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SPECTROPHOTOMETRIC DETERMINATION OF PHENOL AND RESORCINOL BY REACTION WITH *p*-AMINOPHENOL

KARIM D. KHALAF, BERWEEN A. HASAN, ANGEL MORALES-RUBIO and MIGUEL DE LA GUARDIA*
Departamento de Química Analítica, Facultad de Química, Universidad de Valencia, Dr. Moliner 50, 46100 Burjassot, Valencia, Spain

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Summary—Based on the reaction with *p*-aminophenol, a series of procedures have been developed for the spectrophotometric determination of phenol and resorcinol. Three basic approaches have been studied: (i) a batch procedure, using the dissolved oxygen as oxidant, (ii) a stopped-flow procedure by use of KIO_4 as oxidant and (iii) a flow injection (FI) procedure developed in the presence of KIO_4. Phenol can be accurately determined at 626 nm, in the stopped-flow mode, after a reaction time of 45 min in 0.005*M* NaOH, 0.004*M* KIO_4 and 500 μg/ml of PAP. The development of a manifold, which incorporates a sample parking, is a convenient approach which makes it possible to measure, in the stopped-flow mode, four solutions in one hour. The limit of detection of this method corresponds to 64 ng/ml of phenol Resorcinol can be determined by FI at 540 nm in 0.006*M* NaOH, 0.0002*M* KIO_4 and 50 μg/ml PAP with a limit of detection of 6.6 ng/ml and a sample throughput of 300 injections per hour. A combination between the FI procedure for the determination of resorcinol and the stopped-flow procedure for phenol determination provides accurate results in the analysis of spiked samples containing both phenol and resorcinol.

Phenol and resorcinol (*m*-hydroxy benzene) are compounds widely used in the production of formaldehyde resins, such as bakelite, and are also employed in pharmaceuticals, crosslinking agents for neoprene, rubber tackifier, adhesives for wood veneers and rubber to textile composites and on the manufacturing of many other organic products. Industrial plants, in which phenol and resorcinol are employed, are considered as potential sources of pollution. These two phenolic compounds are considered as air and water pollutants due to their biological effects on humans, causing dermatitis, erosion of the skin, eczema, irritation of respiratory organs, digestive disturbances, symptoms of blood degeneration, emaciation, nephritis, gangrene and jaundice.[1] Automobile exhaust, tobacco smoke and petrol refineries also contribute to the emission of phenol and resorcinol to the environment.

The direct contamination by phenols also causes secondary reactions in the environment and, for example, chlorophenols are produced when phenol-polluted waters are chlorinated during their purification process[2] causing an unpleasant taste in potable water supplies.

Many procedures have been developed to carry out the analytical determination of phenol and resorcinol, Gas chromatography (GC) has been used in this field, but difficulties have been experienced, because these compounds are highly polar and have low vapour pressures at ambient temperature. Furthermore, phenolic compounds have similar chemical and physical properties and require high-resolution GC with selective stationary phases for their separation.[3–7]

High performance liquid chromatography has been used for the determination of phenol and resorcinol. Phenol has been eluted and determined in the presence of other phenolic compounds by using the UV-detection (215 nm).[8] It has been determined in indoor air samples using fluorometric detection.[9] Resorcinol has been determined in cream hair dye by HPLC with UV-detection (280 nm)[10] and in human serum and urine by amperometric detection using a vitreous-carbon electrode.[11] Phenol and resorcinol together, have been separated and determined in tobacco smoke by HPLC

*Author for correspondence.

using fluorometric detection[12] and in pharmaceutical formulations with UV-detection (254 nm).[13]

Synchronous fluorescence spectroscopy has been employed for the determination of phenol and resorcinol in pigments[14] and synchronous derivative spectrofluorometry has provided the simultaneous determination of both phenol and resorcinol.[15]

A lot of data have been reported about spectrophotometric methods for the determination of these two compounds by using different derivatization techniques. Resorcinol reacts with cathecol to give coloured species which absorb at 558 nm.[16] Resorcinol and phenol can be treated with 2% HIO_4 and the reaction products measured at 380 nm.[17] Phenol can be diazotized with *p*-nitroaniline to give red complexes which can be measured at 530 nm.[18]

In recent years, the possibilities for increasing the sample throughput and the automation of the analytical procedures have been achieved by using the flow injection analysis (FIA) methodology.[19,20] A FIA spectrophotometric method was used for the determination of phenol in surface water after in-line condensation with 4-amino-antipyrine (4-AAP) with subsequent oxidation to yield a coloured species which absorbs at 470 nm.[21] Other FIA spectrophotometric studies on the determination of phenols in waters with 4-AAP and 3-methyl-2-benzothiothiazoline hydrazone (MBTH) have shown that the sensitivity of the MBTH method is about four times higher than that of the 4-AAP reaction. Also, the reaction product of phenol with MBTH exhibits an absorption maximum at 510 nm which also improves the selectivity of this determination, as compared with the 4-AAP method.[22]

