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Solvent extraction-spectrofluorometric determination of anionic surfactants using acridine orange

66

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A simple and rapid extraction-fluorometric method for the determination of anionic surfactant (AS) at the parts-per-billion (ppb) level is developed. Sodium dodecyl sulfate (SDS), an anionic surfactant after the formation of an ion-associate with acridine orange (ACO), a fluorescent cationic dye was extracted in toluene. The fluorescence intensity (λ_{em} : 530 nm, λ_{exc} : 480 nm) of the complex is a direct measure of the SDS concentration. The calibration graph is linear in the range of 0-1040 ppb of SDS (in toluene). The relative standard deviation is $< \pm 7\%$. The method is applicable to river water, pond water and tap water. It is free from interference from sodium chloride upto 0.5M concentration level, and also from many cations and anions upto a large concentration.

The wide application of surfactants in various industries and everyday life leads to their occurrence in wastewater and evokes intensive pollution of water reservoirs^{1,2}. Waste effluents from textile and organic synthesis production plants and factories, may contain upto 2.5-10 g l⁻¹ of anionic surfactants³. Having penetrated into water, the surfactants change its quality by adding an unpleasant smell and taste to it and cause an intensive foam formation⁴. They exert a solubilising effect on the microbial cell wall that leads to death of the ambient microflora and also increase the carcinogenic impact of some chemical compounds. Thus we require simple and reliable methods for the determination of the surfactant concentration.

Conventional methods for the detection of anionic surfactants (AS) use gas chromatography⁵, high performance liquid chromatography^{6,7}, extraction photometry⁸⁻¹⁰ and extraction fluorometry¹¹⁻¹³ using various dyes such as methylene blue, ethyl violet, rhodamine B, rhodamine 6G, safranin-T etc. The conventional techniques of surfactant detection are mainly based on chromatography and photometric extraction. Many of them are either time consuming or have expensive instrumentation. Thus elaborating simple and rapid method of surfactant detection is still a challenge. Some of the methods are not selective. To overcome the selectivity problem, very recently, methods using microbial sensor¹⁴, amperometric biosensor¹⁵, ion-pair formation with *in situ* flow injection analysis utilising dynamic surface tension

detection¹⁶, ion selective electrode¹⁷ and ion-pairing chromatography with suppressed conductivity detection¹⁸ are also developed. But all these need sophisticated instrumentation and expertise.

Acridine orange (ACO) chemically known as 3,6-bis (dimethylamino) acridine having a colour as well as a strong fluorescence has the potential for being used as an ion-pairing agent with AS. Among many other dyes tried, ACO is the most successful one for spectrophotometric determination of AS¹⁹. But its fluorescence property has still remained unexplored. Fluorometric method as compared with spectrophotometric one, in most cases, is a better choice in terms of selectivity and sensitivity. This has been demonstrated recently when the ion-pairing property in combination with the colour or fluorescence property of ACO has been utilised for arsenic (v) determination^{20,21}. All these facts have prompted the present investigators to undertake an investigation on the potentiality of ACO for being a suitable fluorometric reagent for AS determination. This led, in turn, a very simple and sensitive fluorescence based procedure for AS determination. The method is suitable for real sample analysis also.

Experimental Procedure

All fluorescence measurements were carried out with a Perkin Elmer (Norwalk, CT, USA) LS-50 B spectrofluorimeter equipped with a pulsed 9.9 W xenon lamp excitation source and a signal detected with a photo-multiplier tube with S-20 spectral

response. A HCL (Noida, Delhi, India) personal computer was linked to the spectrometer unit through an interface and appropriate software was written to control the spectrofluorimeter and collect and process the data in graphic form.

Acridine orange (AR grade) was used for the preparation of approximately 5×10^{-3} M solution used as a stock. Sodium dodecyl sulfate (SDS), a representative member of AS was purchased from Fluka and was dried at 50°C under reduced pressure before use. A stock solution of 520 ppm concentration of SDS was prepared. As an extraction solvent toluene (BDH) was used. All chemicals used were of AR grade and used without further purification. Water used was double distilled.

Sample solution (10mL) containing SDS (in the range of 0.026-5.2 μg) was transferred into a separating funnel. Acridine orange and glacial acetic acid 100 μL each was added. Then 5mL toluene was added to it, shaken for 1 min and allowed to settle for 5 min. The aqueous layer was discarded and the toluene layer was used for fluorescence measurements. The fluorescence spectra were recorded using λ_{exc} : 480 nm and slit 2.5/2.5. A fluorescence peak appeared at 530 nm the intensity of which was monitored.

