• Technical

Microdetermination of Anionic and Nonionic Surfactants with Aid of Cholinesterase

KAZURO TSUJI, HIRONORI TANAKA, and KAZUO KONISHI, Industrial Research Laboratories,
Kao Soap Co., Ltd., Minatoyakushubata, Wakayama-shi, Japan

ABSTRACT

The potentiometric method for the microdetermination of anionic and nonionic surface-active agents was studied by using the phenomena that anionics inhibit the enzyme system, cholinesterase-butyrylthiocholine iodide, and nonionics apparently weaken the inhibitory effect of anionics. A constant current is applied across two platinum plate electrodes immersed in the solution containing butyrylthiocholine iodine and surfactant. A cholinesterase solution is added to cause enzymatic hydrolysis of substrate and a change in the electrode potential due to formation of thiol automatically recorded vs time. A decrease in the initial velocity of the enzymatic hydrolysis caused by inhibitor corresponds to the concentration of anionic surfactant. In the case of determining nonionic surfactant, a fixed amount of sodium linear pentadecylbenzenesulfonate is added as inhibitor. The degree of suppression of inhibitory effect is a direct measure of the concentration of the nonionic surfactant. By this method, 5-25 ppm sodium linear dodecylbenzenesulfonate and sodium branched dodecylbenzenesulfonate; 5-100 ppm sodium dodecyl sulate; 1-10 ppm polyoxyethylene dodecyl ether; 5-30 ppm polyoxyethylene sorbitan dodecanoate, and 5-40 ppm polyoxyethylene octylphenyl ether could be determined.

INTRODUCTION

Many workers (1-9) have reported that several anionic surfactants strongly inhibit enzyme reactions at low surfactant concentrations. On the other hand, it is known that nonionic surfactants do not cause appreciable decreases in the rates of enzyme reactions at low concentrations (4,7) or act as activators (1,5,10,11). Furthermore, it has been revealed recently that the inhibition of lipase activity by sodium branched alkylbenzenesulfonate (TBS) and sodium dodecyl sulfate (SDS) is diminished by the addition of certain nonionics (12).

However, an analytical method for surface-active agents based upon these phenomena has been scarcely investigated, except for Guilbault's work (13) on the determination of Triton X-100.

In this paper, the inhibitory action of anionics, such as sodium linear alkyldecylbenzenesulfonate (LAS), TBS, SDS, sodium α-olefin sulfonate (AOS), sodium polyoxyethylene (POE) alkyl ether sulfate (ES), and soap on the enzymatic hydrolysis of cholinesterase-butyrylthiocholine iodide (PrCOSChI) by cholinesterase (ChE) was investigated; and the determination of small amounts of anionic surfactant was carried out. In addition, the protective effect of nonionics, such as POE dodecyl ether, POE sorbitan monododecanoate, and POE octylphenyl ether, was studied, and it was found that the microdetermination of nonionic surfactant became feasible.

EXPERIMENTAL PROCEDURES

Reagent and Samples

Enzyme solution was prepared by dissolving 10 mg horse serum ChE (Sigma Chemical Co., St. Louis, Mo.) in 10 ml 0.1 M Tris buffer.

Substrate solutions were prepared by dissolving the proper amount of acetylthiocholine iodide (MeCOSChI, Sigma Chemical Co.), propionylthiocholine iodide (EtCOSChI, K&K Laboratories, Plainview, N.Y.) and PrCOSChI (Sigma Chemical Co.), respectively, in 0.1 M Tris buffer.

Buffer solution was prepared by dissolving 12.1 g tris-(hydroxymethyl)aminomethane (Sigma 7-9, Sigma Chemical Co.) in 1 liter redistilled water and adjusting the pH to 7.40 with 0.1 N hydrochloric acid.

The C_{12}-LAS, C_{15}-LAS, and C_{12}-AOS derived from 1-olefins were purified with petroleum ether to remove remaining materials, and the residue was extracted with ethanol. The extract was vacuum dried and dissolved in redistilled water.

The TBS derived from tetrapropylene (Wako Pure Chemical Industries, Tokyo, Japan) was dissolved in redistilled water without further purification.

The SDS derived from linear primary dodecanol was recrystallized four times from isopropanol and dissolved in redistilled water.

The C_{12}-ES (from normal dodecanol) and C_{12}-soap (from methyl dodecanoate) were dissolved in redistilled water without further purification. The average mole ratio of ethylene oxide (EO) for C_{12}-ES was 2.9.

