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# Reverse Flow Injection Analysis of Trace Amounts of Anionic Surfactants in Seawater without Liquid–liquid Extraction

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**Abstract**: In order to control the pollution by anionic surfactants in seawater, a simple, accurate and sensitive reverse flow injection (rFIA) spectrophotometric method for the determination of anionic surfactants in seawater is suggested in this paper. Sodium dodecylbenzenesulfonate (LAS) was selected as the reference anionic surfactant. The method is based on the ion associate formed between LAS and Ethyl Violet. The absorbance of the complex is measured by optical detector at 585 nm. Chemical factors and rFIA variables affecting the system are also discussed. Under the optimal conditions, the linear range of this method was 25.0–400.0 μg/l; the detection limit was 2.4 μg/l; the relative standard deviation was 0.38%. This method is suitable for automatic and continuous analysis, and was successfully applied to determine the content of anionic surfactants in seawater.

**Keywords**: anionic surfactants; reverse flow injection analysis; seawater; spectrophotometry

With the extensive application of synthetic detergents in the daily life, the analysis of trace anionic surfactants (AS) in water has become an indispensable topic (Xiao *et al.* 2008) in the environmental monitoring. Anionic surfactants are discharged into the environment, gather on the surface of water and other particulates, and produce foams or emulsions. This results in blocking the exchange of oxygen in water, and may deteriorate the quality of water and cause harm to the aquatic life. In the ocean, the content of anionic surfactants is usually about 30.0 μg/l, but no more than 400.0 μg/l (Standard 1997).

The reference method for AS determination in water is based on the liquid–liquid extraction involving several sample processing steps carried out manually, thus requiring a long time to perform the analysis and generating a big volume of organic waste. These disadvantages were overcome by employing a procedure based on multi-commutation process, where the improvement of throughput and drastic reduction in the consumption of organic solvent were achieved without any significant loss of sensitivity (Eva *et al.* 2005). Although the use of less organic solvent could be considered as an advantage, chloroform presents a very long environmental lifetime, thus requiring laborious and careful waste management. In this sense, analytical procedures without using organic solvents should be useful. This requirement was met by employing methods based on the flow injection analysis (FIA) (Liu & Jiang 2001; Yuan

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*et al.* 2007). Ei-Nemma *et al.* (2009) used FIA to determine anionic surfactant, the lower limit of detection of 1.0 mg/l, however is not suitable for determining anionic surfactant in seawater.

Analytical procedures employing the adsorption on silica gel with the detection by spectrophotometry, and potentiometric titration with ion selective electrodes have also been suggested (Chebotarev *et al.* 2004; Segui *et al.* 2004). These procedures do not use organic solvents but involve tedious manual processing. This drawback could be surpassed by adopting procedures based on FIA.

High performance liquid chromatography-UV detection method of detecting AS has a good detection limit, but it needs 75% methanol to elute some probable interferences and a liquid-liquid extraction in the sample processing steps to overcome the high salinity interference (Wangkarn *et al.* 2005).

Flow injection analysis (FIA), which involves the injection of a small volume of the sample into the stream of reagent flowing through the system, is considered as normal FIA (nFIA) and has the merits of simplicity, automation, and lower risk of contamination. It represents a widespread and well established approach for the determination of AS (Liu 1997; Ei-Nemma *et al.* 2009). Different from this nFIA technique is reverse flow injection analysis (rFIA), where the reagent injected into the flowing system is mixed with the sample and other solutions. In 1982, Johnson and Petty published the first paper on rFIA. The analyte was phosphate with a detection limit of 50nM (Johnson & Petty 1982). In rFIA, with the injection of thereagent into the sample flow, the determination concentration in the reagent zone increases with the increasing dispersion, therefore the determination is carried out with only a slight dilution while higher sensitivity can be obtained. rFIA can be suitable for in situ analysis or industrious processes for the simple sample preparation. The rFIA also has advantageous features such as high selectivity, very low reagent consumption, overcoming of the interference of seawater salinity and chroma. It was applied for the determination of phosphate and other analytes (Teraboon *et al.* 2002; Xie & Zhao 2004; Ma *et al.* 2009), but it has not been applied for the determination of AS in seawater until now.

