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Analysis of Phenols in Sea Water by Fluorometry: Direct Analysis of the Water Phase

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Phenol and phenolic compounds may disturb biological processes like growth (AMER & ALI 1969, SHARMA & GHOSH 1965) and development (KORDYLEWSKA 1980a, 1980b). In order to carry out a study of the effects of phenols on aquatic organisms, it was therefore necessary to find a reliable and reproducible analytical method to determine the concentration of individual phenols in seawater.

The use of HPLC (OGAN & KATZ 1981), gas chromatography (COUTTS et al. 1979, SUPELCO Inc. 1975) and specrophotometry (KOPPE et al. 1977, OCHINSKI 1960) for the analysis of phenols in water has been reported, but these methods were impractical for our purpose, since they both require time-consuming extraction and derivatization steps, whereas we needed a rapid procedure which could utilize small sample volumes. We therefore chose fluorometry.

This method (with extraction steps) has been widely used in oil pollution control (LEVY 1971, KEIZER & GORDON 1973, 1974, HORNIG 1974, FRANK 1975, ZITKO 1975, SHIGEHARA et al. 1979, AWAD 1979 and others). Norwegian and British investigators have demonstrated that it is possible to measure fluorescence emmission directly from the sea water phase for samples containing crude oil (ØSTGAARD & JENSEN 1982, JOHN et al. 1982) and Japanese and French investigators have reported determinations of phenol and cresols in fresh water by fluorometry (UCHIYAMA & YAMAGUCHI 1977, YAMAGUCHI & UCHIYAMA 1979, ANDRÉ et al. 1978). In the present work, which is based on that of the Norwegian and Japanese workers, proceedures have been developed for the fast routine analysis of phenols, and also dimethylphenols, in sea water without an extraction step.

METHODS AND MATERIALS.

Apparatus. A Perkin-Elmer fluorescence spectrometer model LS-5 (Perkin-Elmer Ltd. Beaconsfields, Buckinghamshire, UK.) was used for the spectrometric measurements. Emission was observed at 90° to the incident beam. The excitation and emission wavelengths

were controlled by a microprosessor and the wavelength drive was operated by a stepping motor. A xenon discharge lamp pulsed at line frequency 8.3 W (50 Hz) was used as excitation light source. The detection device was a side-on photomultiplyer with S-5 response and UV glass envelope standard for operation to 650 nm. The fluorescence spectra were recorded on a Perkin-Elmer model 561 strip-chart recorder. The cells were commercial 1 cm path-length quartz fluorescence cells made by Hellma, Germany, stoppered by PTFE stoppers, and the temperature of the cell holder was thermostatically controlled. The S/B ratio is influenced by the slit widths, a 10/5 (excitation/emission slit widths in nm) gave a higher (and better) S/B ratio than wider combinations of slit widths. This combination was selected for the analyses.

All phenols investigated were commercially available to a purity of 97% or better. Phenol and the cresols were purchased from Merck and the dimethylphenols from Aldrich-Europe. Phenol was used without further purification (garanteed purity better than 99.5%), but the other compounds were distilled or recrystallized and purity determined by gas chromathography.

Quinine sulphate of pharmaceutical quality was purchased from the Norwegian Medical Depot and purified (PERRIN et al. 1980). The quinine sulphate was disolved in 0.1N sulphuric acid, diluted to 0.2 mg/l (within ±1%) and stored refrigerated in the dark.

96% ethanol delivered by A/S Vinmonopolet was found suitable in the present work.

Preparation of buffer: This was made from 1.380 g (10.0 mmol) sodium dihydrogenphosphate monohydrate, 4.925 g (13.75 mmol) disodium hydrogenphosphate dodecahydrate and 96.85 g distilled water. Including the crystal water, the total amount of water was 100.00 g. The buffer gave pH 6.96 when diluted to five times the volume with distilled water.

Seawater was filtered through a 0.45 µm filter.

Stock solutions containing 50.00 mg of the phenols were prepared by adding 10 ml of an ethanolic solution of the phenol to seawater at +4 ^{0}C in a volumetric flask and diluting to 1000 ml.

The highest concentration to be measured, 15 mg/l, was made from the stock solution. 2.5 ml of this test solution was added to a 25-ml volumetric flask containing distilled water and 0.20 ml 1.00 N sulphuric acid. 5 ml of the buffer was then added and the con-

tents were diluted to the mark with distilled water. The pH was found to be 6.56. The sample to be measured contained one-tenth of the original concentration. This concentration was denoted the "fluorescence concentration".

