An improved flow system for phenols determination exploiting multicommutation and long pathlength spectrophotometry

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Abstract

A greener and sensitive procedure for spectrophotometric determination of phenols based on a multicommutated flow system with a 100 cm optical path flow cell is presented. The method exploited the oxidative coupling of phenolic compounds with 4-aminoantipyrine in alkaline medium containing potassium hexacyanoferrate(III). Sensitivity was 80-fold higher than that achieved with a 1 cm flow cell, making feasible the determination of phenols in the 10–100 μg l−1 range with a detection limit estimated as 1 μg l−1 phenol. The sampling rate and the coefficient of variation were estimated as 90 determinations per hour and 0.6% (n = 10), respectively. The multicommutation approach allowed a 200-fold reduction of the reagent consumption in comparison with the reference batch method. Moreover, the chloroform extraction for analyte concentration is unnecessary in view of the increase in sensitivity. Recoveries within 93.3 and 106% were achieved for determination of phenol in natural and wastewater samples. Results agreed with the obtained by a reference method at the 95% confidence level.

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Keywords: Flow analysis; Multicommutation; Long pathlength spectrophotometry; Phenols; Green analytical chemistry

1. Introduction

The conscience concerning the use of water has increased as a consequence of the restrict availability of unpolluted freshwater sources and the high costs for obtaining potable water from seawater. Thus, in many countries the legislation has become more severe and reliable analytical methods for fast determination of pollutants are increasingly required. Flow-based methodologies have matched these needs resulting in a large application for water analysis [1]. However, in some situations the sensitivity for direct determination is not suitable and time-consuming procedures for analyte concentration are necessary.

Sensitivity of spectrophotometric procedures can be improved by increasing the measurement optical path. However, the inherent attenuation of the radiation beam has limited the application of conventional materials in long pathlength spectrophotometry. An alternative is the use of liquid core waveguides in which the light propagation is constrained within a liquid medium with a higher refractive index than the surrounding material [2,3]. Liquid core waveguides for measurements in diluted aqueous solutions (refractive index of ca. 1.33) can be constructed from an amorphous fluoropolymer (named Teflon AF-2400), which presents a refractive index of ca. 1.29. This strategy allowed the attainment of detection limits in the nanomole per liter (nmol l−1) range for the determination of iron [2,4], chromium(VI) and molybdenum(VI) [5], nitrate and nitrite [6] in natural waters.

The presence of phenols in aquatic environments indicates anthropic pollution, and the main sources are dyes, petroleum, cellulose and paper industries, tannery and pesticides production and application. Phenols are quite toxic to humans via oral exposure, damaging blood, liver and kidney [7]. As several phenols can be present in aquatic environments, the monitoring can be suitably done by the operational determination of total phenols instead of the quantification of individual species, thus saving time and costs. The American and the Brazilian environmental legislations established the limit of 10 μg l−1 of total phenols in waters [8,9]. However, the official method of the American Public Health Association (APHA), adopted by the control
agencies, requires a tedious and time-consuming extraction of the analytes in chloroform from 500 ml water samples in order to achieve the required quantification limit [10]. Thus, reliable procedures with improved sensitivity are needed for suitable monitoring.

Other hindrance in environmental chemical analysis is the production of large amounts of wastes that in some circumstances are even more toxic than the species being determined [11]. The reagent consumption can be minimized with several flow approaches such as the intermittent addition of reagents [11]. This strategy can be suitably implemented exploiting the multicommutation approach, in which the flow manifolds are designed with computer-controlled discrete commutation devices, allowing all steps of the analytical procedure (including reagent addition) to be independently performed [12].

In this work, a greener and sensitive flow-based procedure is presented for fast determination of phenols in waters, exploiting multicommutation and spectrophotometric detection in a 100 cm optical path flow cell.