PAP has been successfully employed for the derivatization of several carbamate pesticides, which can be hydrolysed to phenolic compounds, under optimum conditions, and have been coupled with PAP to produce different indodyes. Using PAP, a series of spectrophotometric procedures have been developed for the determination of propoxur,[23] formetanate,[24] ethiophencarb[25] and carbaryl.[26]

In the present paper, an automated procedure is proposed for the spectrophotometric determination of phenol and resorcinol by reaction with *p*-aminophenol (PAP), after subsequent oxidation by the dissolved oxygen or by potassium metaperiodate in an appropriate alkaline medium. The reaction can be carried out in batch, in stopped-flow and in FI and in this paper the three approaches are compared. The reaction products have been spectrophotometrically measured at 540 nm for resorcinol and at 626 nm for phenol.

EXPERIMENTAL

Apparatus

A Hewlett-Packard 8452A diode array spectrophotometer with HP 89530A MS-DOS UV/vis software, with a response time of 0.1 sec, and a flow cell with 50-μl internal volume and 1-cm pathlength, was used for the absorbance measurements.

A three-channel manifold (Fig. 1) was used for the flow injection spectrophotometric determination of phenol and resorcinol with PAP. The manifold includes a Gilson P2 Minipulse peristaltic pump to transport the carrier stream, and a Rheodyne type 50 rotary injection for the introduction of samples. Flexible vinyl tubing of 1.52-mm internal diameter was used for the peristaltic pump and it allowed carrier flow rates up to 3.5 ml/min in each channel. The reaction coils, R1 and R2, were Teflon, with an internal diameter of 0.8 mm.

In all cases, Channel 1 was used to transport PAP and Channel 2 to introduce potassium metaperiodate. Channel 3 was employed to transport a sodium hydroxide solution in which samples and standards were injected.

All connections between any two channels were by Y-shaped merging zones to ensure good mixing between reagents.

Because the reaction between phenol and PAP is very slow the manifold shown in Fig. 1(B) was constructed for measurements carried out in the stopped-flow mode to avoid

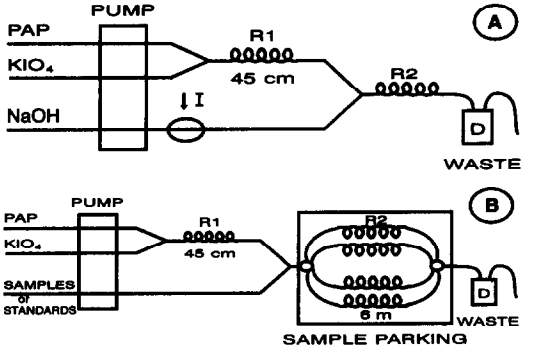


Fig. 1. Manifolds employed for the FI spectrophotometric determination of phenol and resorcinol with PAP (1A) and for the stopped-flow procedure (1B). I: injection valve, D: detector.

the use of long dead times in the spectrophotometric measurement of samples and standards. The manifold employed was modified to include sample parking, which provides the successive measurement of a series of samples and standards after as short as possible time delay.

The manifold incorporates two 5-way directional valves which include one inlet and four alternative outlets. Both directional valves are employed to introduce each solution into the different stand-by coils in which the solutions are allowed to stand in order to complete the reaction between phenol and PAP.

This system is similar to that employed for FI analysis but channel 3 is employed to transport standard solutions or samples. The length of the stand-by coils to store an appreciable amount of sample, which can be used to rinse the detection flow cell and to avoid dispersions during the measuring time was established to be 6 m.

Reagents and solutions

All reagents are of analytical grade: phenol and resorcinol were supplied from Aldrich (Germany), 4-aminophenol (PAP) from Fluka (Switzerland), potassium metaperiodate and sodium hydroxide were obtained from Probus (Spain).

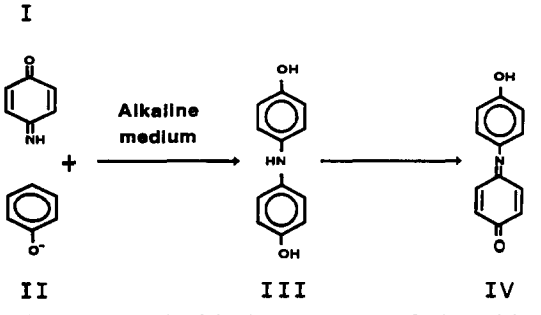
Standard stock solutions of 100 μg/ml phenol and resorcinol were prepared by the dissolution of the reagents in high purity distilled water. Working standard solutions of 50 μg/ml of both compounds were prepared by diluting the stock solutions with distilled water. The standard solution of PAP (1000 μg/ml) was prepared daily by dissolving 0.5 g of PAP in 500 ml of boiled and cooled distilled water. Other solutions with low PAP concentrations were prepared by the same procedure. Boiling the distilled water for 10 min is very important to avoid the oxidation of PAP by the dissolved oxygen.

