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A sensitive procedure to determine *o*-cresol using multicommutation and a portable photometer with a long optical pathway cell

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Abstract

A fast and sensitive procedure was developed for determining *o*-cresol in water samples, by using a homemade light emitting diode (LED)-based photometer (λ= 590 nm), a 10 cm optical pathway cell and the micro-pumping strategy. The method is based on the measurement of the formation of *o*-cresol derivate with 100 mg/L *p*-aminophenol (PAP) and 0.004 mol/L KIO_4 in presence of NaOH 0.06 mol/L. Two different methodologies have been compared; (i) batch analysis in a conventional spectrophotometer using an optical pathway cell of 1 cm, (ii) multicommuted flow injection procedure and a portable LED-based photometer (25 cm x 22 cm x 10 cm, 3 Kg) with a 10 cm cell length. *O*-cresol reaction requires *ca.* 17 min for the full development of the colour with PAP. Nevertheless, the developed procedure was carried out in the stopped-flow mode using a reaction time of 3 min. Good results were obtained and the calibration equation was $I = (170 \pm 5)\ C_{o\text{-cresol}} + (0 \pm 9)$ with R = 0.9993. Limits of detection of 10 and 80 ng/mL and repeatability of 2.8 and 1.9 % relative standard deviation were obtained for the 10 cm cell and for the conventional 1 cm cell, respectively. Multicommutation, using a long pathway cell (10 cm), has shown a higher sensitivity, but it only permitted a maximum of 20 determinations per hour, as low as batch procedure. *o*-Cresol can be determined in water samples and recoveries from 92 to 105 % were found in spiked tap, waste and irrigation waters. The paired Student's t test compared with batch data was lower than the theoretical t value (2.306) for a 95% probability level and 8 degrees of freedom.

Key words: Light emiting diode; multicommutation analysis; long optical pathway; *o*-cresol; photometer.

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Un procedimiento sensible para determinar o-cresol empleando multicommutación y un fotómetro portatil con celda de paso óptico largo

Resumen

Se ha desarrollado un procedimiento rápido y sensible para la determinación de *o*-cresol en aguas empleando un fotómetro fabricado en nuestro laboratorio con un diodo emisor de luz (LED) (λ= 590 nm), una celda de medida de paso óptico de 10 cm y minibombas para la introducción de reactivos y muestras. El método se basa en la medida de la absorción del derivado del *o*-cresol con 100 mg/L de *p*-aminofenol (PAP) y 0,004 mol/L KIO_4 en medio NaOH 0,06 mol/L. Se han comparado dos metodologías diferentes: (i) Análisis en regimen estacionario en un espectrofotómetro convencional usando una celda con paso óptico de 1 cm, y (ii) un procedimiento por inyección en flujo multicommutado en un fotómetro portatil de LED (25 cm x 22 cm x 10 cm, 3 Kg) con una celda de 10 cm de longitud. El *o*-cresol necesita un tiempo de reacción de 17 min para el completo desarrollo del color con el PAP. No obstante, el procedimiento desarrollado se llevó a cabo en flujo parado empleando un tiempo de reacción de solo 3 minutos. Se obtuvieron buenos resultados y la ecuación del calibrado fue I= (170 ± 5) $C_{o\text{-cresol}}$ + (0 ± 9) con un R= 0,9993. Se obtuvieron límites de detección de 10 y 80 ng/mL y precisiones de 2,8 y 1,9 % en términos de desviación típica relativa para las celdas de 10 cm y de 1 cm, respectivamente. La multicommutación, empleando la celda de paso óptico largo (10 cm), ha mostrado una mayor sensibilidad y permite, al igual que el procedimiento en régimen estacionario, 20 determinaciones h^{-1}. El *o*-cresol se puede determinar en muestras de agua y las recuperaciones obtenidas para muestras aditivadas de agua potable, residual y de riego fueron de entre el 92 y el 105%. El test de Student's por parejas, aplicado a la comparación de los resultados dió un valor menor que el teórico para un 95% de probabilidad y 8 grados de libertad (2,306).

