

Rapid determination of total phenols in seawater by 4-aminoantipyrine colorimetry

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A rapid and efficient 4-aminoantipyrine (4-AAP) colorimetric method without any cleanup step to determine total phenols in seawater is described. Efficiency of the method for seawater using external addition of phenol concentrations with working range of $5\text{--}100\ \mu\text{g.l}^{-1}$ is 86.7-102.9%. Efficiency at the lowest detection limit of $1\ \mu\text{g.l}^{-1}$ is $81.5 \pm 18.5\%$. Efficiency of 4-AAP with distillation method is comparatively low. Several water samples from Ulhas estuary having salinity in 5.3-35.8 ppt range were analysed to evaluate suitability of the method for pollution monitoring programme. The method is found to be rapid, convenient and efficient for analysing coastal and estuarine water samples during a marine pollution monitoring programme.

Phenols are categorised as highly persistent and toxic pollutant in marine environment^{1,2}. They enter coastal waters through decomposition of attached algae and phytoplankton and wastewater discharges from a variety of chemical industries³⁻⁵. Phenolic compounds have significant detrimental effects on water quality and they are toxic to aquatic life^{3,6-8}. Individual phenols in seawater are determined by UV, differential UV, fluorescence, GLC, GCMS and HPLC⁹⁻¹⁷. Total phenols in seawater are determined using several cleanup and preconcentration steps such as liquid-liquid extraction, adsorption on macroporous resins and distillation preferably followed by colorimetry^{10,18-27}. As these methods are time consuming due to complicated workup procedure and instrumentation, they may not be suitable for the analysis of large number of samples during a marine pollution monitoring programme. In view of this, a rapid, convenient and efficient method of 4-aminoantipyrine (4-AAP) colorimetry without performing any cleanup or preconcentration step is being proposed and the results are described in this communication.

Materials and Methods

Assay of phenol was done by bromate-bromide method²⁸. Several phenol concentrations ($1\text{--}100\ \mu\text{g.l}^{-1}$) which were prepared using phenol free distilled water, were analysed for phenol content as follows:

Reagents²⁷ NH_4OH (12 ml), phosphate buffer solution (10 ml), 4-aminoantipyrine (4-AAP, 3 ml) and $\text{K}_3\text{Fe}(\text{CN})_6$ (3 ml) were added to the sample (500 ml) in

sequence with stirring after each addition. pH of the solution was adjusted to 7.9 ± 0.1 during addition of the buffer solution. The orange coloured complex thus formed after standing for five minutes was extracted using chloroform (25 ml). The separated organic layer was dried over anhydrous sodium sulphate and its absorbance was measured at 460 nm on Perkin Elmer Lambda 5 UV/VIS spectrophotometer. The variations in absorbance values were ± 0.002 . The concentration factor was calculated on the basis of average absorbance values.

Efficiency of recoveries of phenol ($5\text{--}100\ \mu\text{g.l}^{-1}$) from seawater was determined by external addition as described above. The recoveries for 1 and $2\ \mu\text{g.l}^{-1}$ solutions were determined¹⁰ by putting additional contamination of phenol ($20\ \mu\text{g.l}^{-1}$). Efficiencies of 4-AAP with distillation method²⁷ for distilled water and seawater samples were also determined for comparison.

Surface and bottom water samples (500 ml) from Ulhas estuary were collected on 13/14 November 1992 at 8 stations (Fig. 1) using Niskin water sampler and analysed immediately as described above. Salinity of the water mass was recorded using Kahlsico, induction salinometer.

Results and Discussion

Coastal seawater contains a variety of salts in dissolved state and suspended particulate matter such as clay, sediment, vegetation and pelagic organisms along with several pollutants namely petroleum hydrocarbons, phenols and urea but it is fairly clean as compared to industrial and domestic wastewaters of which

