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Sequential determination of anionic-type detergents by complexation with methylene blue using dual high speed counter-current chromatography

Eiichi Kitazume[a,*], Saki Koikawa[a], Lu Hui[a], Syou Sannohe[a], Yanjun Yang[a], Yonosuke Maki[a], Yoichiro Ito[b]

[a] Faculty of Humanities and Social Science, Iwate University, Ueda, 3-18-37 Morioka, Iwate 020-8550, Japan
[b] Laboratory of Biophysical Chemistry, National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892, USA

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ABSTRACT

A new dual high-speed counter-current chromatographic system using organic extraction phase and aqueous mobile phase containing methylene blue was applied to the analysis of anionic-type detergents. After selecting appropriate conditions such as flow rate of each mobile phase and sample volume, the new system was successfully applied to the analysis of anionic detergent in river water. As all the analytical procedures can be made in a closed system, the method has no health hazard. The present method is safe, precise, and highly sensitive, and can be applied for sequential determination of multiple samples in a short analysis time.

1. Introduction

Surfactants are a major anthropogenic organic component in raw wastewaters and sludges. Environmental analysts often determine the amount of anionic detergents when assessing surface water pollution. Also the determination of anionic surfactants is often accomplished by measuring the color change using a solution of methylene blue dye, which reacts with anionic-type surface active detergents to form a blue colored complex that is extracted into an organic solvent, such as chloroform. [1] As the pollution level in river and sea water caused from detergents and other chemical substances is increasing, quick response, precision monitoring system is required to replace the traditional chemical analysis method. In the past, concentration and separation for a certain inorganic element based on chemical reaction using a ligand have been studied with high-speed counter-current chromatography (HSCCC) in our laboratory [2]. More recently a new system has been developed for the analysis of oil pollution using a dual flow system using true countercurrent movement of organic and aqueous mobile phase [3]. If one phase containing a small amount of detergent is extracted into the other phase with a ligand, it may be applicable to a sequential analysis for detergents. After selecting appropriate conditions such as flow rate of each phase and sample volume, this new system was successfully applied to the analysis of anionic-type detergent in river water.

2. Experimental

2.1. Apparatus

A Hitachi Tokyo Electronics countercurrent chromatograph (HSCCC-R1, prototype) was used to hold a single multilayer coil separation column on the rotary frame at a distance of 10.0 cm from the central axis of the centrifuge. The column was prepared from a single piece of 1865 mm long, 5 mm i.d. polytetrafluoroethylene (PTFE) tubing by winding it directly onto the holder hub (10 cm diameter). β, an important parameter governing hydrodynamic distribution of the two solvent phases in the rotating coil, was about 0.5 for the monolayer column. $\beta = r/R$, where r is the distance from the column holder axis to the coil, and R is the distance from the column holder axis to the centrifuge axis. The total coil capacity is 36 mL. Fig. 1 shows two small bore feed tubes (0.5 mm i.d.) drawn out from the small hole (B) and (C) on the 5 mm i.d. tube, wound around the aluminum drum (10 cm o.d.). On the other hand, a 0.3 mm i.d. return tube is connected to the connector (A) at the tail end of the column. Column head (D), not visible in Fig. 1, is on the other side of the drum to which a 0.3 mm i.d. thin return tube is connected. Inner column volume is about 7 ml, between (A) and (B), and 12 ml (B) and (C). All four feed and return flow tubes are twisted together and drawn outside of the HSCCC case through the center shaft of

* Corresponding author. Tel.: +81 019 621 6825; fax: +81 019 621 6825.
E-mail address: kitazume@iwate-u.ac.jp (E. Kitazume).

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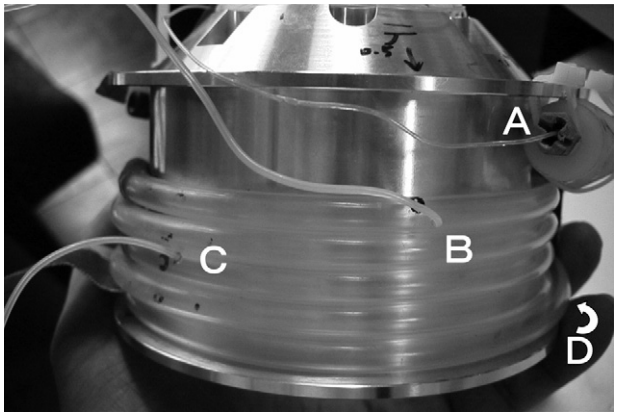