Key words: Analysis por multicommutación; fotómetro; *o*-cresol; paso óptico largo.

Introduction

Phenol and phenolic compounds, such as cresols, resorcinol and aminophenol, are considered as air and water pollutants due to their biological effects on humans (1). However, these products are widely employed in industrial manufacturing for the production of photographic developers, explosives, synthetic resins, pharmaceuticals, disinfectants, fumigants or adhesives. Automobile exhaust, tobacco smoke and petrol refineries also contribute to the emission of phenols to the environment. So, phenolic products must be controlled in environmental samples.

Cresolic compounds occur at many production plants as secondary products. The toxic effects of these products are similar to those of phenol, but *o*- and *m*-cresol are more poisonous (2). Different national and local norms for safety in working areas have stated that the maximum tolerable time-weighted average (TWA) values for cresolic compounds are 5 mg/L and 22 mg/m^3 (3).

The most commonly used methods for the determination of *o*-cresol compounds are chromatographic methods (4-6). Sometimes spectrofluorometric and infrared methods have also been used (7-9). Few results have been reported for the ultraviolet-

visible spectrophotometric determination of cresols, because these compounds usually absorb in the UV region, which involves serious problems from matrix interferences. The spectrophotometric determination of cresols therefore requires a derivatization procedure that yields products which absorb in the visible range (10-12).

In the last years, our research group has developed a flow analysis strategy for the spectrophotometric determination of *o*-cresol, *m*-cresol, phenol and resorcinol, in waters (13, 14), based on the reaction between phenols and p-aminophenol (PAP) in an alkaline medium and in the presence of KIO_4. It is well known that PAP is a phenol derivative reagent that is very sensitive for cresol and resorcinol determination, which provides different absorbance maxima for each one of the phenolic compounds. Moreover, all these properties were taken in advantage of carrying out a simultaneous kinetic spectrophotometric determination of the aforementioned phenols using partial least squares data treatment (15).

However, all the proposed techniques are expensive and no portables because of the size and weight of the instruments required. Peristaltic pumps are the most expensive equipment required to implement flow systems, and, for this reason, in this paper we have developed a portable, non-expensive and sensitive photometric method using micro-pump devices to impel the solutions, for the determination of *o*-cresol based on reaction with *p*-aminophenol (PAP) in an alkaline medium to produce a coloured indophenolic compound which present absorption maximum at 590 nm using an orange light emitting diode (LED). The study has been carried out in batch and in multicommutation modes for the analysis of natural water samples without preconcentration or preliminary extraction steps.

Materials and methods

Apparatus

The flow system comprised four solenoid micro-pumps Bio-chem 090SP Valve Inc. (Boonton, NJ, USA) with a nominal volume of 8 μL per pulse to introduce KIO_4, PAP, NaOH and samples or standards respectively, a three-way solenoid valve NResearch 161T031 (West Caldwell, USA) to aspirate sequentially the samples inside the measurement cell or the bubbles formed in the system, flow lines of 0.8 mm i.d. PTFE tubing and a four line confluence connector made on acrylic were used to transport and mix solutions. A microcomputer equipped with an electronic interface card Advantech, PCL-711S (San José CA, USA) and a homemade electronic interface (see reference (16) for details) were used to drive the solenoid devices. The flow system control and data acquisition were performed by the microcomputer running software written in Quick Basic 4.5.

Spectrophotometric measurements were made with a Hewlett-Packard Model 8452A diode array spectrophotometer (Waldbronn, Germany), equipped with a 1 cm optical path and 50 μL inner volume flow cell, and alternatively using a homemade LED-based photometer, designed to work employing a flow cell of 10 cm long pathlength and 315 μL inner volume flow cell. The electronic diagram and the structure of the LED-photometer are shown in Figure 1a.