Stock solutions of potassium metaperiodate (0.01286M) and 1M NaOH were prepared by dissolving the corresponding solid products in distilled water.

RESULTS AND DISCUSSION

Reaction of PAP with phenol and resorcinol

The reaction between phenol 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.[27] Therefore, the corresponding phenolate (II) reacts with (I) through the electrophilic attack on C4 of the phenolate to produce the corresponding leucodye (III) which is oxidized to an indodye (IV) and presents a maximum absorption intensity at 626 nm according to the following scheme:



As compared with the spectrum of phenol in 0.005M sodium hydroxide, which has two absorption bands at 236 and 288 nm, the spectrum of the reaction product of phenol with 500 μg/ml of PAP provides an absorption maximum of 626 nm with a 2-fold greater sensitivity than that obtained for phenol, thus indicating that the above reaction provides a higher selectivity and sensitivity than that corresponding to the direct spectrophotometric determination of phenol in alkaline medium. Because of that, based on the reaction between PAP and phenol, a spectrophotometric procedure for the determination of phenol has been developed which has been assayed both in batch and in flow modes, considering stopped-flow and FIA.

Resorcinol (1,3-dihydroxybenzene) reacted very rapidly with PAP producing a coloured species which gave an absorption maximum at 540 nm. The presence of two hydroxyl groups on the benzene ring increases the electron density and facilitates the electrophilic attack by PAP. This reaction has been investigated using highly diluted concentrations of reagents, in batch, stopped-flow and FI modes, in the same way as indicated before for phenol.

Spectrophotometric determination of phenol and resorcinol with PAP

The best experimental conditions for the determination of phenol were established for sodium hydroxide (from 0.005 to 0.6M) and PAP (from 25 to 400 μg/ml) by altering one variable at a time, using the dissolved oxygen as oxidizing agent, and studying the absorbance at 626 nm as a function of time. The obtained results show that 0.02M NaOH and 50 μg/ml PAP are the concentrations that can give a

Table 1. Analytical features of the procedures developed for the determination of phenol and resorcinol with PAP

Parameter	Batch procedure		Stopped-flow procedure		FI procedure	
	Phenol	Resorcinol	Phenol	Resorcinol	Phenol	Resorcinol
Regression equation	$A = 0.0142 + 0.00926C$	$A = 0.009 + 0.0386C$	$A = 0.0165 + 0.0969C$	$A = 0.004 + 0.0397C$	$A = 0.001 + 0.00345C$	$A = 0.004 + 0.0274C$
Concentration range ($\mu g/ml$)	0.7–20	0.06–8	0.6–20	0.07–8	1–20	0.06–8
Regression coefficient	0.9996	0.9999	0.9999	0.9999	0.9997	0.9991
LOD ($k = 3$) (ng/ml)	71	6	64	7.5	0.97	6.6
RSD (C)	0.96% (15)	0.4% (4)	4.7% (15)	0.5% (4)	0.94% (15)	0.3% (4)
Sample throughput (hr^{-1})	0.7	12	4	90	80	300

(C): Concentration in μg/ml.
LOD: Limit of detection for a probability level of 99.6% ($k = 3$).
RSD: Relative standard deviation for 5 independent analysis of a sample containg C in μg/ml. The concentration is indicated in brackets.

higher absorption intensity at 626 nm in the minimum interval of time.

The development of the colour of the phenolate-PAP indodye from a mixture containing 20 μg/ml phenol and 50 μg/ml PAP in 0.02*M* NaOH gave evidence that the colour develops during the first 90 min and remains stable for a long time. The absorption intensity of the reaction product provides twice the enhancement of the sensitivity corresponding to the direct spectrophotometric determination of phenol as phenolate.

The regression equation obtained, from a series of phenol standards, and the analytical figures of merit of this procedure are summarized in Table 1 in which are also summarized the main performances of the flow procedures developed for phenol determination and those of the procedures assayed for resorcinol, in order to make an effective comparison between all approaches.

The batch procedure allows the measurement of phenol in waters at a concentration level of parts per million and is very economical when compared to other methods, such as those based on the use of HPLC. The only disadvantage of the method is the long time required to achieve maximum absorption. Increasing the concentrations of both NaOH and PAP, in an attempt to accelerate this reaction, caused a rapid degradation of PAP which affected the absorption intensity of the reaction product.

In the batch method, for the spectrophotometric determination of resorcinol with PAP, the effect of NaOH concentrations (from 0.0009 to 0.004*M*) on the absorbance at 540 nm as a function of time, showed that the reaction yield increases when the NaOH concentration increased (Fig. 2). However, the stability of the indodye in the presence of a NaOH concentration of 0.004*M* was very poor and so 0.002*M* NaOH seemed more appropriate to obtain an adequate sensitivity and a reasonably stability of the reaction.