Results and Discussion

Anionic surfactants form 1:1 complexes with cationic dyes and depending on the property of the dye the method for their determination could be either spectrophotometric or fluorometric. The main criteria for this type of method is that the dye should be sufficiently soluble in water so that the excess dye can be disposed off easily and the ion-association complex should have large extractability in a suitable organic solvent. It was possible when the pH of the water solution during extraction was maintained in the acidic range. Various organic solvents such as benzene, toluene, chloroform, dichloromethane, chlorobenzene, 1,2-dichloroethane, a binary mixture of benzene and isobutyl methyl ketone etc. were tested for extraction purpose. Finally toluene has been chosen. Among all solvents mentioned, toluene is the least toxic and has the best extracting power.

Since extraction of the ion-association complex is an important step in the procedure, an extensive study was carried out on the shaking and standing time during extraction. The effect of time was examined using the variation of time for shaking and standing from 10-30 min. It has been observed that even 30s

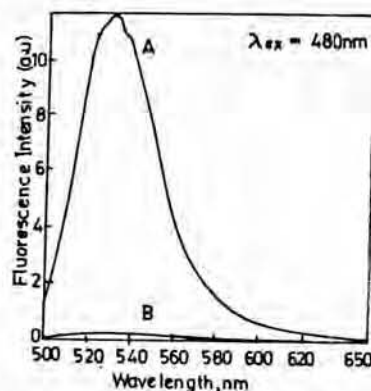


Fig. 1—Fluorescence spectra in toluene of ion-association complex formed between SDS (due to 1040 ppb) and ACO (curve A) and reagent blank (curve B)

shaking and 3 min standing were sufficient to obtain reproducible and optimum fluorescence intensity. Thus the procedure is advantageous because the extraction is quick as compared to other methods often requiring 10-90 min shaking and/or standing^{9,10,12}. In some cases tedious centrifugation step is also necessary¹¹. Moreover, in the procedure discussed no washing of the organic layer is needed as it is needed for ethyl violet method⁹. Phase separation is so quick here that no salting-out agent^{9,10} such as Na_2SO_4 even was needed.

The optimum concentration range (final) of acridine orange for 0-5.2 μg of SDS present in 10mL of water was 1×10^{-5} - 1×10^{-4} M. Above and below this range a decrease of fluorescence intensity is observed. Hence 5×10^{-5} M ACO was used throughout the experiment.

In the present work acetic acid was used to maintain the pH during extraction. It has been observed that glacial acetic acid 20-150 μL per 10 mL of sample solution was optimal for our purpose. The pH of the water solution was thus ~ 2 . In presence of lower dose of acetic acid quantitative recovery of the dye in aqueous medium was not possible.

To assign the efficiency of toluene for the quantitative recovery of the ion-association complex, a study was performed using different volumes of toluene keeping that of water constant. The water: toluene (v/v) ratio was varied from 1:1-1:0.1. Four different mixtures having water: toluene (v/v) 1:1, 1:0.5, 1:0.25 and 1:0.1 were tried. Under our described procedure it is observed that the mixtures having water: toluene (v/v) 1: 0.25 and 1: 0.1 is not suitable for complete recovery of the complex, where

Table 1—Effect of co-existing substances on AS determination

Ion	Added as	Concentration / M
I ⁻	KI	1×10^{-4}
Mg ²⁺	MgCl ₂	1.5×10^{-1}
SO ₄ ²⁻	Na ₂ SO ₄	1×10^{-1}
Na ⁺	Na ₂ SO ₄	1×10^{-1}
Cl ⁻	NaCl	5×10^{-1}
NO ₃ ⁻	NaNO ₃	2.5×10^{-3}
Br ⁻	KBr	3×10^{-3}
Fe ³⁺	FeCl ₃	5×10^{-4}
Ca ²⁺	CaCl ₂	1×10^{-1}
HCO ₃ ⁻	NaHCO ₃	5×10^{-1}
Fe ²⁺	FeSO ₄	1×10^{-3}
BO ₃ ³⁻	H ₃ BO ₃	1×10^{-2}
CO ₃ ²⁻	Na ₂ CO ₃	1×10^{-3}
HPO ₄ ²⁻	Na ₂ HPO ₄	5×10^{-3}

Table 2—Determination of AS in natural water

Sample	Solution taken	AS concentration found (ppb)* \pm standard deviation in organic layer using Proposed method ⁹	Other method ⁹
River Ganges water	10 mL	38.3 \pm 5.5%	36.8 \pm 3.1%
Pond (local) water	0.1 mL + 9.9 mL distilled water	237 \pm 4.5%	245 \pm 6.1%

* Average of three determinations

as those containing water: toluene 1:1 and 1: 0.5 (v/v) show complete recovery.