The flow cell was made from a borosilicate glass tube with an inner diameter of 2.0 mm and was machined to provide two plane windows of approximately 0.15 cm^2 surface close to which both, the LED source and the detector, were mounted. The flow cell is shown in Fig. 1b. The cell was installed in a PVC block that was machined to allow that both, LED and photodiode, were attached in front of flow cell observation windows. When the photometer was switched

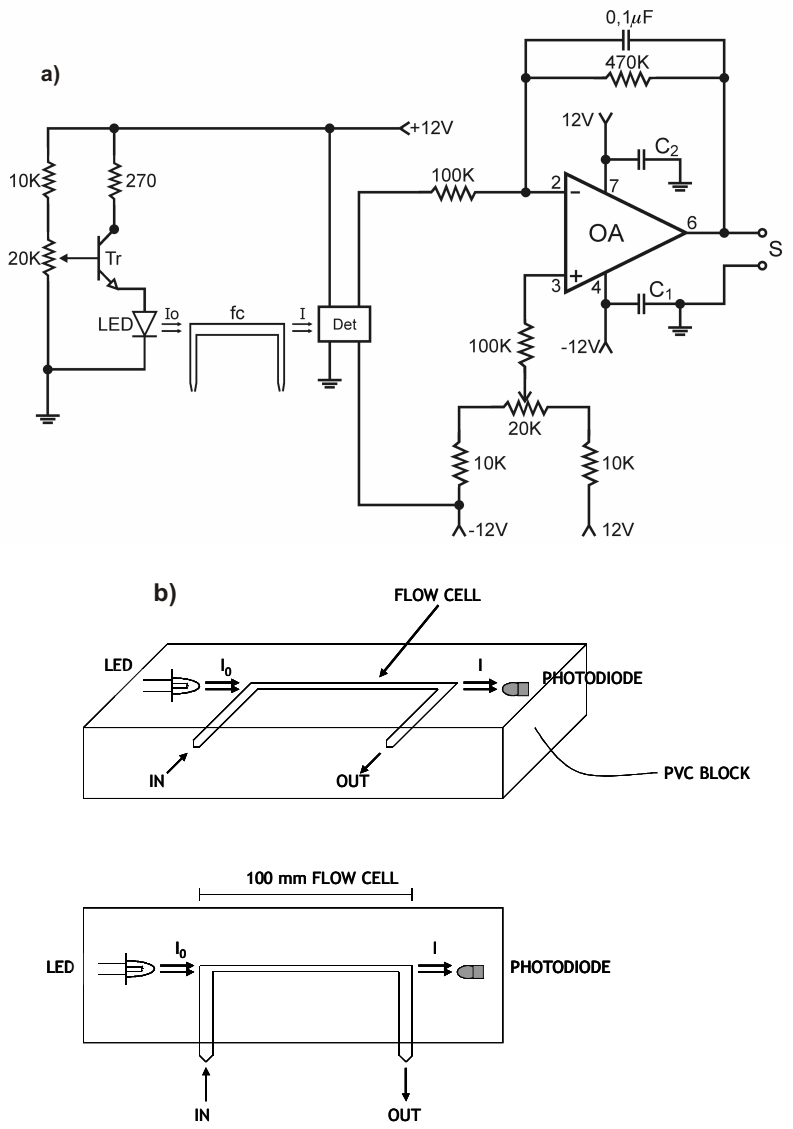

Figure 1. Structure of the LED photometer. a) Electronic diagram of the homemade photometer. Tr= transistor BC547. (LED) = 590 nm. I_0 and I= radiation beam coming and leaving the flow cell, respectively. fc= flow cell. Det= photodiode (IPL 10530 DAL). OA= OP07 operational amplifier. C_1 and C_2= 2 μF Tantalum capacitor. S= output signal. b) Flow cell, LED and photodiode arrangement. Glass tube 10 cm long and 2.0 mm inner diameter.