Figure 3 shows the effect of different concentrations of PAP on the absorbance at 540 nm, using 0.002*M* NaOH and 5 μg/ml resorcinol. From this figure it can be concluded that 50 μg/ml PAP provided a high sensitivity and good stability of the reaction product. The inset in this figure shows that using a 50 μg/ml concentration of PAP the reaction required less than 5 min and thereafter the reaction product remained stable.

A typical calibration graph obtained from a series of standard solutions of resorcinol, and the main figures of merit of the procedures developed for the spectrophotometric determination of resorcinol are summarized in Table 1. From these results it can be concluded that the reaction with PAP provided good sensitivity and selectivity as compared with the direct spectrophotometric determination of resorcinol in alkaline medium, which provided a maximum absorbance at 286 nm with an extinction coefficient nine times lower than that found for the determination with PAP at 540 nm.

Stopped-flow determination of phenol and resorcinol

The batch methods for determination of phenol and resorcinol were adapted, by using the stopped-flow approach, and introducing KIO_4 as oxidizing agent, instead of the use of the dissolved oxygen.

An intensive study was carried out on the effects of NaOH concentrations (from 0.005 to 0.1M), PAP (from 50 to 1000 μg/ml), and potassium metaperiodate (from 0.001 to 0.01826M) on the absorption intensity at 626 nm of the indodye of phenol with PAP.

Results obtained show that 0.005M NaOH, 500 μg/ml PAP and 0.004M KIO_4 are the best conditions which provided the highest absorbance measurements. The colour development was studied by using a phenol concentration of 20 μg/ml in the above conditions and the results indicated that the colour developed within the first 45 min and remained constant for more than 15 min providing a 10-fold enhancement of the analytical sensitivity compared with the batch procedure. The introduction of KIO_4 changed the experimental conditions required to obtain the maximum absorbance at 626 nm in the minimum required time.

The use of the sample parking in which all the reaction mixtures are stored and then sequentially measured, in order to avoid long dead times, allows all the measurements to be carried out in less than one hour.

The characteristic features of the procedure developed are summarized in Table 1.

On comparing the batch and stopped-flow methods for the determination of phenol with PAP, it can be seen that when using potassium metaperiodate, both the reaction velocity and the maximum absorption intensity increased, providing a faster and more sensitive analytical procedure. The only drawbacks are that the higher value of the intercept of the calibration line and the poorer repeatability of the absorbance measurements do not provide a better limit of detection.

As it has been mentioned before, the reaction between PAP and resorcinol takes place in the presence of the dissolved oxygen. However, this reaction can be improved by using KIO_4 as oxidizing agent. The effect of NaOH concentrations (from 0.002 to 0.008M), KIO_4 (from 5×10^{-6} to $5 \times 10^{-3}M$) and also the effect of the PAP concentrations (from 25 to 200 μg/ml) were studied in a univariate manner as a function of time in order to develop a stopped-flow procedure for the spectrophotometric determination of resorcinol.

Figure 4, shows the simultaneous effect of both NaOH and KIO_4 on the absorbance of the reaction product between 5 μg/ml resorcinol and 50 μg/ml PAP and, as can be seen, KIO_4 concentrations higher or equal than 0.0002M and NaOH concentrations of the order of 0.006M provide the highest sensitivity.

As indicated in Fig. 5, 50 μg/ml PAP provides the highest absorbance values at 540 nm in the shorter reaction time.

From the results an appropriate set of experimental conditions can be selected which permit the spectrophotometric determination of resorcinol in the stopped-flow mode using a reaction time of 40 sec.

The analytical figures of merit of the recommended procedure are summarized in Table 1.

FI determination of phenol and resorcinol with PAP

The experimental conditions obtained in the stopped-flow mode have been used as a basis to develop FI procedures, using the manifold indicated in Fig. 1(A). In the case of phenol determination, the signal to noise ratio at 626 nm has been improved, by studying the effect of the flow rates of reagents, the injection volume of samples and standards, the length of the reaction coils (R1 and R2) and the effect of temperature (from room temperature, approximately 20–40°). The results show that a flow rate of 2.1 ml/min in each channel, values of R1 = 45 cm and R2 = 12 m and 800 μl of the injection volume gave the maximum absorption intensity at room temperature.

The increase in the temperature of the reaction coil does not increase the absorbance at

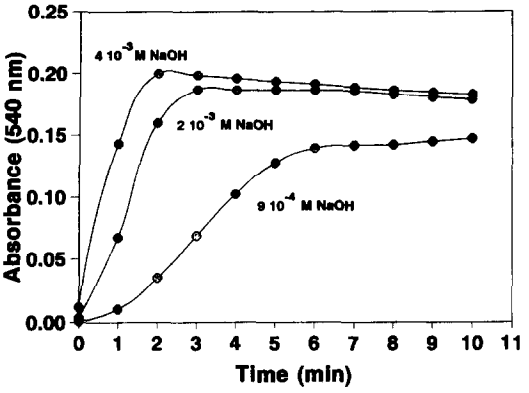


Fig. 2. Effect of NaOH on the reaction between resorcinol and PAP. Experimental conditions: Resorcinol 5 μg/ml, PAP 200 μg/ml, absorption measurements carried out at 540 nm.