Fig. 1. Coiled CCC column connected with two small bore tubes between head and tail. (A) Tail end of the column; (B) tube connected at 300 mm from A (about 7 ml between A and B); (C) tube connected at 620 mm from B (about 12 ml between B and C, about 19 ml between C and head end of the column); (D) head side of the column.

the sun gear. Then the column is mounted around the sun gear in the HSCCC system. The column holder undergoes the type-J synchronous planetary motion horizontally around the vertical axis of the centrifuge. In the present study, the column was operated at 600 rpm.

The flow diagram of the experimental assembly is shown in Fig. 2. A Shimadzu LC-10Ai pump (Pump 1) was used to circulate aqueous methylene blue solution. Air trap is set between Pump1 and column head (D) to prevent entering air into the line. Methylene blue solution introduced in the column at (B) flows toward the head of the column, exits the column at (D), and returns to the Pump 1 to circulate through the column. An appropriate amount of sample is intermittently injected into the circulating methylene blue solution by the injector valve. Chloroform is introduced by a Shimadzu LC-6A pump (Pump 2) through a coiled small bore stainless steel tube (0.13 mm i.d. × 5 m) for pressure adjustment, entered the column at (C), flows toward the tail of the column (A) and is monitored with the detector after mixing with ethanol pumped from an FMI LAB PUMP Model RH (Pump 3) while absorbance is measured by a TOSOH UV-8020UV-VIS spectrophotometric detector at 650 nm. Ethanol is introduced into extracting line to stabilize the base line signal.

2.2. Reagents

To prepare the two-phase solvent system, HPLC grade of chloroform and sodium hydride were purchased from KANTO Chemical Co., Inc. (Tokyo, Japan), and methylene blue and sodium tetraborate, anhydrous were purchased from MERK Inc. (Darmstadt, Germany). To prepare the anionic detergent sample, analytical reagent grade of sodium *n*-dodecyl sulfate (SDS) and sodium dodecylbenzensulfonate (DBS) (KANTO Chemical) were dissolved in water, purified by circulation through a train of columns filled with carbon and mixed resins in a Millipore Super-Q System. All other reagents were of analytical reagent grade.

2.3. Preparation of solvent system

Methylene blue solution used as the aqueous mobile phase was prepared as follows: 5 ml of 1 mM methylene blue solution and 10 ml of 25 mM sodium tetraborate solution containing 0.05 M sodium hydroxide were diluted with 50 ml of water. Then, the solution was shaken with 10 ml of chloroform in a 1000 ml-capacity separatory funnel. If the chloroform phase showed purple color, it should be discarded and the process was repeated several times until it became clear. Next, several hundred ml of chloroform was added to the mixed solution and used as the organic mobile phase.

2.4. Procedure

After filling the HSCCC column with water, all column lines, from (A) to (D) in Fig. 2 were disconnected from the HSCCC system while connectors at (C) and (D) were plugged. Next, 3 ml of chloroform was introduced from (A) by a syringe draining water through (B). Shortly after rotating the HSCCC column at 600 rpm, connectors at (A) and (C) were plugged and Pump 1 was connected to (B) and a connector at (D) was unplugged. Then, using Pump 1,