ON the base line can be adjusted maintaining the dark condition (LED disable for radiation emission) and flow cell filled with carried solution. Next, the output signal (S) was adjusted to 0.0 mV by mean of the variable resistor wired to the no inverting input of the operational amplifier. Afterwards, the LED was enabled to emit radiation turning the variable resistor wired to the base of the transistor (BC547). The radiation emission intensity was increased up till the output signal (S) attained a potential difference of 2000 mV.

Reagents, solutions and samples

All solutions were prepared with deionized water (18.2 M cm) and analytical grade chemicals.

A standard stock solution of 100 mg/L of *o*-cresol was prepared from the solid product obtained from Aldrich (Germany). Working standards were prepared by diluting the stock solution with deionized water in order to obtain calibration curves from 0 to 10 mg/L and from 0 to 4 mg/L, for the spectrophotometric and photometric measurements, respectively. A stock solution of 100 mg/L of PAP (Panreac, Spain) was prepared daily using boiled and cooled distilled water and was stable for more than 8 h (boiling the distilled water for 10 min is very important to avoid oxidation of PAP by dissolved oxygen). Stock solutions of 0.004 mol/L potassium metaperiodate and 0.06 mol/L sodium hydroxide were prepared by dissolving the solids obtained from Panreac in distilled water.

Tap, domestic waste and Turia river waters were collected in polyethylene bottles from the Valencia metropolitan area. Samples were filtered with 0.45 μm nylon filters to remove the suspended solids, which could impair the diaphragm of the solenoid micropumps, and stored at 4ºC until analysis.

Photometric recommended flow procedure

The flow diagram of the manifold is shown in Figure 2. It comprises four solenoid micro-pumps, P_1, P_2, P_3 and P_4, to handle *o*-cresol standards or samples, NaOH, PAP and KIO_4, respectively, a confluence point (*x*) to merge the aforementioned solutions and a reaction coil of 100 cm (B1). A three way solenoid valve was used to drive the final solution after debubbling in a debubbler (Bs).

When the solenoid coil of the micropump was energized (ON) a sucking action was carried out, thus permitting the solution insertion into the micro-pump chamber through the input channel. When the applied voltage was turned OFF, the inner diaphragm go back to rest position and the fluid was dispensed through the output channel.

The solenoid micro-pumps were arranged as shown in Figure 2, employing one device for each solution handled, during 0.1/0.1 s (ON/OFF) intervals of time. The devices were operated at 5 Hz.

The multicommutated system was operated as described in Table 1. Each step was repeated until to complete the number of pulses indicated. Volumes of *o*-cresol standard/sample, NaOH, PAP and KIO_4 so-

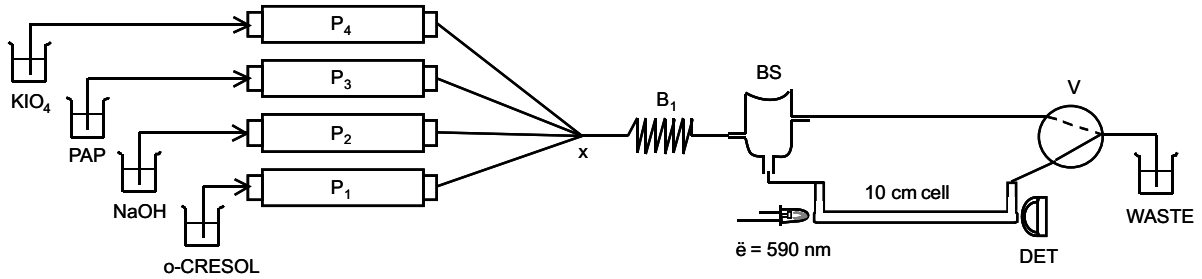

Figure 2. Manifold of the flow system. P_1, P_2, P_3, P_4 μ-pumps employed to introduction of sample or standards, NaOH, PAP and KIO_4 respectively. *x*= confluence point. B_1= 100 cm reaction coil. BS= debubbler. Det= homemade photometer with 10 cm measurement cell. 590 nm LED source light. V= 3-ways solenoid valve.