626 nm and caused a rapid degradation of PAP which provided a high background and a low sensitivity and stability of the reaction products.

The analytical features of the procedure are summarized in Table 1. On comparing the FI method with the batch and stopped-flow procedures it can be seen that, due to the slow reaction velocity between phenol and PAP, the detection limit obtained by FI is poorer than those obtained in the previous two methods. However the FI procedure is faster than the batch and stopped-flow procedures.

The experimental conditions, previously established in the stopped-flow mode, for the determination of resorcinol with PAP have also been used as a basis for the development of a FI method of this compound. Using the manifold indicated in Fig. 1(A) the effect of the different FI parameters on the reaction between resorcinol and PAP; such as the flow rates of reagents (from 0.5 to 3.5 ml/min in each channel), length of the reaction coil R2 (from 1 to 4 m) for a fixed length of R1 corresponding to 45 cm, and injection volume (from 100 to 700 μl) were studied. The results showed that a flow rate of 3.5 ml/min in each channel, a R2 length of 3 m and an injection volume of 500 μl were the best conditions which provided the highest absorbance at 540 nm with the lowest noise.

A standard calibration line, obtained for a series of resorcinol standards and the main analytical figures of merit of the developed procedure are indicated in Table 1.

Study of the interferences of other phenols on the determination of resorcinol

In the experimental conditions, previously established for the determination of resorcinol, by FI, the effect of several common phenolic compounds (such as phenol, *o*-cresol, *m*-cresol and 2-naphthol) on the FI absorbance peaks height of resorcinol was studied.

Figure 6 shows the effect of increasing concentrations of each one of the interferent compounds studied on the peak height at 540 nm of 5 μg/ml resorcinol and, as it can be seen, small interferences were found only for interferent to resorcinol proportions equal or higher than 10:1 indicating that, although PAP is a general reagent for the derivatization of phenolic compounds, the differences in reactivity between

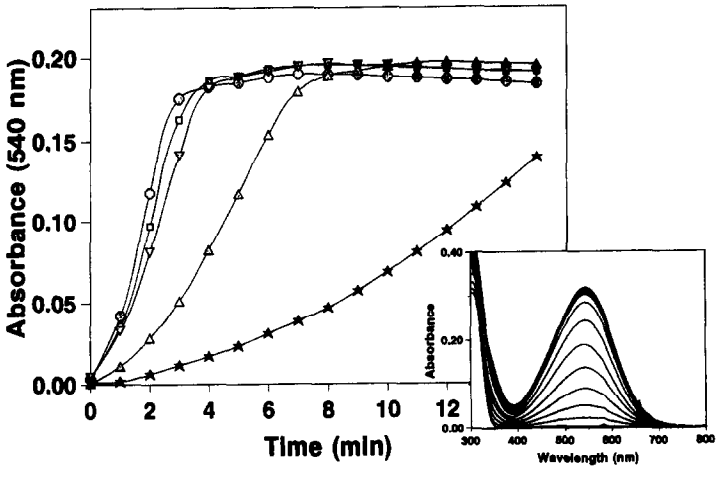


Fig. 3. Effect of PAP concentration on the absorbance of the reaction product with resorcinol as a function of time. Experimental conditions: Resorcinol 5 μg/ml, NaOH 0.002*M*. Absorbance measurements were carried out at 540 nm (★ 10 μg/ml, ▲ 25 μg/ml, ▼ 50 μg ml, ■ 75 μg ml, ● 100 μg/ml PAP). Inset: Development of the colour of the reaction product of 8 μg/ml resorcinol in 0.002*M* NaOH and in the presence of 50 μg/ml PAP. Scans were obtained at 1-min intervals of time.

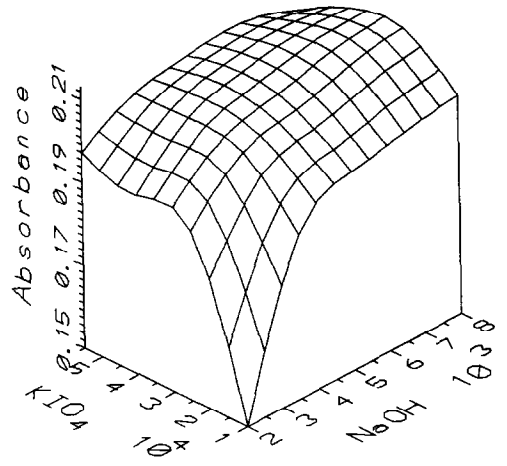


Fig. 4. Effect of the KIO_4 and NaOH concentration on the reaction between 5 μg/ml resorcinol and 50 μg/ml PAP measured at 540 nm after 50 sec.