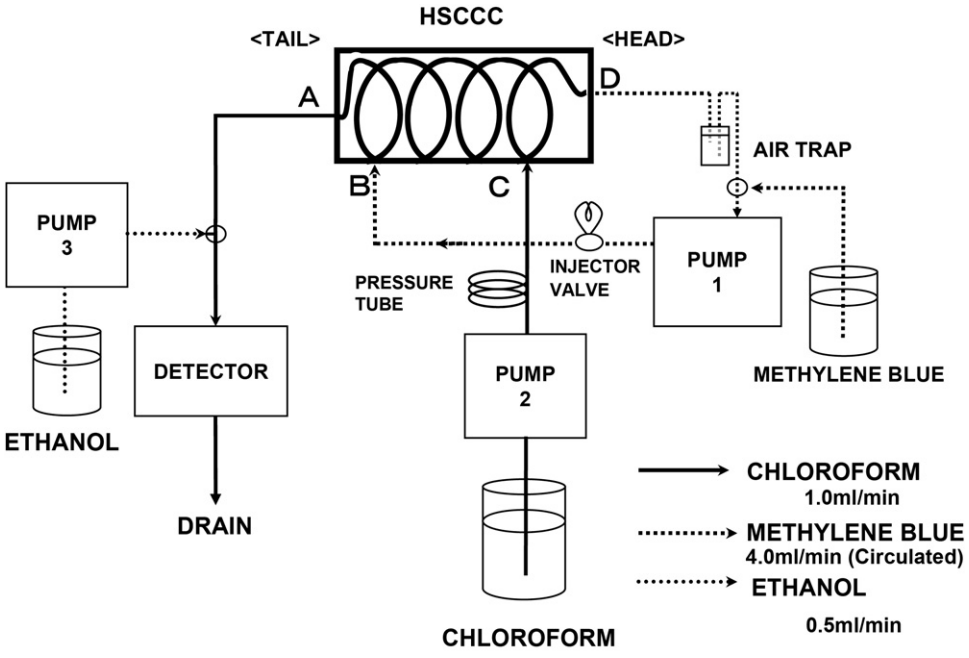

Fig. 2. Flow diagram of the instrumentation assembly.

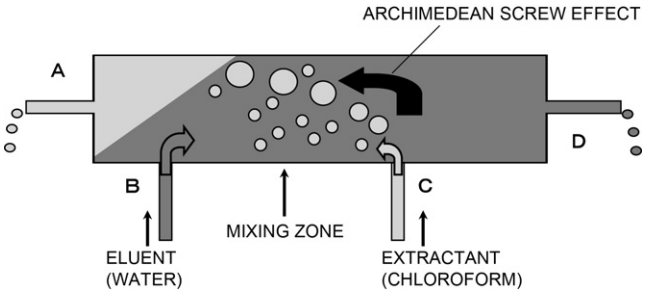

Fig. 3. Schematic diagram of countercurrent extraction process in HSCCC column.

methylene blue solution was introduced into the column at a flow rate of 5 ml/min. When the methylene blue solution emerged from (D), Pump 1 was stopped and (D) was connected to the AIR TRAP line. The connector at (C) was unplugged and connected to the line from Pump 2 while the unplugged connector at (A) was connected to the DETECTOR line. Then, chloroform was pumped by Pump 2 at a flow rate of 1 ml/min and drained through the DETECTOR, while ethanol was introduced to the line between the detector and the column outlet by Pump 3 at a flow rate of 0.5 ml/min for about 15 min. After the baseline at 650 nm of the spectrophotometric detector was adjusted to zero, all pumps were stopped once and all lines were confirmed to be connected as shown in Fig. 2.

For online sequential determination of the detergent for several samples including the detergent standard, Pump 1 was started at a flow rate of 4 ml/min to circulate the methylene blue solution through the column followed by starting Pumps 2 and 3 for about 10 min until the baseline signal was stabilized to a near zero level. Next, 10 ml of water sample was introduced into the line through the injector valve. After the peak signal was detected, the next sample was injected.

3. Results and discussion

Fig. 3 shows a schematic diagram of counter-current extraction process in the revolving HSCCC column. When operating with 600 rpm, chloroform as an extractant mobile phase introduced from (C) and the aqueous mobile phase containing detergent injected from (B) is mixed in the zone between (B) and (C). By using these additional two ports for inlets in HSCCC column in the figure, real counter-current movement between immiscible two phases becomes possible [4]. However, one phase must circulate in the system to stabilize the volume ratio of two phases in the column. If both phase did not return to the column and drained, the volume ratio of each phase would be changed during operation and eventually the column would be completely occupied by a single phase. However, if one of the mobile phase was circulated through the column as in the present experiment, the volume of the circulating phase could be kept almost constant except for a little loss by the mutual solubility for each phase.

At the first stage of the experiment, chloroform was not drained but recirculated through the column by inserting the flow line from the outlet of the DETECTOR into the reservoir of Pump 2. In this complete dual circulatory system, 0.1 ml of 50 μg/ml sodiumdodecyl sulfate (SDS) was injected three times into the system at the intervals of 30–60 min. The results are shown in Fig. 4. After each injection of SDS, the absorbance was increased like a staircase pattern. Though each height of SDS signal was almost the same, the baseline signal was not stable and slowly decreased. To stabilize the base line, chloroform was drained from the outlet of the detector as shown in Fig. 2 in the following experiment. Then, the absorbance of the base line became more stable and quickly returned to the base line after elution of each peak.