lutions were simultaneously inserted in the reaction coil B1 after be mixed in the confluence point *x* using 10 pulses. The valve V was maintained in ON position to expel the air from the debubbler (step 1). Sample zone was transported by 300 pulses of NaOH carrier towards the 10 cm flow cell (step 2). In this step V was in position OFF all time to allow the pass of the solution to the detector. After that, in steps 3 and 4, micro-pumps were stopped for 3 min, in order to complete the reaction inside the flow cell and to make the transient signal measurements. After obtaining three replicates of each sample it was introduced a cleaning step (step 5) throughout the micro-pump P_2, during 300 pulses. Measurements of *o*-cresol were established at wavelength of 590 nm and no correction of the baseline was necessary.

Batch mode procedure

Solutions of *o*-cresol, NaOH, PAP and KIO_4 were directly mixed inside a volumetric flask and let to stand for colour development, making the absorbance measurements were at 590 nm. Samples were measured in the same way as standards and data interpolated in the external calibration line.

Results and Discussion

Reaction of PAP with o-cresol

The reaction between *o*-cresol and PAP is based on the reactivity of the benzoquinoneimine form of PAP (I), in an alkaline medium, with phenolic compounds in which the *para* position is not blocked (17), as it is shown in Figure 3. Therefore, the corresponding phenolate (II) reacts with (I) through the electrophilic substitution on C4 of the phenolate to produce the corresponding leucodye (III) which is oxidized to an indodye (IV) and presents a maximum absorption at 590 nm.

Previous studies made in batch and in classical flow injection analysis (FIA) evidenced that a 100 mg/L PAP, a 0.004 mol/L KIO_4 and a 0.06 mol/L NaOH were the best concentrations to be used for the determination of cresolic compounds at mg/L concentration levels (13).

The compound formed in the studied reaction show absorption maxima at 614 nm and to accomplish this requirement an orange LED (590 nm) was employed as light source. The absorption spectrum of the monitored compound is shown in Figure 4. As it can be seen, a suitable matching between product absorption and LED emission spectra was achieved, thus indicating that it

Table 1
Operational conditions of the multicommutation flow system for the *o*-cresol determination[a]

Step	Description	P1	P2	P3	P4	V	Pulses/Time
1	Introduction of *o*-cresol, NaOH, PAP and KIO_4	ON	ON	ON	ON	ON	10 pulses
2	Transport to the cell	OFF	ON	OFF	OFF	OFF	300 pulses
3	Stop time	OFF	OFF	OFF	OFF	ON	180 s
4	Measuring time	OFF	OFF	OFF	OFF	ON	1 s
5	Cleaning time	OFF	ON	OFF	OFF	OFF	300 pulses [b]

[a] ON/OFF indicates a pulse of the solenoid device. In case of solenoid valve (V), ON and OFF indicate the dotted and the continuous lines, respectively.
[b] Just after three replicates.