Washing of the organic layer with water did not show any change in the fluorescence intensity. Hence this step is not necessary as it was reported earlier⁹ to be an obligatory step. Drying of the toluene layer with dry Na₂SO₄ causes decrease in the fluorescence intensity without altering the position of the peak. A separate calibration curve was generated after the drying step is performed. The LDR (linear dynamic range) and LOD (limit of detection) remained the same in this case as observed for the process without drying. The precision (RSD, 5 determinations) is found to be $\pm 6.9\%$ at 1040 ppb level of SDS concentration. The correlation coefficient was 0.992. The equation for the straight line is $I_f = 0.160 \times C$ (ppb) + 1.44.

The fluorescence spectra of the ion-associate formed between SDS (due to 1040 ppb) and ACO has

Table 3—Determination of AS in tap water

Sample	Solution taken	Anionic Surfactant (AS)		Recovery %
		Added (μ g) in water (10 mL)	Found (ppb)* in toluene layer (5 mL)	
Tap water	10 mL	0	5.0	-
	10 mL	5.2	1023	98
	10 mL	2.6	527	101
	10 mL	0.52	108	104

* Average of three determinations

been shown in Fig. 1, curve A. The fluorescence intensity is measured at 530 nm (λ_{exc} : 480 nm) where the fluorescence of the reagent blank is very small (Fig. 1, curve B). The calibration curve obtained by the standard procedure was linear in the range of 0 – 1040 ppb (which corresponds to $0 - 3.6 \times 10^{-6}$ M) (in toluene) of SDS. The equation for the straight line is $I_f = 0.18 \times C$ (ppb) + 1.37. The correlation coefficient found is 0.995. The limit of detection ($3 S_B/m$; where m represents the slope of the calibration curve) is 3 ppb. The precision (RSD, 5 determinations) is 6.7% as determined for 1040 ppb of SDS. The standard deviation of the blank is 3.3 % (before drying) and 5.7 % (after drying).

Effect of coexisting ions was examined by the standard procedure. The limiting concentrations of the constituents those do not interfere in the determination of SDS are reported in the Table 1. The ions were added to the water solution (10 mL) spiked with 5.2 μ g of AS (so that the concentration in the water remains as 520 ppb, a particular concentration within the LDR described) and the AS concentrations were determined using the described procedure and compared with that obtained from the water sample spiked with the same amount of AS but no interfering ions. The fluorescence intensity variation upto 5% was allowed.

Application of the method to the determination of AS in natural water- It was examined that whether in a practical sample of water such as domestic water, our procedure holds good for the determination of AS or not. For water sample collected from a river into which large amounts of domestic wastewater flows, or in case of a pond water sample which contains large amounts of colloidal materials and suspension, emulsion may occur between the aqueous and organic

phases by vigorous shaking. Hence time required for separating the two phases after shaking may sometimes be longer. However, the settling time, in our case, for real sample, remains the same as described in the standard procedure. The samples were collected from the river Ganges and a local pond on December 6, 1998, stored at 4°C and used within 48 h. The results were compared with those obtained by other method⁹ and are presented in Table 2.

The recovery tests were carried out using tap water (source: Kangshabati River) by adding known amounts of AS in it and determining AS concentration according to the described procedure. The results are shown in Table 3. The recovery of AS was good and ranged within 97 – 105%. In the determination of AS in tap water, the coefficient of variance was measured. The standard deviation (for 5 determinations) was $\leq \pm 6.0\%$.

The method was virtually unaffected by the presence of NaCl (upto a concentration level of 0.5 M) and other constituents of sea-water at a concentration level much higher than those present in sea. Thus the method can potentially be applied for the determination of AS in sea-water (Table 1). Presence of non-ionic surfactant TX-100 and cationic surfactant such as hexadecyltrimethylammonium chloride did not show interference 10^{-2} M concentration level.

Conclusion

In this study a fluorescent ion-association complex is allowed to be formed between an anionic surfactant (AS) and a fluorescent dye acridine orange (ACO). The complex has then been extracted in toluene and used for the fluorometric determination of AS. The method is very simple, rapid, reliable and applicable to natural water.

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