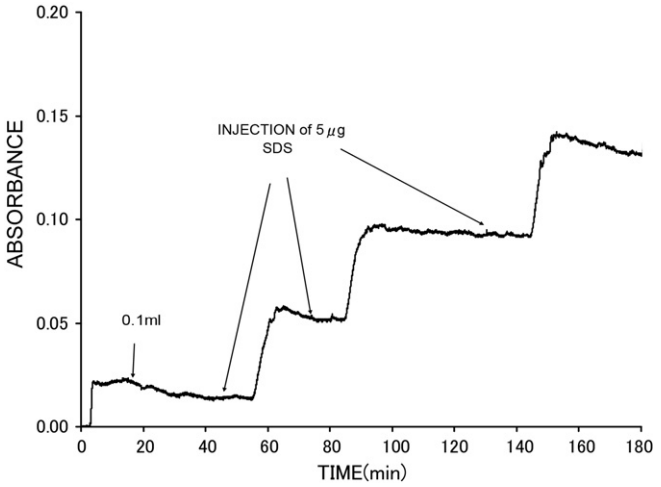

Fig. 4. Results of dual countercurrent circulatory system after three injections of SDS standard. 0.5 ml aliquot of 50 ppm SDS standard solution was injected three times while chloroform drain tube was directly connected to the Pump 2 in Fig. 2.

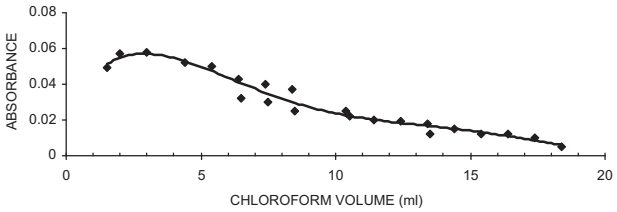

Fig. 5. Relationship between chloroform volume in the column and absorbance peak height. Chloroform volume initially filled up between A and C was decreased from 18.4 ml to 1 ml when 0.1 ml of 12.5 ppm SDS sample was injected.

Fig. 5 shows the relationship between the volume of the chloroform initially introduced in the HSCCC column and the absorbance peak observed when 0.1 ml of 12.5 μg/ml SDS sample was injected. As the volume of chloroform in HSCCC column was decreased, the peak height of absorbance was increased. Therefore, 3 ml of chloroform was used as the standard procedure for the present studies.

Fig. 6 shows the relationship between peak height for 0.1 ml of 12.5 μg/ml SDS sample and flow rate of chloroform, changing flow rate from 3.5 to 4.5 ml/min. As the flow rate increased, the absorbance of peak signal increased. However, as the noise was often observed at a flow rate of over 4.5 ml/min, 4 ml/min flow rate was used in this experiment.

As the sample volume injected into the methylene blue line was increased, the peak height of absorbance signal was linearly

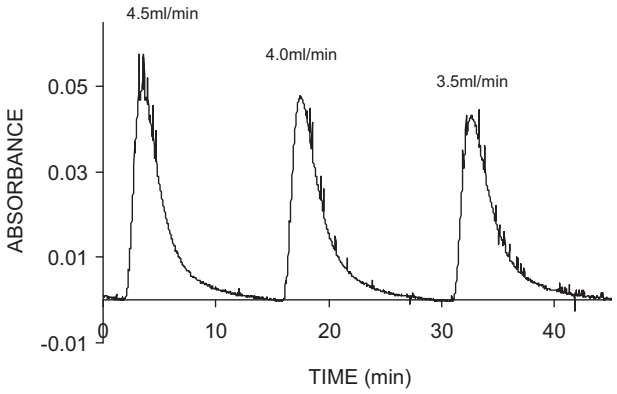

Fig. 6. Relationship between peak height and flow rate of chloroform. 0.1 ml of 12.5 ppm SDS standard sample solution was injected.