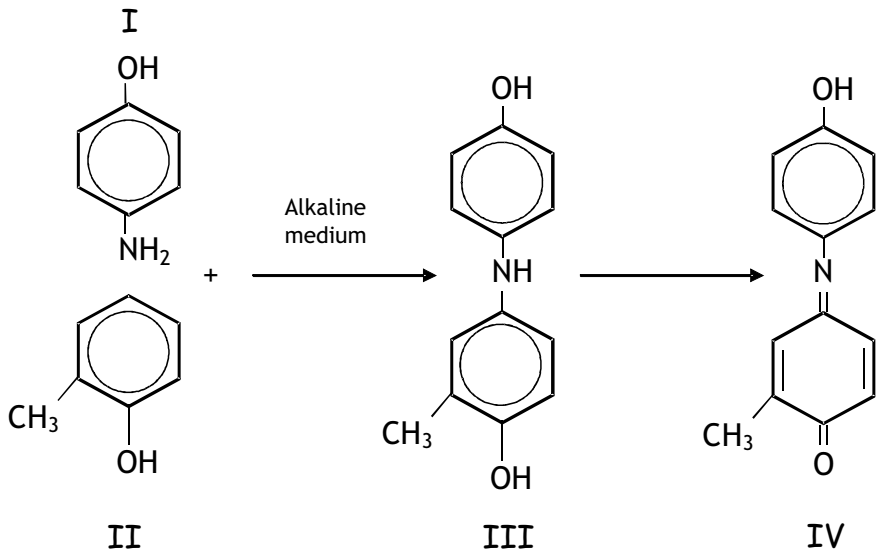

Figure 3. Chemistry scheme of the *o*-cresol reaction with PAP.

was possible to perform the measurements employing the corresponding radiation source.

In preliminary experiments, it was verified that the emission intensity of the LED affected both, sensitivity and dynamic range of the photometer. This drawback was overcome employing the electronic network (Figure 1a) comprised by the transistor and the variable resistor. Under these conditions, the LED emission intensity was easily adjusted controlling the electric current that was drained through the variable resistor and the base of the transistor. The photometer must be switched ON at least 15 min before. Working continuously for six hours no significant baseline variation was observed. This feature was always observed, thus indicating that the long-term stability of the photometer was very good. In this sense, we can affirm that the performance of the proposed photometer was suitable to carry out measurements for chemical determinations.

Stop time study

The reaction of *o*-cresol with PAP needs ca. 17 min to end. Figure 5a shows the relation between the *o*-cresol absorbance on the reaction time using the 1 cm optical path length measurement cell and the diode array spectrophotometer. As can be seen, over 1000 seconds were indispensable as the most suitable time to achieve the maximum sensitivity.

In order to reduce this time, it was necessary to introduce the stop time concept. Figure 5b shows that, using the photometer and the 10 cm optical path length flow cell, only 180 seconds (3 min) were required to achieve the same behaviour. So, *o*-cresol determinations were made using a stop time of 3 minutes after the insertion of the solutions, in order to complete the reaction inside of the 10 cm flow cell (Table 1).

Analytical features

Table 2 shows the analytical features of the different procedures assayed, batch using a 1 cm cell and photometric multicommutation using a 10 cm flow cell. Moreover, there is presented data obtained in FIA by Khalaf et

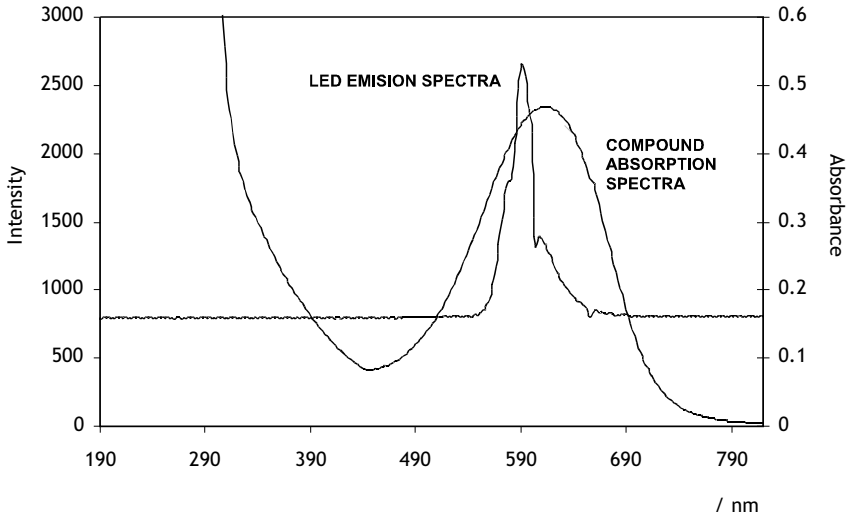

Figure 4. Absorption spectrum of the chemical products formed between *o*-cresol and PAP and the emission spectrum of the orange LED used in this study.