2. Experimental

2.1. Apparatus

The flow setup comprised five three-way solenoid valves (NRResearch, 161T031), a coiled reactor and transmission lines made of 0.7 mm i.d. polyethylene tubing and Perspex joint points. An Ismatec IPC peristaltic pump was employed for fluid propelling. An AMDK6 500 MHz microcomputer equipped with an electronic interface (Advantech Corp., PCL-711S) was employed to control the commutation devices. Control signals were generated at TTL pattern and an electronic interface based on ULN 2803 integrate circuits was employed to generate the electric potential and current required to switch the valves (12 V, ca. 100 mA). Signals were measured with a multi-channel PC-2000 fiber-optic spectrophotometer (ocean optics) with an LPC-1-FL flow cell with 100 cm optical path and ca. 280 µl inner volume (ocean optics). Optical fibers with 600 µm core were employed to transport radiation from the source to the flow cell inlet and from the flow cell outlet to the detection system. The software for system controlling and data acquisition was developed in Visual Basic (Microsoft).

2.2. Reagents and solutions

All solutions were prepared with distilled and deionized water; and analytical grade chemicals. Phenol reference solutions within 10.0 and 100 μg l−1 were daily prepared by appropriate dilutions of a 1.00 g l−1 stock solution. Reagent R1 was prepared by dissolving 50.0 mg of 4-aminoantipyrine (4-AAP) in 50 ml of a buffer containing 5.2 g l−1 NaHCO3, 5.8 g l−1 H2SO4 and 6.2 g l−1 KOH (pH 10.0). Reagent R2 was a 0.20% NH4 H3BO3 and 6.2 g l−1 K3 [Fe(CN)6] solution prepared in water. Water was used as carrier.

Fig. 1. Flow diagram of the system for determination of phenols. V1, three-way solenoid valves; B, reaction coil (80 cm); D, long pathlength flow cell (100 cm optical path); C, water carrier (5.4 ml min−1); S, sample (6.0 ml min−1); R1, 0.10% (v/v) 4-AAP buffered at pH 10.0 (0.8 ml min−1); R2, 0.2% (v/v) K3[Fe(CN)6] (0.8 ml min−1); W, waste. Dashed lines represent the flow paths when the valves are switched on.

Solutions containing 7.0 μmol l−1 phenol, m-cresol, p-cresol, p-chlorophenol, catechol, hydroquinone, p-amino-phenol and p-nitrophenol were employed for evaluation of the relative response for different phenols.

2.3. Flow system and procedure

The flow diagram of the system is shown in Fig. 1. In the initial status, all valves are switched off and the carrier is flowing through the analytical path while other solutions are recycling to their vessels. Sample and reagent solutions are introduced by simultaneously switching the valves V1-V4 on for 7.0 s. Afterwards, all valves are switched off and data acquisition is started. For sample replacing, valves V2 and V5 are simultaneously switched on for 10 s and the path between these valves is washed with the sample. Measurements were carried out at 500 nm.

The detection limit was estimated by absorbance measurements (n = 5) for solutions obtained from sequential dilutions of a solution 10 μg l−1 phenol in order to evaluate the lowest phenol concentration that produce an analytical signal statistically different of the blank at the 95% confidence level. Relative responses for different phenolic compounds were estimated by five measurements carried out at 500 nm with a 1 cm flow cell. The results were related to the phenol response.

Natural river waters, treated and untreated industrial wastewater samples were collected in São Carlos in polyethylene bottles and acidified to 3.6 × 10−2 mol l−1 H2SO4. Samples were distilled before analysis according to the cleanup procedure recommended by APHA [10].

3. Results and discussion

3.1. Flow system

The flow system was designed exploiting the multicommutation approach in order to minimize the reagent
consumption that should produce positive effects on both a reduction of effluent generation and minimization of the contact time with the Teflon AF-2400 tube. Although it is not expected that the employed reagents could deteriorate the polymeric material in a short period, the minimization of the contact time can help to avoid tube degradation or adsorption of reagents and products. There are two usual alternatives to design multicommmuted flow systems. The former consists in a flow system with a single pumping tube for asparging the solutions and comprises the introduction of sample and reagent aliquots in tandem [12]. However, adopting this strategy, the flow-rate cannot be higher than ca. 3 ml min\(^{-1}\) in order to avoid degassing of the solutions and the consequent liberation of air bubbles that could disturb spectrophotometric measurements. This drawback could be magnified by the high gas permeability of the Teflon AF-2400 that can be more than three orders of magnitude higher than conventional Teflon [13]. Alternatively, the flow system was designed exploiting the intermittent addition of pumped sample and reagent solutions resulting in an inner pressure slightly higher than the ambient pressure, thus circumventing the formation of air bubbles in the analytical path. With the employed setup, sample and reagent volumes are defined by the flow-rates and the sampling time. Under the selected conditions (Table 1), the volume of the sample zone is 860 \(\mu\)l that provides limited sample dispersion for the 80 cm analytical path (680 \(\mu\)l, including the flow cell volume).