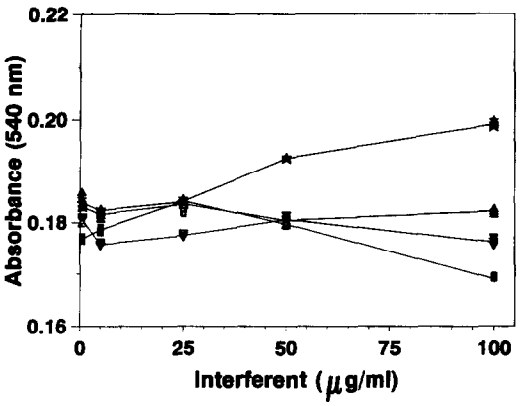


Fig. 6. Interference of phenolic compounds on the FI-spectrophotometric determination of resorcinol. Experimental conditions: NaOH 0.006M; KIO_4 0.0002M, PAP 50 μg/ml (★ o-cresol, ▲ m-cresol, ▼ 2-naphthol and ■ phenol).

different phenols and the characteristic wavelength of the reaction products obtained with PAP provide a convenient selectivity for the spectrophotometric determinations of these compounds. In this sense, data summarized in Table 2 indicates the different maximum, absorbance wavelength of the reaction products between PAP and different phenolic compounds.

Determination of phenol in the presence of resorcinol

Because the reaction product between resorcinol and PAP, in the presence of high concentrations of KIO_4 and PAP, decomposes rapidly the slow reaction speed of phenol with PAP and the fast reaction of resorcinol should allow determination of phenol, in the stopped-flow mode, in the presence of resorcinol.

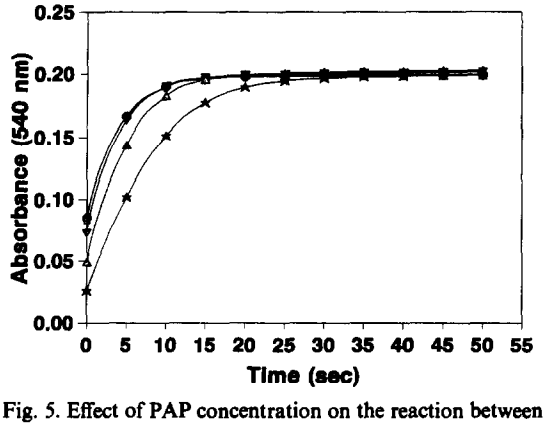


Fig. 5. Effect of PAP concentration on the reaction between PAP and resorcinol as a function of time. Experimental conditions: resorcinol 8 μg/ml, NaOH 0.006M, KIO_4 0.0002M (★ 25 μg/ml, ▲ 50 μg/ml, ▼ 75 μg/ml, ■ 100 μg/ml, ● 200 μg/ml PAP).

To investigate the determination of phenol in the presence of resorcinol a series of synthetic mixtures of phenol and resorcinol were prepared in distilled water and the absorbance measured under the best condition established for phenol by the stopped-flow procedure, using the manifold indicated in Fig. 1(B). The calibration graph used was that obtained from standards containing phenol only. Data in Table 3 show that low concentrations of resorcinol did not interfere in the determination of phenol and recovery percentages of the order of 100% can be found for phenol concentrations ranging from 5 to 20 μg/ml.

The least squares fitting of calibration curves obtained with pure phenol standards and phenol standards containing additional concentrations of resorcinol, in an order of magnitude of 10% (w/w) of the phenol content, provides comparable regression equations:

$$A = 0.277 + 0.0712C \text{ (C in μg/ml phenol)}$$
$$A = 0.291 + 0.0719C \text{ (C in μg/ml phenol in the presence of resorcinol).}$$

Table 2. Maximum absorbance wavelength of the PAP derivatives of different phenolic compounds

Compound	Wavelength (nm)
Resorcinol	540
m-aminophenol	575
1-naphthol	596
2-isopropoxyphenol	600
8-hydroxyquinoleine	610
o-cresol	614
phenol	626
m-cresol	632

Table 3. Spectrophotometric determination of phenol with PAP in the stopped-flow mode in the presence of resorcinol

Phenol added ($\mu g/ml$)	Resorcinol added ($\mu g/ml$)	Phenol found ($\mu g/ml$)	SD (%)	Recovery (%)
5	0.5	5.2 ± 0.1	2	104
	1.5	4.84 ± 0.03	0.6	97
10	1.0	10.3 ± 0.1	1	103
	3.0	9.43 ± 0.06	0.6	94
15	2.0	15.4 ± 0.1	0.6	103
	4.5	13.5 ± 0.1	0.7	90
20	1.5	20.3 ± 0.4	1.8	102
	6.0	17.6 ± 0.1	0.6	88

SD: Standard deviation of 5 independent analyses.