In the present work, a simple rFIA system was used to determine trace AS without liquid-liquid extraction in seawater by optical detector. Ethyl Violet was used as the complexing agent to overcome the salinity interference of seawater, while it decreased the detection limit of AS. The method is based on the disappearance of colour, so the absorbance signals decrease with the increasing concentration of AS. The method is rapid, reproducible, and eminently suitable for on-line monitoring applications.

## MATERIAL AND METHODS

***Apparatus.*** Model HFY6-1 automatic metallic element analyser equipped with a six-way automatic injection valve, optical detector, and optical flow cell has the functions of flow injection analysis (Xie *et al.* 2008). The six-way automatic injection valve was controlled by the time-delay device. The flow system employed one peristaltic pump (Shanghai Huxi Analytical Instrument Plant, China), which delivers all flow streams. Poly tetra fluoro ethylene (PTFE) tubing (0.5 mm i.d.) was used to connect all components in the flow system. Ethyl Violet solution was injected into the carrier stream by the six-way injection valve. The absorbance of the solutions was detected by means of the optical detector, in the course of their flowing into the optical flow cell. Data acquisition and processing were performed with HW-2000 Chromatography software (Version No. 2.13, Qianpu software Co. Ltd., Shanghai, China) running under Windows XP.

***Reagents.*** All reagents were of analytical-reagent grade and the solutions were prepared with deionised water with a resistance of 18 MΩ (Molecular, Shanghai, China).

According to the Chinese marine monitoring standards (Standard 2007), Sodium dodecylbenzenesulfonate (LAS) was selected as the single standard material for the determination of AS in seawater. In some studies, LAS was selected as the standard material for the detection of AS (Liu & Jiang 2001; Xiao *et al.* 2008). Therefore, LAS was also selected as the standard material in the present research method.

Sodium dodecylbenzenesulfonate standard solutions of 100 mg/l:0.010 g LAS was dissolved in 100 ml of deionised water. The working solutions were obtained by appropriate dilutions with deionised water.

Ethyl Violet solution of 500 mg/l:0.050 g Ethyl Violet was dissolved in 100 ml of deionised water. The working solutions were obtained by appropriate dilutions with deionised water.

Seawater standard product: In each 1000 ml, the composition of Seawater standard product was NaCl 26.518 g, $MgCl_2$ 2.447 g, $MgSO_4$ 3.305 g, $CaCl_2$ 0.752 g, $NaHCO_3$ 0.202 g, and NaBr 0.083 g.

***Procedure.*** A series of working standard solutions with different concentrations were prepared by diluting the concentrated fresh standard solution of LAS. As shown in Figure 1, flow paths were fed with the sample or standard solution (S), Ethyl Violet solution (R), and carrier stream (deionised water, C), respectively. With the six-way automatic injection valve in the load position, the pump was started to wash the whole flow system until a stable baseline was recorded. When the switching valve was in the analysing position, the pump propelled R to the waste, at the same time propelled C and S into the reaction coil (the analysing step needing 3 min). The switching valve was then automatically turned to the injection position, R and S were injected into the reaction coil (the injection step needing 2 min), and C was propelled to the waste. The absorbance signals (peak, mV) were obtained by the optical detector at 585 nm, when the reaction solutions flowed into the optical flow cell. The relative absorbance intensity (relative peak height, mV) versus LAS concentration was used for the calibration. The peak height of the working solution containing LAS was $H_1$. The relative peak height ($Hr$) was the difference between the peak height of Seawater standard product ($H_0$) and $H_1$. Hence, the result should be $Hr = H_0 - H_1$.

***China's official testing method.*** The official method for determining anionic surfactants is based on the formation of the ion pair complex between the LAS and cationic dye methylene blue, followed by extraction with chloroform and spectrophotometric measurements at 652 nm (Standard 2007). Its detection limit was 50 μg/l LAS and relative standard deviation was 2.3%. It provided possible measurements of 1–2 samples/h.

## RESULTS AND DISCUSSION

The working solutions were prepared with Seawater standard product (purchased from China Oceanic Administration, exclude LAS). The best experimental conditions were selected by the results of the relative peak heights ($Hr$). The peak height of the working solution containing 200 μg/l LAS was $H_1$. With $Hr$ providing the highest value the best conditions for the experiment were obtained.