The intermittent addition of reagents resulted in relatively high blank signals caused by light absorption by reagents at the measurement wavelength. Blank signals that were insignificant with conventional 1 cm flow cells (A < 0.003) were magnified with the increase of the optical path and values as high as 0.300 were measured with the long path flow cell. Thus, system optimization was carried out in order to reduce blank values and improve sensitivity aiming the determination of low phenol concentrations. These criteria were employed for selecting the parameters in Table 1.

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Evaluated range</th>
<th>Selected value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(K_t[^{\text{Fe(CN)}_6}]) concentration (% m/v)</td>
<td>0.10–0.50</td>
<td>0.20</td>
</tr>
<tr>
<td>4-AAP concentration (% m/v)</td>
<td>0.050–0.15</td>
<td>0.10</td>
</tr>
<tr>
<td>Sampling time (s)</td>
<td>3.0–4.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Flow-rates (ml min(^{-1}))</td>
<td>2.0–4.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Sample</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(R_1)</td>
<td>0.1–1.2</td>
<td>0.8</td>
</tr>
<tr>
<td>(R_2)</td>
<td>0.5–3.6</td>
<td>0.8</td>
</tr>
</tbody>
</table>

### 3.2. Chemical aspects

The procedure was based on the oxidative coupling of phenols with 4-AAP in alkaline medium as represented by the Eq. (1). The reaction proceeds instantaneously by employing hexacyanoferrate(III) as oxidant [14]. This was compatible with the obtained results in which better sensitivity was achieved for a 7 s residence time (80 cm reactor coil). However, responses for different phenols for residence times of 30 and 60 s (implemented by stopping the sampling zone after the sampling step) were also investigated in order to evaluate the influence of kinetic effects on the formation of the reaction products with substituted phenols. Slight variations were observed only for hydroquinone and measurements were carried out with 7 s residence time to maximize the sampling rate.

![Reaction scheme](image)

Reagent concentrations were varied in order to minimize blank values without affecting sensitivity. Better results were obtained for concentrations shown in Table 1, that are in agreement with those previously employed [14]. However, due to differences in flow-rates, the sample/4-AAP and sample/hexacyanoferrate(III) volumetric fractions were three- and sixfold higher in the proposed system, respectively. Blank values are caused by absorption of radiation by the hexacyanoferrate(III) ion as well as the product of the oxidation of 4-AAP at higher oxidant concentration [15]. Thus, the increase of sample/reagent volumetric fraction allowed obtaining blank values 65% lower than those observed by using the previously reported conditions [14]. A solid-phase reactor with hexacyanoferrate(III) immobilized in an anionic-exchange resin placed before or after the reactor coil was also evaluated to reduce blank values. However, sensitivity was strongly lessened.

The reaction with 4-AAP can be used for determining phenol, ortho- and meta-substituted phenols and, under proper pH conditions, phenols \(p\)-substituted by groups such as halogen, carboxyl, sulphonic acid, hydroxyl and methoxy. However, the reaction is not efficient to determine phenols \(p\)-substituted by aryl, alkyl, nitro, benzoyl and aldehyde groups [10,14]. In spite of this, the result obtained by employing 4-AAP is usually referred as total phenols concentration. Relative responses for different phenols commonly present in industrial effluents are shown in Table 2. Values agreed with those presented in the literature exploiting the same reaction, except the response for \(m\)-cresol that was lower than the reported values (70–85%).
and p-cresol and p-nitrophenol that presents higher relative responses than the reported values [14,16]. An extensive evaluation of the relative response of phenols by employing several chromogenic reagents (including 4-AAP) was previously presented [16]. As the total phenols’ results are often related to the species with the highest response (phenol), the oxidative coupling with 4-AAP allows the determination of the minimum concentration of phenols in the sample, which is beneficial to the preventive control of the pollutants in environmental samples.