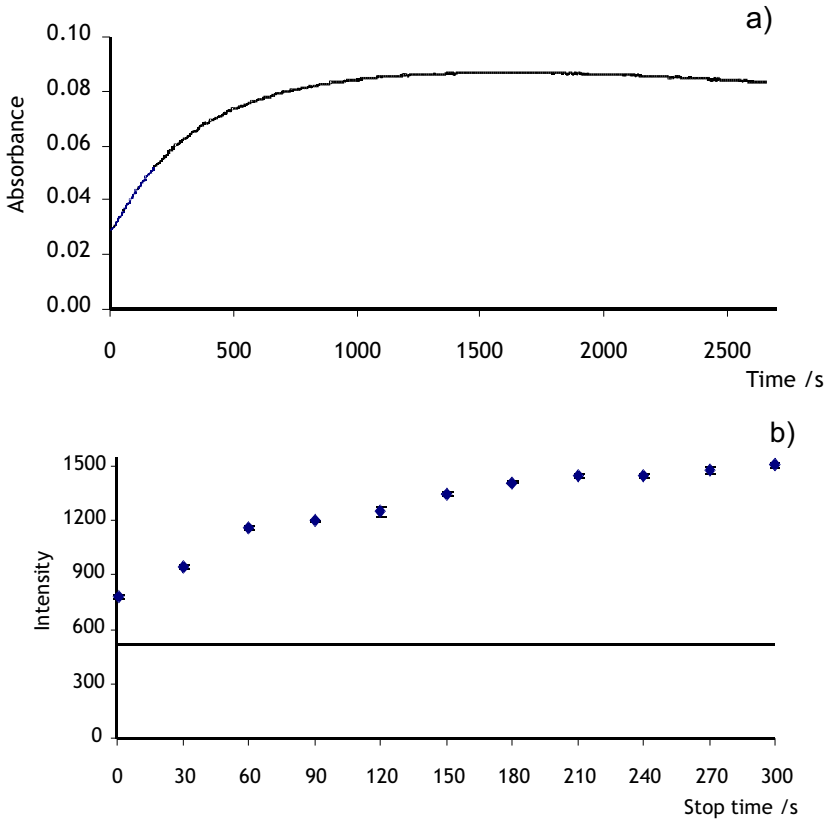

Figure 5. Effect of the stop time. a) 10 mg/L *o*-cresol spectrum measured in batch in a conventional spectrophotometer. b) 10 mg/L *o*-cresol spectrum measured using multicommutation in the homemade photometer. 100 cm coil. 10 pulses of P_1, P_2, P_3 and P_4 to fill the cell and 300 pulses of P_2 to clean.

Table 2
Analytical parameters of *o*-cresol determination by using the reference and the proposed multicommuted methods.

	Batch Spectrophotometer 1 cm cell	Flow injection [a] Spectrophotometer 1 cm cell (13)	Multicommutation developed method [b] Photometer 10 cm cell
Calibration line[c]	A = (0.085 ± 0.002) + (0.0196 ± 0.0005) $C_{o\text{-cresol}}$	A = 0.0257 + 0.0705 $C_{o\text{-cresol}}$	I = (0 ± 9) + (170 ± 5) $C_{o\text{-cresol}}$
Correlation coefficient (R)	0.998	0.9994	0.9993
Linear range (mg/L)	0 – 10	0 – 6	0 – 4
RSD (%)	1.9	0.7	2.8
LOD (ng/mL)	80	10	10
Sample/standard consumption (mL/det)	0.5	0.2	0.004
Reagent consumption (mL/det)	1.5	90.0	0.244
Waste (mL/det)	2.0	90.2	0.248
Throughput (h^{-1})	20	5	<20

[a]12 minutes of stopped reaction time.
[b]3 minutes of stopped reaction time.
[c]A: absorbance. I: intensity, $C_{o\text{-cresol}}$: *o*-cresol concentration in mg/L.