***Detection wavelength.*** In order to find the most appropriate wavelength for the analysis in the rFIA, the effect of wavelength on the peak height was studied in the range from 350 nm to 700 nm by the Spectrum UV2800 (You Nike Ltd. Co., Shanghai, China). Figure 2 shows the absorbance spectra of two different solutions: (1) Mixed solution of 2 ml 8.0 mg/l R and 8ml deep seawater; (2) Mixed solution of 2 ml 8.0 mg/l R and 8ml deep seawater of 400.0 μg/l LAS. In the second solution, LAS and Ethyl Violet formed an ion associate, so the R was light in colour. The greatest absorbance intensity of the solutions was at 585 nm, which was chosen for further work.

***Optimisation of flow injection variables.*** Obtaining higher sensitivity, the least noise of baseline and the shortest analysis time being the aim, the process of optimisation was carried out, and the optimised results of the flow injection variables are shown in Table 1.

***Effect of chemical reaction variables.*** The reagent solution is the most important factor in rFIA. Therefore, the effect of Ethyl Violet concentration was examined in the range of 3.5–11.0 mg/l. Figure 3 shows that the relative peak height increased sharply with the increase of Ethyl Violet concentration changing from 3.5 mg/l to 8.0 mg/l. In the contrary, it decreased quickly when the EV concentration changes from 8.0 mg/l to 11.0 mg/l. Consequently, 8.0 mg/l Ethyl Violet was chosen for further study.

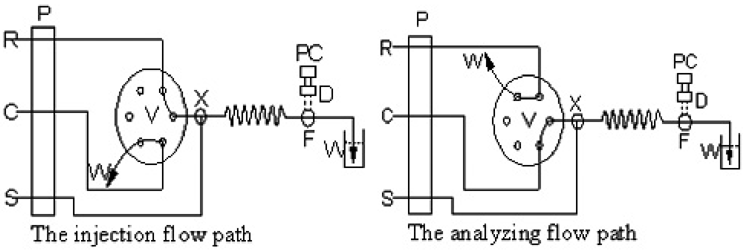

Figure 1. Schematic diagram of the rFIA system

S – sample or LAS solution; C – carrier; R – Ethyl Violet solution; P – peristaltic pump; V – six-way automatic injection valve; X – mixer; L – reaction coil; F – flow-through cell; D – optical detector; PC – computer; W – waste

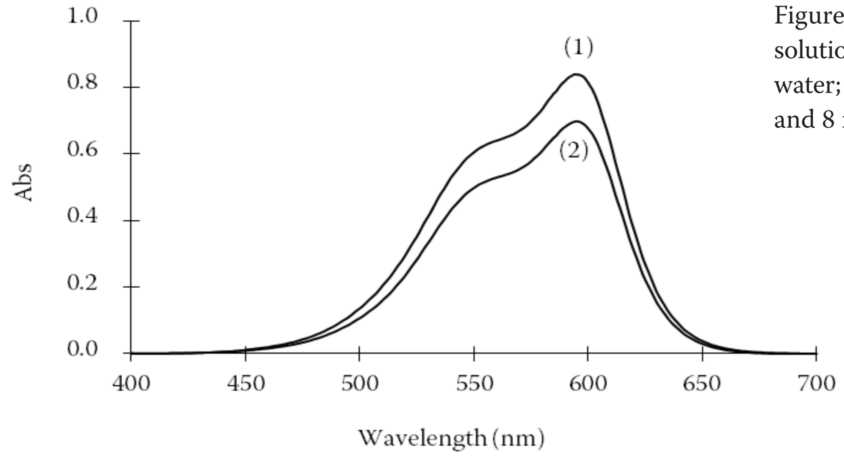

Figure 2. Absorption spectra; (1) – mixed solution of 2 ml 8.0 mg/l R and 8 ml deep seawater; (2) – mixed solution of 2 ml 8.0 mg/l R and 8 ml deep seawater of 400.0 μg/l LAS

***Calibration curve, reproducibility, and detection limit.*** In order to assess the reproducibility and accuracy of the method, tests of linearity and precision were conducted under the selected conditions given above. The linearity test was carried out by analysing a series of working standard solutions. The relative peak height ($Hr$, mV) was truly linear versus LAS concentration (C, μg/l) within the range of 25.0–400.0 μg/l (Figure 4). The regression equation was $Hr = 0.331C + 5.63$ ($n = 5$, correlation coefficient $R^2 = 0.9991$). The standard deviation of 10 measurements of the blank solution was 0.27 mV.