However, when experiments were carried out with synthetic mixtures of phenol and resorcinol measured in the FI mode, using the best conditions for the determination of phenol the fast reaction of resorcinol, which takes place with a high yield in only few seconds, interfered with the recovery of phenol.

Determination of resorcinol in the presence of phenol

As previously indicated the FI spectrophotometric determination of resorcinol with PAP is free for interference of phenol and other phenolic compounds.

Under the optimum conditions found for the resorcinol determination, synthetic mixtures of resorcinol and phenol were measured by FI, against a standard calibration graph obtained with standards containing only resorcinol by using the manifold indicated in Fig. 1(A). Average recoveries ranging from 99 to 104% were found for solutions containing from 1.5 to 6 μg/ml resorcinol, in the presence of phenol concentrations ranging from 0.5 to 2.5 μg/ml (see Table 4); which indicated that the phenol did not interfere.

Simultaneous determination of phenol and resorcinol

Based on the results reported in the previous sections, a method is proposed for the determination of both phenol and resorcinol in the same sample by a combination of the FI and the stopped-flow procedures.

Table 4. Spectrophotometric determination of resorcinol with PAP in the FI mode and in the presence of phenol

Resorcinol added ($\mu g/ml$)	Phenol added ($\mu g/ml$)	Resorcinol found ($\mu g/ml$)	Recovery (%)
1.5	0.5	1.54 ± 0.03	103
3	2	3.11 ± 0.04	104
4.5	2.5	4.57 ± 0.01	101
6	1.5	5.92 ± 0.01	99

The methodology consists on the selective FI determination of resorcinol at 540 nm in 0.006*M* NaOH 0 0002*M* KIO_4 and 50 μg/ml PAP. The samples are then allowed to stand for 90 min (in these conditions the reaction between phenol and PAP is extremely slow), in the manifold of Fig. 1(B) and then measured at 626 nm in the stopped-flow mode against two separate calibration graphs of phenol and resorcinol, obtained in the same conditions and for which the following two regression equations were obtained

$A_{626} = 0.050 + 0.00652C$ (C in μg/ml of resorcinol).

$A_{626} = 0.014 + 0.0189C$ (C in μg/ml of phenol).

The reaction conditions employed in this case are the better ones found for the resorcinol determination and therefore the reaction product between resorcinol and PAP did not decompose in the measurement time.

The above equations provide the corresponding coefficients for the relation between the absorbance of the samples and the concentration of resorcinol and phenol. The general expression

$$A_{626} = 0.032 + 0.00652C_R + 0.0189C_{Ph}$$

in which C_R and C_{Ph} are the concentrations of resorcinol and phenol respectively can be used to determine the concentration of phenol from the experimental values of the absorbance at 626 nm measured in the stopped-flow mode and from the concentration of resorcinol, previously determined by FI.

Data in Table 5 summarizes the results found in the analysis of synthetic samples containing both phenolic compounds and, shows that good recovery percentages were found in all cases.

Comparison of the different procedures developed for the spectrophotometric determination of phenol and resorcinol with PAP

From the figures of merit of the different approaches to the determination of phenol and resorcinol with PAP (see Table 1) it can be concluded that the spectrophotometric determination of resorcinol is more sensitive than that of phenol because the reaction with PAP takes place quantitatively in less than one minute, whereas the slow reaction between phenol and PAP requires a long reaction time.

Table 5. Spectrophotometric determination of phenol and resorcinol in a same sample by reaction with PAP

Resorcinol added ($\mu g/ml$)	Resorcinol found ($\mu g/ml$)	Recovery (%)	Phenol added ($\mu g/ml$)	Phenol found ($\mu g/ml$)	Recovery (%)
1.5	1.39 ± 0.07	93	5	4.84 ± 0.03	97
3	2.87 ± 0.04	96	10	9.43 ± 0.06	94
4.5	4.25 ± 0.05	94	15	13.5 ± 0.1	90
6	5.3 ± 0.1	89	20	17.6 ± 0.1	88

Note: Resorcinol was determined selectively in the FI mode, using a 0.006*M* NaOH, 0.0002*M* KIO_4 and 50 μg/ml PAP concentrations againts a series of standards containing only resorcinol. After that the phenol concentration was determined in the same medium but in the stopped-flow mode using a reaction time of 90 minutes, and a calibration line with coefficients obtained from series of pure phenol and pure resorcinol standards.

The differences between the reaction speed of both compounds with PAP is the reason for obtaining so different analytical features by stopped-flow and FI procedures. For the determination of resorcinol, the FI procedure is the more convenient method because of its speed and the small loss of sensitivity. Moreover it provides a limit of detection comparable to those obtained by the stopped-flow and batch procedures, for reaction times of 40 sec and 13 min respectively.