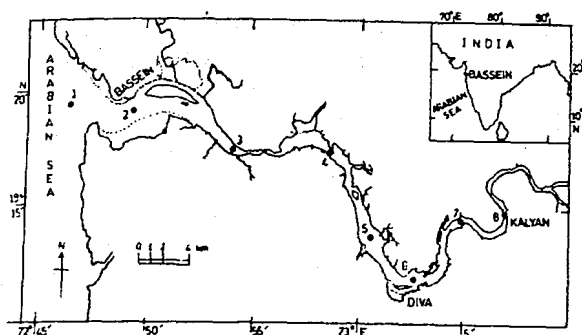


Fig. 1—Map of Ulhas estuary showing sampling stations

constituents generally interfere determination of phenols. Naley¹⁴ reported fluorimetry of seawater without distillation. Therefore, it was proposed to perform 4-AAP colorimetry without any cleanup procedure. It has been reported that phenol concentration of $20\text{--}75\ \mu\text{g.l}^{-1}$ would introduce pathological changes in gills and tissues of fish²⁹, and it was noted³⁰ that a level below $3.5\ \text{mg.l}^{-1}$ would be necessary to prevent most toxic effects to salt water and fresh water aquatic species. It was also estimated³⁰ that a level below $0.3\ \text{mg.l}^{-1}$ would be necessary to control undesirable taste and odour in ambient water. As there occurs multifold dilutions during tides, normally concentration of phenols which would be present in coastal seawater may fall below $100\ \mu\text{g.l}^{-1}$ which was considered as the highest concentration value of interest (C_H) in present study. Assuming that seawater contents may interfere the determination, the lowest concentration of interest (C_L) was decided as $5\ \mu\text{g.l}^{-1}$ while the lowest detection limit (L_D) was kept as $L_D = 0.2(C_L) = 1\ \mu\text{g.l}^{-1}$ which is the L_D for 4-AAP with distillation method also²⁷. Efficiency of 1 and $2\ \mu\text{g.l}^{-1}$ solutions was evaluated from the point of view of criterion of detection. The 4-AAP colorimetry which is one of the rapid and most recommended method has been incorporated in the study^{18,27,31-33}.

Presuming that the recoveries of phenol concentrations from distilled water were 100%, the recoveries of 5, 10, 20, 40, 70 and $100\ \mu\text{g.l}^{-1}$ concentrations from seawater were 93.3 ± 6.7 , 100 ± 2.9 , 92.6 ± 1.2 , 96.4 ± 1.2 , 98.6 ± 0.4 and $98.2 \pm 0.1\%$ respectively (Fig. 2) having standard deviation of $0.918\ \mu\text{g.l}^{-1}$ at $10\ \mu\text{g.l}^{-1}$. The recoveries of $5\text{--}100\ \mu\text{g.l}^{-1}$ phenol solutions from distilled water and seawater by 4-AAP with distillation method²⁷ were 70.6-91.4, and 46.9-89% respectively indicating comparatively low efficiency (Fig. 2). Norwitz *et al.*³⁴ noticed decomposition of some phenols and Strohl³⁵ reported 10-20% loss of volatile phenols, during distillation process. In concurrence with Afghan *et al.*¹⁰ it was observed here that the

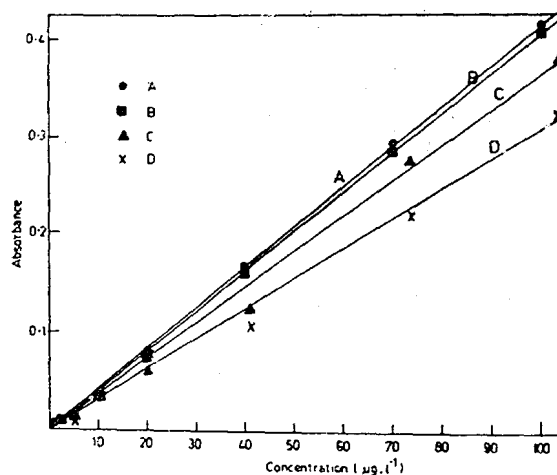


Fig. 2—Recoveries of phenol from (A) distilled water by 4-AAP method, (B) seawater by 4-AAP method, (C) distilled water by 4-AAP with distillation method, (D) seawater by 4-AAP with distillation method

concentrations $< 5\ \mu\text{g.l}^{-1}$ were difficult to determine due to low response of 4-AAP complex. In present study they were determined¹⁰ by addition of contaminant ($20\ \mu\text{g.l}^{-1}$) to extend the method up to $1\ \mu\text{g.l}^{-1}$. Percent recoveries in present study were 81.5 ± 18.5 and 77.3 ± 4.5 for 1 and $2\ \mu\text{g.l}^{-1}$ concentrations respectively. As seen from Fig. 2 interference from contents of seawater appears to be minimum and a minor average difference of 2.24% in efficiencies for distilled water and seawater indicated that technically 100% efficiency may be possible for seawater.

Random error for the working range of $5\text{--}100\ \mu\text{g.l}^{-1}$ was 0.1-6.7%, while bias (systematic error) based on average recoveries was 0-7.5% (Table 1). Thus total error observed in the study was in the range of 1.7-13.4% (Table 1). However, total error which is expressed in terms of the proportion of the result for 1 and $2\ \mu\text{g.l}^{-1}$ solutions i.e. when concentration approaches the lowest detection limit (L_D) was high, i.e. 43.5 and 22.7% respectively (Table 1). Comparatively large variations in low absorbance values may be responsible for amplifying the errors multifold. The method may be performed in 10 minutes as against 50 minutes required for analysis of one sample using 4-AAP with distillation method²⁷. It is necessary to run blanks and standards simultaneously to achieve optimum results.