Table 3
Recovery values (n=3) for *o*-cresol measurements in tap, waste and irrigation water, using both, the batch and the multicommutation methods.

	RECOVERY %		
Water source	Spiked level (mg/L)	Batch 1 cm cell	Multicommutation 10 cm cell
Tap	0.1	98 ± 20	96 ± 18
	1	94 ± 1	105 ± 8
	4	101 ± 2	98 ± 5
Waste	0.1	101 ± 20	100 ± 4
	1	102 ± 3	92 ± 5
	4	101.9 ± 0.3	96 ± 3
Irrigation	0.1	98 ± 10	98 ± 15
	1	96 ± 5	100 ± 4
	4	102 ± 2	98 ± 2

al. (13) in the middle of the table. As can be seen, flow injection procedure was more sensitive than batch mode. Measurements made in multicommutation using a 10 cm cell were obtained in intensities by the photometer, and they also presented a good correlation coefficient and reduced the linear range up to 4 mg/L. Moreover, this proposed method reduces the sample, standard and reagent consumptions and the waste generation in factors of 125, 6 and 8, as compared to batch method and 50, 370, 360, as compared to flow method in the literature (13).

On the other hand, multicommutation using a long flow cell allows reducing the limit of detection to 10 ng/mL, eight times better than it obtained in batch method, maintaining the throughput in 20 determinations per hour. This value is higher than that obtained by Khalaf et al. (13).

So, it can be concluded that multicommutation approach, using a 10 cm flow cell and measuring in the homemade photometer, offers a sustainable alternative to the batch and FIA methods in terms of analytical parameters, environmental procedure and less contact with the operator (18, 19).

Analysis of water samples spiked with o-cresol

In order to demonstrate the applicability of the proposed method for the determination of *o*-cresol in real samples, water from different sources (tap, domestic waste and irrigation water) with cresol concentrations below the limit of detection of the photometric methods, were spiked with known quantities of *o*-cresol. Sodium hydroxide was added to the samples in order to obtain an alkaline medium of 0.06 mol/LNaOH, and then they were directly analysed in batch and multicommutation modes at 590 nm.

Table 3 shows the recoveries for the determination of *o*-cresol in spiked samples by batch and multicommutation. For added concentrations from 0.1 to 4 mg/L, the average recoveries were 99.3% and 98.1% by batch and multicommutation, respectively. The paired Student's t test for both groups of data was lower than the theoretical t value (2.306) for a 95% probability level and 8 degrees of freedom. So, it can be concluded that the accuracy of the multicommutation procedure is comparable to that found in batch on using the same reaction.

The good recoveries obtained show that the developed procedure is selective and free from matrix interferences. The main part of cations, anions and uncoloured organic molecules present in water do not interfere. However, it must be recognized that PAP can also react with other phenolic compounds. Results from studies in the literature (13) showed that different phenolic compounds require different experimental conditions with NaOH concentration which varied between 0.005 and 0.4 mol/L, PAP concentration varying from 50 to 500 mg/L and KIO_4 concentration which varied between 0.002 mol/L and 0.004 mol/L, and also provide different maximum absorbance wavelengths between 540 and 638 nm. All these factors could be important in achieving convenient selectivity for the determination of phenols with PAP. Another possibility to determine o-cresol in the presence of phenolic compounds which can react with PAP is the coupling online of the LED photometer with a micro capillary electrophoresis in order to maintain the portability of the instrumentation and to improve the selectivity of determinations.

Conclusions

The proposed method for the photometric determination of *o*-cresol, using a 10 cm flow cell, after reaction with PAP in an alkaline medium, presents many obvious advantages compared with published methods, as high sensitivity, high throughput, low reagent and sample consumptions and low waste generation. Moreover, the proposed method is very economical, portable and does not require the use of a prior sample clean-up.

On the other hand, multicommutation permitted us to do the on-line reaction of samples and reagents, avoiding the manipulation of the solutions and their dangerous contact with the operator.

Acknowledgements

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