The detection limit of the proposed method was 2.4 μg/l LAS, which was deduced by the following formula:

$$D = (3A)/B$$

where:
D – detection limit
A – standard deviation of 10 measurements of the blank solution
B – slope of the calibration curve

The precision test was carried out by eleven injections of the working standard solution with the LAS concentration of 400.0 μg/l. The relative standard deviation (RSD) obtained was 0.38% (Figure 5).

The detection limit, RSD and sample throughput of the proposed method were 2.4 μg/l, 0.38% and 10–20 samples/h, and the detection limit, RSD

Table 1 Optimization of rFIA variables

| Variables | | Studied range | Optimum value |
|---|---|---|---|
| Pump tube flow rate (ml/min) | S | 0.20–0.85 | 0.75 |
| | C | 0.20–0.85 | 0.75 |
| | R | 0.15–0.50 | 0.20 |
| Reactor coil length (m) | | 1.0–6.0 (i.d. 0.5 mm) | 5.0 (i.d. 0.5 mm) |
| Radiation diode wavelength (nm) | | 350–700 | 585 |
| Optical path length of flow through cell (mm) | | 16/28 | 16 |
| Inner diameter of flow through cell (μl) | | 2 | 2 |
| Sample coil (ml) | | 0 | 0 |
| Sampling time (min) | | calculated according to the flow rate, the immobile tubes | 2 |
| Analysing time (min) | | calculated according to the flow rate, the volume of reactors and immobile tubes | 3 |

S – samples; C – carrier stream; R – Etyl Violet solution

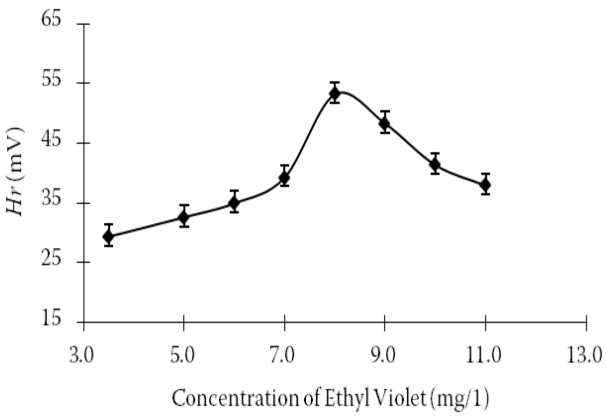

Figure 3. Effect of Ethyl Violet concentration on the relative peak height; conditions: sample loop 0 µl; reaction coil 5.0 m (0.5 mm i.d.); flow rate of C 0.75 ml/min; flow rate of S 0.75 ml/min; flow rate of R 0.20 ml/min

and sample throughput of the official method were 50 µg/l, 2.3% and 1–2 samples/h. Therefore, the proposed method proves to be simple and it shows a better sensitivity, precision, and accuracy.

***Effects of accompanying ions.*** The effect of the accompanying substances was tested by analysing the standard solution of LAS. The tolerable concentration ratios with respect to 400.0 µg/l LAS for interference at ± 5% level were, respectively, over 2 times for aniline, phenols and sulfide; over 5 times for $Fe^{3+}$ $Cr^{3+}$, $Fe^{2+}$, $Cd^{2+}$, $Zn^{2+}$, $Cu^{2+}$, $Al^{3+}$; over 1000 times for $Ca^{2+}$, $K^+$, $Mg^{2+}$, $Na^+$, $Cl^-$, $SO_4^{2-}$, $HCO_3^-$, $CO_3^{2-}$, $NO_3^-$ and $PO_4^{3-}$. The results showed that the most common seawater ions (Standard 1997) did not interfere with LAS. Thus the proposed method has a good selectivity.