Alekseeva²⁰ reported that 4-AAP with distillation method for seawater has a linear range of $20\text{--}80\ \mu\text{g.l}^{-1}$ while Goulden *et al.*²¹ mentioned lower detection limit of $0.2\ \mu\text{g.l}^{-1}$ and coefficient of variation of 3.6% at a concentration of $5\ \mu\text{g.l}^{-1}$ for manual as well as automated 4-AAP with distillation method. EPA¹⁸ reported determination of phenols in saline water

by 4-AAP with distillation method at 10.1 ± 0.1 pH for $5-1000 \mu\text{g.l}^{-1}$ concentration range having standard deviation of ± 0.99 and $\pm 4.2 \mu\text{g.l}^{-1}$ at 9.6 and $93.5 \mu\text{g.l}^{-1}$ respectively. APHA³⁶ automated method consists of 4-AAP with distillation technique at 10.1 ± 0.1 pH having lower detection limit of $2 \mu\text{g.l}^{-1}$ with standard deviation of ± 0.5 and $\pm 1 \mu\text{g.l}^{-1}$ at 3.8 and $89 \mu\text{g.l}^{-1}$ respectively. Gouldberg & Weiner³⁷ reported low concentration efficiencies (23-87%) having reproducibilities of 10% for individual phenols by liquid extraction using Kuderna-Danish concentrator followed by GLC determination. Comparison of these reports with present study showed that 4-AAP determination without any cleanup step is the rapid, convenient and efficient method for seawater analysis.

Water samples collected at 8 stations (Fig. 1) from Ulhas estuary, which possesses wide salinity range (5.3-35.8 ppt) were analysed to evaluate utility of the

method for determining phenol content for marine pollution monitoring. All the samples except one corresponding to st. 8 could be analysed with ease. Regarding sample collected at st. 8 during ebb, it was observed that organic layer containing 4-AAP complex did not separate immediately, possibly due to presence of high content of sewage sludge. The concentrations were observed in $0-23.5 \mu\text{g.l}^{-1}$ range having the highest value for extreme upstream station (Table 2). The values decrease with increase in salinity downstream. However, marginally high values were observed near the mouth of the estuary. The phenol concentrations in surface waters during flood were in $0-21.5 \mu\text{g.l}^{-1}$ range which decrease to $1.7-9.8 \mu\text{g.l}^{-1}$ during ebb. Comparatively low values in bottom water and consistently high concentrations at upstream stations (sts 6-8) were observed. It was noticed that average concentration values increased from 0 to $16.4 \mu\text{g.l}^{-1}$ towards upstream stations.

The method was also successfully employed to determine phenols in waters of northwest coast of India during marine pollution monitoring programme³⁸. Surface water samples from 3/4 stations (0-12 km distance from shore) each at 11 transects possessing a varied range of chemical characteristics were analysed. The phenol contents observed were in $0-18.7 \mu\text{g.l}^{-1}$ range. It was noticed that samples from Veral and Versova creeks, having a load of sewage sludge were difficult to analyse.

The method is handy, rapid and efficient enough for employing in marine pollution monitoring programme. Most of the coastal and estuarine water samples can be analysed using the method. However samples having heavy load of sewage are difficult to analyse.

Table 1—Error in the method during efficiency experiments using seawater

Conc. ($\mu\text{g.l}^{-1}$)	Error (%)		
	Random	Bias	Total
1	18.5	25.0	43.5
2	4.5	18.2	22.7
5	6.7	6.7	13.4
10	2.9	0.0	2.9
20	1.2	7.5	8.7
40	1.2	3.6	4.8
70	0.4	1.4	1.8
100	0.1	1.6	1.7

Table 2—Salinity and concentrations of phenols in seawater at various stations from Ulhas estuary

St. no.	Maximum depth (m)	Flood tide				Ebb tide				Average phenol conc. ($\mu\text{g.l}^{-1}$)
		Surface		Bottom		Surface		Bottom		
		Sal. (ppt)	Phenol ($\mu\text{g.l}^{-1}$)	Sal. (ppt)	Phenol ($\mu\text{g.l}^{-1}$)	Sal. (ppt)	Phenol ($\mu\text{g.l}^{-1}$)	Sal. (ppt)	Phenol ($\mu\text{g.l}^{-1}$)	
1	10.5	35.8	ND	35.8	ND	NA	NA	NA	NA	ND
2	6.9	33.9	ND	34.6	6.1	29.6	6.8	35.3	10.8	5.9
3	16.5	24.6	5.1	27.2	5.4	24.4	6.4	26.5	2.7	4.9
4	5.1	23.6	11.2	24.5	5.4	20.1	1.7	22.1	ND	4.6
5	19.0	16.5	4.4	17.3	7.1	16.8	8.3	16.8	ND	6.6
6	4.1	13.1	4.4	15.2	8.3	12.4	9.8	15.7	8.1	7.7
7	10.8	11.8	15.4	13.1	9.3	10.5	6.8	11.1	5.6	9.3
8	26.2	6.7	21.5	8.3	11.5	5.3	9.1	7.5	23.5	16.4

ND—Not detected

NA—Not analysed.

se. The method may be considered as the suitable alternative for 4-AAP with distillation method. Baseline concentrations of total phenols corresponding to unexposed and moderately exposed areas along north-west coast may be considered in the range of 0-22 $\mu\text{g.l}^{-1}$.

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