used for performing the elution. The experiments were carried out maintaining a flow rate of 1.0 mL min^{-1} and using a 0.5 mg L^{-1} Zn(II) standard solution. The assays showed that with acid concentrations lower than 0.5 mol L^{-1}, the analyte elution was not completed, while for concentrations higher than this value, the solid phase lifetime underwent a decrease. Furthermore, it was observed that there was a close relationship between the volume of sample or standard solution pumped through the solid phase (flow cell) and the volume of acid solution (eluent) necessary for complete elution. This relationship was found to be 84 and 17 µL for the sample and eluent solutions, respectively. This pattern was supplied to the microcomputer software as a parameter used to carry out the further assays.

Effect of the surfactant concentration

The surfactant Triton X-100 has been used to improve TAN solubility.[4] In order to verify its effect on the sensitivity, a set of experiments was carried out with TAN solutions prepared using several Triton X-100 concentrations, yielding the results shown in Table 3. Analyzing these results, it was possible to observe that as Triton X-100 concentration was increased, the intersect and slope presented decreasing values.

The results show that the slope obtained using a 5% Triton X-100 solution was 35% lower than that obtained using a 1% solution. Nevertheless, the precision of the results was better when using the 5% concentration, which was, therefore, selected. It was observed that for surfactant concentrations lower than 5% (m/v) TAN dissolution was not complete, and that the useful life of the immobilized reagent underwent a reduction.

Table 3. Effect of the surfactant concentration

Parameter	Surfactant concentration / %				
	1	3	5	7	9
Intersect / (mg L^{-1})	0.1373	0.188	0.1186	0.1117	0.0970
Slope	0.7931	0.5209	0.4876	0.4339	0.2648
Linear coefficient (r)	0.9932	0.9956	0.9966	0.9980	0.9988

These parameters were calculated using the average of three consecutive measurements; standard solution and immobilized solid phase as indicated in previous sections.

Effect of the TAN concentration

The results discussed in the previous sections were achieved using a TAN solution with concentration of 10 μg mL^{-1} to perform immobilization on the solid phase (C_{18}). In order to verify whether the reagent concentration could affect signal response and useful life of the immobilized reagent, a set of experiments was done by varying the solution concentrations from 10 up to 20 μg mL^{-1}, yielding the results shown in Table 4.

Table 4. Effect of the TAN solution concentration

Concentration / (μg mL^{-1})	Intersect / (mg L^{-1})	Slope	Linear coefficient (r)
20	0.1640	0.8133	0.9756
16	0.1416	0.6843	0.9831
13	0.1196	0.5671	0.9882
10	0.1224	0.5557	0.9867

Results are average of three consecutive measurements; standard solutions were equal to those used in Table 3.

As can be seen, the highest slopes were achieved for TAN concentrations of 16 and 20 μg mL^{-1}. Nevertheless, both curves presented a linearity worsening compared with those achieved using lower concentrations. While running the experiments, a baseline drift was observed, indicating that the chromogenic reagent was being removed from the flow cell channel. It was concluded that the linearity lessening occurred because the amount of immobilized chromogenic reagent decreased while the assays were performed. Since the results achieved using TAN concentrations of 10 and 13 μg mL^{-1} were similar, the latter concentration was selected.

Effect of time for TAN immobilization

As indicated in the Experimental section, the chromogenic reagent (TAN) was immobilized on the solid phase (C_{18}) off line, prior to putting the solid phase into the flow cell channel. In order to verify whether the time for immobilization exerted significant effects, a set of experiments was done by setting time intervals of 1.0, 2.0, 4.0 and 6.0 h, yielding slope values of 0.5895, 0.6429, 0.6569 and 0.6890, respectively. Considering that the linear regression curves were similar, and that the increase in slopes was not significant, the time interval of 2 h was selected.

Effect of the optical pathlength

According to the Lambert-Beer law, absorbance varies directly with the length of the space crossed by the light beam through the sample. This effect has been exploited to improve sensitivity in spectrophotometry, using sample solution as an absorbing medium. To the best of our knowledge, this effect had not been yet exploited for analytical purposes in solid phase photometry. Solid phase photometry has usually been performed using a flow cell with an optical pathlength of around 1 mm.[4,18,19] In this work, the flow cell (Figure 1) was designed to allow the channel (optical path) filled with solid phase material to be varied from 2 up to 6 mm, while also varying the amount of C_{18} packed into the flow cell. The assays were carried out employing a flow rate of 1.0 mL min^{-1}, a loading time interval of 40 s and using the following standard solutions: 0.0, 0.05, 0.15, 0.30, 0.55 and 0.85 mg L^{-1} Zn(II), yielding the results shown in Figure 3.

Analyzing these curves, it was observed an increasing of the signal up to the optical pathlength of 4 mm, while results achieved when the flow cell length was 5 mm was practically equal to those generated when its length was of 4 mm. Considering signal magnitude, it was observed that the worst result was that related to the optical pathlength of 6 mm. In this case, the light scattering became predominant, thus worsening the conditions for taking accurate measurements. The curves a, b and c were achieved using flow cell with inner volume of 6.4, 9.6 and 12.8 μL, respectively, while the amount of C_{18} packed into the flow cell channel were 7.2, 10.7 and 14.0 mg, respectively, thereby both flow cell length and C_{18} amount increased twice. The linear regression coefficients and as well as slopes related to curves a, b and c are 0.9873,

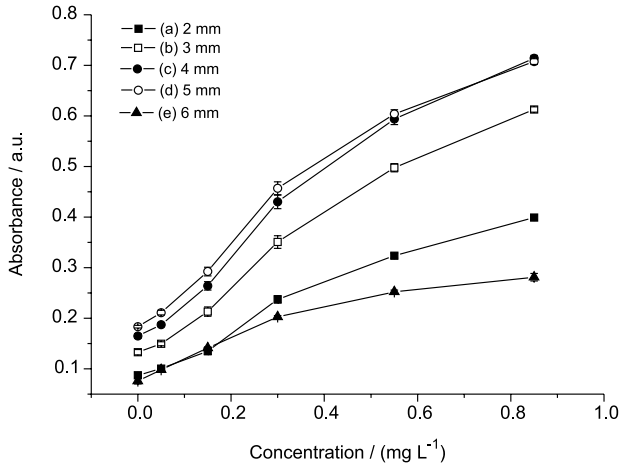

Figure 3. Effect of the optical pathlength: (a), (b), (c), (d) and (e) correspond to the flow cell length of 2, 3, 4, 5 and 6 mm, respectively. Results are average of three consecutive measurements.