***Effects of salinity.*** The seawater salinity is the scale of the salt content in the sea. In all regions of the oceans, some differences occur in terms of the seawater salinity. Commonly, the seawater salinity is about $w(NaCl) = 30 \times 10^{-2}\%$ in the ocean, but in coastal regions, where rivers dilute the sea, it is about $w(NaCl) = 10 \times 10^{-2}\%$ somewhat lower than in other oceanic areas. The salinity of Seawater standard product is $w(NaCl) = 30 \times 10^{-2}\%$. The seawater salinity of $w(NaCl) = 20 \times 10^{-2}\%$ and $w(NaCl) = 10 \times 10^{-2}\%$ prepared using standard seawater was obtained by appropriately diluting Seawater standard product with deionised water. In the selected conditions, three different kinds of salinity standard seawater were, respectively, prepared into a series of working solutions (25.0 to 400.0 µg/l), and their regression equations were $Hr = 0.331C - 5.63$ [$w(NaCl) = 30 \times 10^{-2}\%$], $Hr = 0.334C - 5.71$ [$w(NaCl) = 20 \times 10^{-2}\%$] and $Hr = 0.336C - 5.73$ [$w(NaCl) = 10 \times$ ression curves almost overlapping. Therefore, the proposed method could be applied to various seawater samples without salinity correction.