The cell temperature was regulated at 18 $\pm 0.1^{o}$C. Before measuring the emission, the sample was held in the cellholder for 5 min to attain temperature equilibrium. Slit widths were set to 10 and 5 (nm) for the excitation and emission beam, respectively.

The fluorescence response of 15 mg/l from each phenol (fluorescence concentration 1.5 mg/l) and that of a blank solution, was found. This relationship is called the signal-to-background ratio, or the S/B ratio. The quinine sulphate standard solution was used as a reference and the fluorescense response of this was found at 344/445 nm for excitation and emission wavelength, respectively, under the same conditions.

RESULTS AND DISCUSSION.

In sufficiently dilute solutions, it is known that under non-quenching conditions, fluorescence emission intensity is lineary related to the concentration obeying the relationship:

$$I_f = I_o \Phi_f (1-e^{-\varepsilon CB})$$

where I_o is the incident intensity of radiation, ε is the molar absorptivity, Φ_f is the apparent quantum yield and C is the concentration of the compound contained within a pathlength B in cm. Linearity holds true for concentrations with an absorbance less than 0.05 (89% transmittance) (RHYS WILLIAMS 1980). This means a concentration of maximum 2.47 mg/l for an ethanolic solution of phenol.

For the fluorescence analysis of phenols in water, it can be seen that the emission is nearly constant at pH 3-8 and at temperatures ranging from 10^{o} to 20^{o}C (YAMAGUCHI & UCHIYAMA 1979). In the present work, it was decided to operate at pH´s near neutral and at a temperature of 18^{o}C, since problems with condensation on the sample cells were encountered at temperatures lower than 15^{o}C.

A linear response was found at the maximum excitation wavelength (r=0.9999) for phenol and p-cresol in fluorescence concentrations ranging from 0 to 2 mg/l and 0 to 1.5 mg/l, respectively. The maximum excitation and emission wavelengths of the phenols found under these conditions are given in Table 1.

Table 1. Maximun excitation and emission wavwelengths of phenols in water, buffered at pH 6.55.

Compound	λ(ex/em) (nm)	Raman 1) (nm)	A-max (log ε)	
Phenol	269/298	298	271 (3,28)	al
o-Cresol	270/300	298	275 (3,22)	w
m-Cresol	271/300	298	271 (3,20)	hx
m-Cresol	-	-	277 (3,27)	hx
p-Cresol	276/307	306	280 (3,23)	cy
2,3-DMP	270/299	298	273 (3,15)	hx
2.3-DMP	-	-	278 (3,19)	hx
2,4-DMP	275/309	305	- -	
2,5-DMP	273/302	301	276 (3,28)	cy
2,6-DMP	269/298	298	271 (3,19)	al
3,4-DMP	276/307	306	277,5 (3,28)	hx
3,5-DMP	273/300	303	281,5 (3,21)	cy

1) The maximum Raman emission of the blank.
al=ethanol, w=water, cy=cyclohexane, hx=hexane and DMP= dimethylphenol.

The Raman emission of the blank coincides with the emission wavelength of the samples, thus lowering the sensitivity of the analysis by giving a high background emission level and a lesser S/B ratio (Table 1).

Figure 1a illustrates the fluorescence spectra of p-cresol. Separation of the emission- and Raman bands can be achieved by lowering the excitation wavelength, but this also results in a lowering of the fluorescence quantum yield. In this way, the S/B ratio can be maximized. The background level will overcome the effect of shifting the Raman emission to shorter wavelength. This is illustrated in Figure 1b.

In the present work, the excitation wavelength which gave the maximum S/B value is denoted as "optimum emission wavelength", λ(opt) (Table 2).

Dilution of analysis samples (in addition to buffering) permit the use of a test concentration higher than 1.5 mg/l and reduces the possibiliy of light scatter from solid particles from the test organisms. The latter is a problem for instruments where emission is measured at 90° to the excitation beam (EWING 1969).