3.3. Analytical features and applications

A comparison between the sensitivities obtained with 1 and 100 cm optical path flow cells can be done from the transient signals shown in Fig. 2. A 80-fold increase in sensitivity was estimated as the lowest concentration of phenols in the sample, which is beneficial to the preventive control of the pollutants in environmental samples.

The proposed procedure was applied for the determination of phenols in freshwaters (from lakes and river), wastewaters and respiratory samples, interferences and matrix effects caused by color of the solutions are efficiently removed by the cleanup procedure [10]. A detection limit of 1 µg l$^{-1}$ (10 nmol l$^{-1}$) phenol was verified, that is suitable for determination of phenols in natural water samples considering the limits established by the American and the Brazilian legislations [7,9]. A detection limit of 1 µg l$^{-1}$ (10 nmol l$^{-1}$) phenol was estimated as the lowest concentration that generated an analytical signal significantly different of the blank at the 95% confidence level.

The sampling rate and the coefficient of variation were estimated as 90 determinations per hour and 0.6% ($n = 10$), respectively. The multicommutation approach allowed reducing the reagent consumption from 8- to 670-fold in comparison with the batch procedure [10] (Table 3). Moreover, the chloroform extraction for analyte concentration is unnecessary in view of the improvement in sensitivity provided by the increase in the optical path. Thus, the proposed procedure is inherently greener than the spectrophotometric ones previously reported and can produce highly beneficial effects by reducing both costs and waste generation.

Analytical features of several spectrophotometric procedures for phenols determination with 4-AAP are presented in Table 4. In addition to the lower reagent consumption, the proposed procedure presents better sensitivity than the other methods without extraction [10,14] and allows attaining detection limits comparable with the extraction batch method [10]. Repeatability and sampling rate are notably better than that reported in previous procedures. For natural and wastewater samples, interferences and matrix effects caused by color of the solutions are efficiently removed by the cleanup procedure [10].

The proposed procedure was applied for the determination of phenols in freshwaters (from lakes and river), wastewaters

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**Table 2**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Relative response (%)</th>
<th>Proposed method</th>
<th>Literature [16]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>o-Cresol</td>
<td>64</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>p-Cresol</td>
<td>10</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>p-Chlorophenol</td>
<td>40</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>Catechol</td>
<td>18</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Hydroquinone</td>
<td>10</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>p-Anisophenol</td>
<td>8</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>p-Nitrophenol</td>
<td>8</td>
<td>&lt;1</td>
<td></td>
</tr>
</tbody>
</table>

NA: not available.

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**Table 3**

<table>
<thead>
<tr>
<th>Consumption of reagents and sample per determination in different procedures for determination of phenols</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proposed method</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>4-AAP (mg)</td>
</tr>
<tr>
<td>K$_3$[Fe(CN)$_6$] (mg)</td>
</tr>
<tr>
<td>CHCl$_3$ (ml)</td>
</tr>
<tr>
<td>Sample (ml)</td>
</tr>
</tbody>
</table>

* Flow-injection system with continuous reagent addition.
from paper/cellulose and wood industries as well as labora-
tory wastes treated by a photocatalytic procedure involving
TiO₂ and solar radiation. As shown in Table 5, recoveries
within 93.3 and 106% were obtained. Results agreed with
that obtained by the literature method [14] at the 95% con-
fidence level (Table 6).

4. Conclusions

The proposed flow system yielded better analytical fea-
tures in comparison to previously reported procedures for
phenols determination, associating the sensitivity achieved
by the procedures that exploit analyte preconcentration with
the advantages of those without concentration (precision
and sample throughput). The detection limit is suitable for deter-
miming phenols in natural and wastewaters, attending the re-
quirements of environmental controlling agencies. Reagent
consumption and toxic-waste production are quite reduced
in comparison to both flow-based and batch methods. In ad-
inition, the employment of toxic organic solvents is avoided.

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