The phenol determination by FI is very fast but it provides a 30-times lower sensitivity and 15-times higher limit of detection than the stopped-flow procedure and the presence of resorcinol interferes. Therefore the stopped-flow procedure is the most appropriate for the determination of low concentrations of phenol and the use of the sample parking, in the stopped-flow mode is an indispensable tool to reduce the time required for the analysis of samples.

An intelligent combination between both, the FI procedure for the determination of resorcinol and the stopped-flow approach for phenol determination can be used in order to determine phenol and resorcinol in a same sample, as evidenced in the aforementioned sections.

Comparison of the developed procedures with other spectrophotometric methods

The developed methodology is very adequate for the determination of both, phenol and resorcinol in water samples at a concentration level of traces and without requiring any previous separation step nor a carefully "clean up" of samples.

As compared with previously published papers, involving the FI-spectrophotometric determination of phenolic compounds with other reagents, such as 4-AAP[21] and MBTH,[22] the reaction with PAP provided a poorer limit of detection but a higher selectivity not requiring a solvent extraction nor a steam distillation of samples, as indicated in the previous papers, and the dynamic range employed for the determination of phenol with 4-AAP (0.05 to 15 ppm), MBTH (0.02 to 5 ppm) and PAP (0.6 to 20 ppm) are of the same order.

On the other hand, PAP is a very sensitive reagent for resorcinol determination and, as can be seen in Fig. 6 and in Table 2, the reaction between PAP and phenolic compounds is more selective than those of other reagents employed for the derivatization of phenols.

REFERENCES

1. M. B. Jacobs, *The Analytical Chemistry of Industrial Poisons, Hazards and Solvents*, Interscience Publishers Inc., New York, 1949.
2. E. F. Jr. Mohler and L. N. Jacobs, *Anal. Chem.*, 1957, **29**, 1369.
3. G. Dielman, S. Meier and U. Rapp, *J. High Resoln. Chromatogr. Chromatogr. Commun.*, 1979, **2**, 343.
4. F. P. DiSanzo, *ibid.*, 1981, **4**, 649.
5. M. T. Dimitriev and Yu. B. Suvorova, *Gig. Sanit.*, 1991, **2**, 85.
6. L. N. Amankwa and W. G. Kuhr, *Anal. Chem.*, 1991, **63**, 1733.
7. E. J. Nanni, M. E. Lovette, R. D. Hicks, K. W. Fowler and M. F. Borgerding, *J. Chromatogr*, 1990, **505**, 365.
8. A. Brega, P. Prandini, C. Amaglio and E. Pafumi, *ibid.*, 1990, **535**, 311.
9. C. H. Risner and S. L. Cash, *Environ. Technol.*, 1990, **11**, 345.
10. D. Ehlers, *Lebensmittelchem. Gerichtl. Chem.*, 1983, **37**, 75.
11. F. Palmisano, A. Guerrieri and P. G. Zambonin, *Ann. Chim. (Rome)*, 1986, **76**, 419.
12. C. H. Risner and S. L. Cash, *J. Chromatogr. Sci.*, 1990, **28**, 239.
13. A. K. Sen, *Indian-Drugs*, 1990, **27**, 316.
14. Z. Li and R. Yu, *Yaoxue-Xuebao*, 1989, **24**, 127.
15. R. Xu and J. Xu, *Fenxi-Huaxue*, 1991, **19**, 32.

16. R. T. Sane, B. D. Bamane and J. G. Mhalas, *J. Indian-Chem. Soc.*, 1983, **60**, 381.
17. J. P. Rawat and K. P. S. Muktawat, *Microchem. J.*, 1984, **30**, 289.
18. M. Ernszt, *Lebensmittelindustrie*, 1990, **37**, 206.
19. J. Růžička and E. H. Hansen (eds.), *Flow Injection Analysis*, 2nd Edn. Wiley, New York, 1988.
20. M. Valcarcel and M. D. Luque de Castro, *Flow Injection Analysis*. Principles and Applications, Ellis Horwood, Chichester, 1987.
21. J. Möller and M. Martin, *Z. Anal. Chem.*, 1988, **329**, 728.
22. W. Frenzel and J. Oleksy-Frenzel, *Anal. Chim. Acta*, 1992, **261**, 253.
23. K. D. Khalaf, J. Sancenón and M. de la Guardia, *ibid.*, 1992, **266**, 119.
24. K. D. Khalaf, J. Sancenón and M. de la Guardia, *Talanta*, 1993, 40, 1173.
25. K. D. Khalaf, J. Sancenón and M. de la Guardia, *J. Anal. Chem.*, 1993, 347, 52.
26. K. D. Khalaf, A. Morales-Rubio and M. de la Guardia, *Anal. Chim. Acta*, 1993, **280**, 231.
27. K. C. Brown, J. F. Corbett and R. Labinson, *J. Chem. Soc., Perkin Trans.*, 1978, **2**, 1292.