0.9903, 0.9865 and 0.3823, 0.5937, 0.6795, respectively. Comparing the slope of curve a with those of curves b and c, it was found an increase of 55 and 78%, respectively. These results would be considered as an indication that the relationship between optical pathlength and sensitivity does not follow the Lambert-Beer law. Taking into account that the volume of standard solution was maintained while performing these assays, this resource can be used to improve the sensitivity. Considering these results, the optical path length 4 mm was selected.

Effect of a potential interference

Because the chemical elements Co(II), Cu(II), Ni(II), Mn(II), Ca(II), Fe(III), Mo(VI), Al(II) and Mg(II) can form a compound with TAN,[4] a set of experiments was carried out in order to evaluate the extent to which each of these elements interferes with the results. The assays were done by mixing a volume of a 0.5 mg L^{-1} Zn(II) standard solution with an equal concentration of one of these potentially interfering elements. Interference was not considered significant when the absorbance variation was within the range of ± 5%. The Cu(II) presented a significant interference effect, which was suppressed by masking with thiosulfate, as suggested elsewhere.[24-26]

Results comparison and figures of merit

Once the values of the main variables that can affect the proposed system were selected, the effectiveness of the proposed setup needs to be proved. For this reason, a set of pharmaceutical formulations was processed. Aiming to assess accuracy, samples were processed for Zn determination using the induced coupled plasma and optical emission spectrometry (ICP OES), yielding the results shown in Table 5.

Table 5. Results comparison

Sample	ICP OES / mg[a]	Proposed procedure / mg[a]	Labeled value / mg[a]
1	0.11 ± 0.01	0.11 ± 0.01	0.10
2	0.55 ± 0.02	0.56 ± 0.01	0.58
3	0.92 ± 0.03	0.75 ± 0.03	0.86
4	0.20 ± 0.04	0.23 ± 0.01	0.19
5	0.41 ± 0.02	0.33 ± .02	0.29
6	0.39 ± 0.01	0.40 ± 0.01	0.48
7	0.85 ± 0.01	0.72 ± 0.01	0.58
8	27.02 ± 0.02	23.78 ± 0.01	23.90
9	26.52 ± 0.01	22.25 ± 0.02	23.90
10	7.84 ± 0.01	7.02 ± 0.03	7.00

Results are average of three consecutive measurements; [a]values are related to 1.0 g of dry material.

Applying the paired *t*-test between the results at the 95% confidence level produced the value t_{calc} = 1.753, while the theoretical value was t_{tab} = 2.262. Therefore, there is no significant difference between the results at this confidence level.

The optical pathlength of the flow cell was fixed at 4 mm, thus a C_{18} amount ca. 14 mg could be packed into them. The reagent immobilization was done using 1.5 mL of a 13 μg mL^{-1} TAN solution and 100 mg of solid phase material, then the amount of reagent used when packing them into the flow cell was 3.2 μg. It was observed that 100 determinations can be performed without significant variation of the signal magnitude, whereby it is possible to deduce that the reagent consumption *per* determination was 0.032 μg.

The parameters selected in order to evaluate the overall performance of the proposed setup are shown in Table 6, where it can be seen that they compare very well with those of the referred work.[9]

Table 6. Performance comparison

Parameters	Proposed procedure	Reference 9
Linear range / (mg L^{-1})	0.05-0.85	0.04-4.00
Linear coefficient (r)	0.995	0.999
Slope	0.546	0.310
Limit of detection / (μg L^{-1})	9.3	10
Variation coefficient (n = 10)	1.4	3.3
Sampling throughput / h^{-1}	36	45
Reagent consumption (TAN) / (μg *per* determination)	0.03	< 1
Sample volume / (μL *per* determination)	680	625
Eluent volume / (μL *per* determination)	133	400
Waste volume / (mL *per* determination)	1.7	6.0

While the referred work presents a wider analytical range and a higher throughput, the setup used in the present work afforded lower reagent consumption and waste generation. For these reasons, we consider very well the overall performance of the equipment setup and the proposed analytical procedure.

Conclusion

A new flow cell design for solid phase photometry was created for use in the experiments reported here. Such a flow cell can easily be loaded with solid phase material, allowing inner variation of the optical pathlength, avoiding leakage of solution, and maintaining entrapped into the optical pathway all solid material inserted into it. Such flow cell geometry can, therefore, become a reliable tool for solid phase photometry.

The LED-based photometer presented a good performance, thus indicating that this setup can become an effective option for solid phase photometry for analytical purposes. When a solid phase device is coupled to a multicommuted flow analysis module, the inner pressure becomes higher than that of the usual systems, and as a consequence the usual solenoid valves can fail. This drawback was avoided by using pinch solenoid valves.

The low volume of waste generation *per* determination shows that the reduced dimension of the proposed equipment setup is an effective option when developing analytical procedures in accordance with the Analytical Green Chemistry guidelines.[1,2]

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