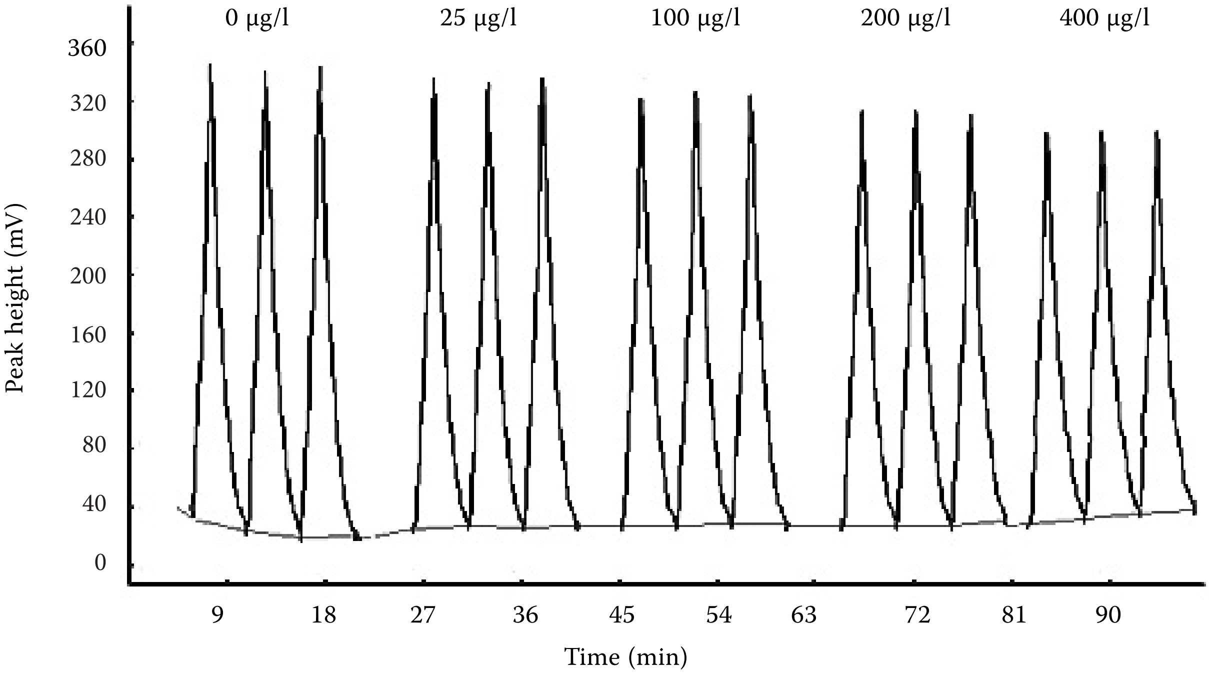

Figure 4. Spectrophotometric flow injection signals of standard solution

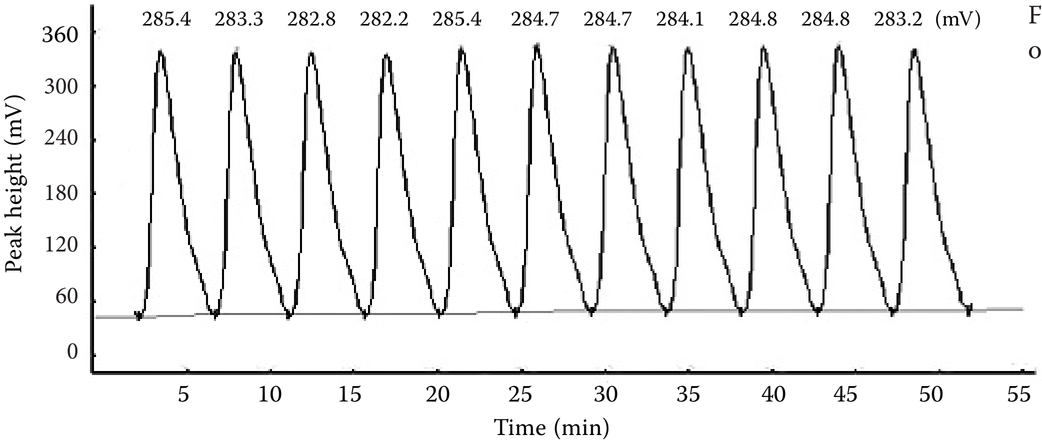

Figure 5. rFIA gram of the precision test

***Application.*** The proposed method was further applied to the determination of AS in seawater. The samples were as follows: (1#) the sample came from Qingdao city, China; (2#) the sample came from Dalian city, China; (3#) the sample came from Shanghai city, P.R. China.

To prove the applicability of the method, a test of recovery was conducted by accurately adding known concentration of LAS into each sample whose original LAS concentration had been determined. The recoveries of the different concentration levels varied from 98.43% to 104.02%, as shown in Table 2. The analytical results of the proposed method and official method were very similar (–3.1% < relative error < 3.1%) and satisfactory (correlation coefficient = 0.9999). No statistically significant difference was observed between the proposed and the official method with the paired Student's *t*-test at the 95% confidence level. Therefore, the proposed method proved to be suitable for the determination of AS in seawater.

## CONCLUSIONS

Ideal standard curves with concentrations in the range of 25.0–400.0 μg/l can be achieved under optimum conditions with the method of rFIA for AS determination. The detection limit, RSD, and sample throughput were 2.4 μg/l, 0.38% ($n$ = 11), and 10–20 samples/h, which was better than with the official method. The method is rapid and simple, and adaptable to on-line automatic and continuous analysis. The model HFY6-1 automatic metallic elements analyser employed in this study not only has a low price ($3000 per instrument) and a small volume (dimension of 30 × 30 ×10 cm) but is also portable. This procedure is very sig-

Table 2 Results of samples analyses (proposed method, $n$ = 3)

| Sample | LAS (μg/l) | | | | Recovery (%) | Relative error (%) | Correlation coefficient |
|---|---|---|---|---|---|---|---|
| | determined value | official method | added | recovery | | | |
| | | | 50.0 | 101.22 | 104.02 | | |
| 1# | 49.21 | 50.11 | 150.0 | 199.13 | 99.94 | –1.79 | |
| | | | 300.0 | 350.11 | 100.30 | | |
| | | | 50.0 | 75.26 | 101.90 | | |
| 2# | 24.31 | 25.07 | 150.0 | 174.86 | 100.37 | –3.03 | 0.9999 |
| | | | 300.0 | 324.59 | 100.09 | | |
| | | | 50.0 | 111.57 | 102.10 | | |
| 3# | 60.52 | 61.93 | 150.0 | 208.17 | 98.43 | –2.28 | |
| | | | 300.0 | 360.96 | 100.15 | | |

LAS – sodium dodecylbenzenesulfonate

nificant, in view of the environmental protection, for on-line monitoring of AS levels in seawater.

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