It was not found necessary to prepare standard curves, since linearity was demonstrated for phenol and p-cresol within the range of the analysis. It is clear from the log ε 's for the methylated phenols given in Table 1 that these compounds should show a similar

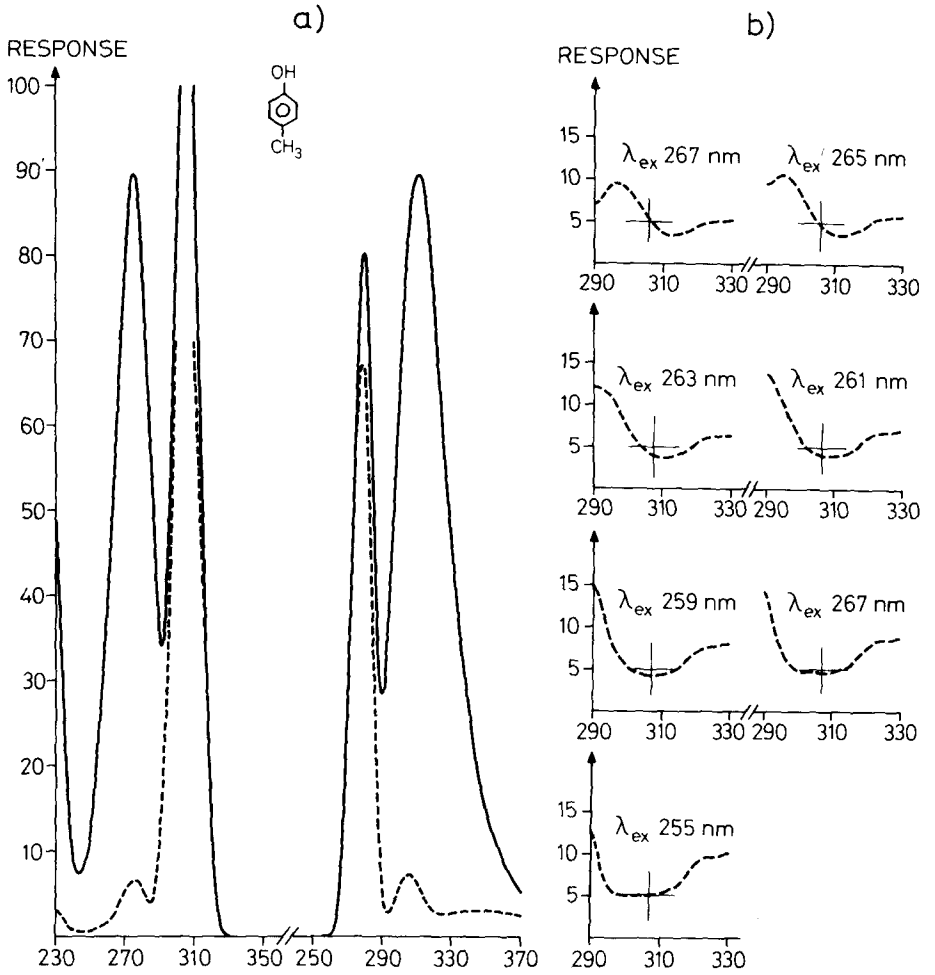

Figure 1.

a) Excitation; and emission spectra of p-cresol in water. The sample was prepared as explained in the text. Fluorescence concentration 0.5 mg/l. Excitation spectrum (left) at λ(em)=307 nm, slits 10/10, and emission spectrum (right) at λ(ex)=275 mn, slits 10/5. The scale factors were 2.304 and 11.54, respectively. Whole line: sample. Dotted line: blank.

b) This; part of the figure illustrates the background emission curve when shifting λ(ex) to shorter wavelengths to avoid the background response from Raman emission. Only the curve from the blank is shown, since the response of the sample is adjusted to 90.0 at λ(em)=307 nm at all excitation wavelengths. Slits 10/5.

linear response, and it was therefore only necessary to measure the emissions from these phenols using 15 mg/l (1.5 mg/l fluorescence concentrations) and blank solutions.

Table 2. The optimum excitation and emission wavelengths found in the present work.

Compound	λ(ex/em) (nm)	Lower detection limit (mg/l) 1)
Phenol	254/298	0,10
o-Cresol	253/300	0,084
m-Cresol	254/302	0,089
p-Cresol	263/307	0,21
2,3-DMP	253/297	0,22
2,4-DMP	265/310	0,23
2,5-DMP	261/305	0,13
2,6-DMP	254/298	0,24
3,4-DMP	263/309	0,14
3,5-DMP	255/298	0,14

1) The lower detection limit is defined as the concentration where the response of a blank is one-half of the response of a sample.
DMP=dimethylphenol.

For the measurement of blank solution, no significant difference was found between blanks made from distilled water or from seawater and those made from seawater containing 1% ethanol.

In the results, the spread, $\sigma(n-1)$, was less than 2%. Error in the volumetric equipment was negligible (0,04 - 0,15%) compared with the spread in the measurements.

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