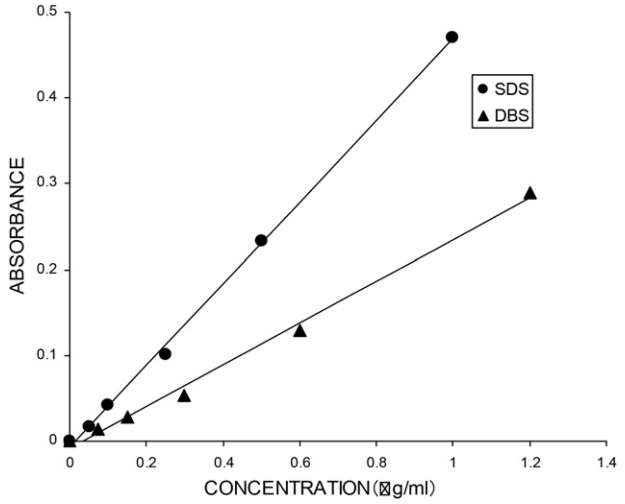

Fig. 7. Calibration curves for SDS and DBS.

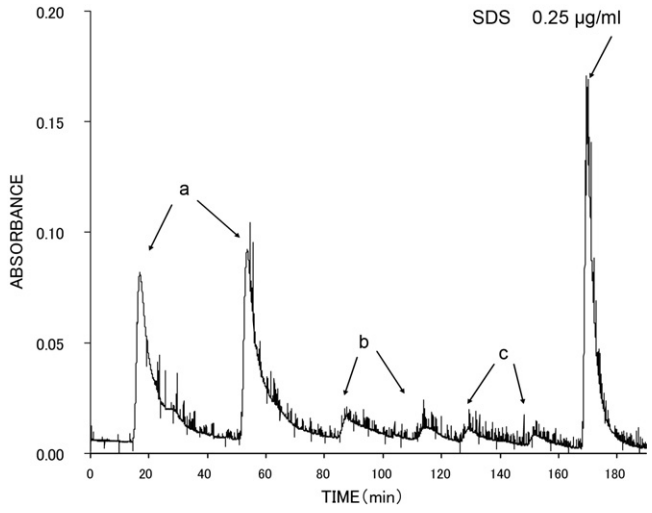

Fig. 8. Peak profiles for surfactant in water from KITAKAMI River in MORIOKA city. Sample size: 10 ml (a) sampled in a brook, just joined to main stream, including raw sewage from home; (b) from the main stream; (c) from another main stream several km upstream from point (b).

increased. But, in order to prevent the dilution of methylene blue solution in the column for at least several sequential measurements with good reproducibility, 10 ml of the sample volume was used in the experiment.

Fig. 7 shows typical calibration curves for SDS and DBS. Each curve shows good linearity up to about 1 μg/ml concentration. The reproducibility was 5.8% for 5 sequential determination of 0.25 μg/ml of the SDS standards sample.

The coexistence of sodium salts such as 10 μg/ml NO_3^-, 5000 μg/ml Cl^-, 1 μg/ml SCN^-, 10 μg/ml Br^-, 20 μg/ml NO_2^- was not affected to the determination of anionic detergents under the present conditions.

Fig. 8 shows the peak profiles for anionic surfactant materials in river water, each determined from 10 ml aliquots of river surface sample from KITAKAMI River in MORIOKA city, the fourth longest river in Japan, was injected in this HSCCC system. Each peak labeled (a)–(c) shows the results from different sampling point; (a) a brook including raw sewage from home, just joined to main stream; (b) main stream sample in the downstream of MORIOKA City; (c) another main stream sample, several km upstream from point (b). The last peak profile shows a signal from 0.25 μg/ml of SDS standard sample. The signal of (a), a sample from brook shows higher peak than those in main streams (b) and (c). The determination results for (a), (b) and (c) were 0.149 μg/ml, 0.011 μg/ml and 0.007 μg/ml as SDS, respectively. The detection limit was about 0.002 μg/ml as SDS, which is almost one order lower than that of conventional method using separatory funnel.

4. Conclusions

The detergent sample is efficiently concentrated and detected by the present method. Aqueous sample phase and organic extractant phase undergo the counter-current stream by setting two extra ports in HSCCC column. We have confirmed that the system has the following advantages over the conventional method: (1) precise, safe and high-performance for determination of anionic detergents; (2) high sensitivity; and (3) sequential analysis of multiple samples.

Acknowledgements

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