The POE dodecyl ether (from linear primary dodecanol, EO = 6.5), POE sorbitan monododecanoate (EO = 26), and POE octylphenyl ether (from diisobutylene, EO = 8.5) were dissolved, respectively, in redistilled water without further purification.

All these solutions were prepared freshly before use.

Apparatus

The apparatus proposed by Kramer, et al., (14) was used

TABLE I

Effect of Inorganic Compounds on Ratio of Initial Velocity

Inorganic compounds	Concentration, M		
	10^{-4}	10^{-3}	10^{-2}
$CaCl_2$	1.75	2.32	2.55
$MgCl_2 \cdot 6H_2O$	1.01	1.15	1.37
NaCl	1.12	1.00	0.98
Na_2SO_4	1.15	1.13	1110
$Na_3PO_4 \cdot 12H_2O$	0.99	0.98	1.12
$Na_4P_2O_7 \cdot 10H_2O$	1.00	0.94	0.90
$Na_5P_3O_{10}$	1.11	0.97	0.95
$FeCl_3$	0.62	0.80	0.54
$K_2Cr_2O_7$	0.99	0.58	0.09
$HgCl_2$	0.24	0.00	0.00

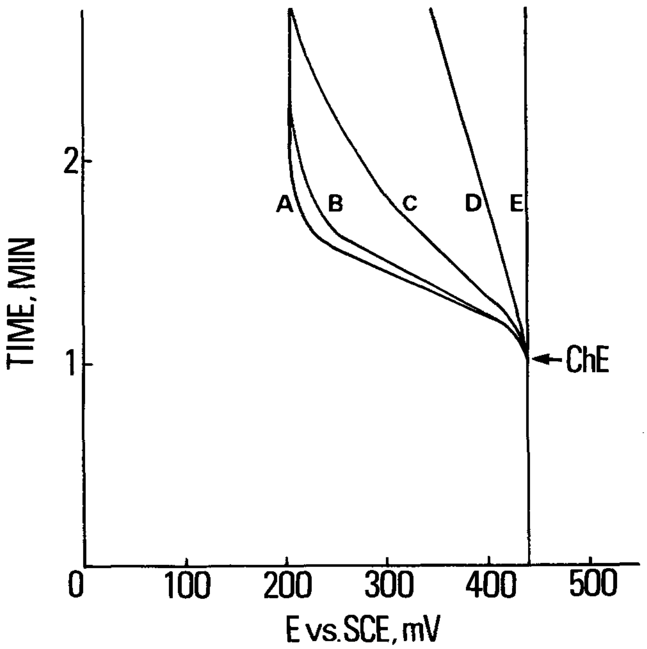

FIG. 1. Potential time curves for enzymatic hydrolysis of butyrylthiocholine iodide by cholinesterase (ChE), inhibited by C_{12}-sodium linear alkylbenzenesulfonate. A. No surfactant. B. 10 ppm. C. 20 ppm. D. 30 ppm. E. 50 ppm.

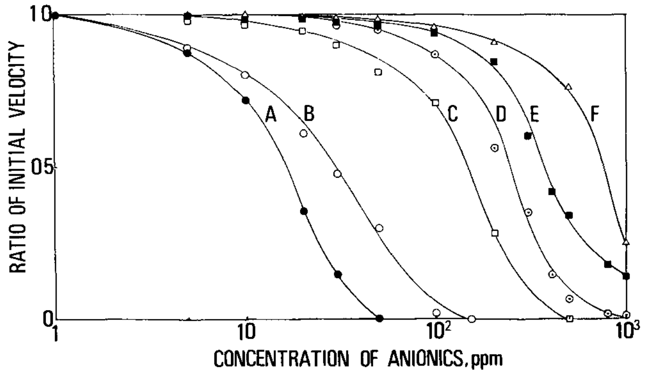

FIG. 2. Effect of anionic surfactants upon initial velocity. A. C_{12}-sodium linear alkylbenzenesulfonate. B. C_{12}-sodium branched alkylbenzenesulfonate. C. sodium dodecyl sulfate. D. C_{12}-α-olefin sulfonate. E. C_{12}-ether sulfate. F. C_{12}-soap.

in the present study.

A constant current was applied across two platinum plate electrodes (4 x 4 mm, 0.3 mm thickness) by two 67.5 volt radio B batteries (Fuji Electrochemical Co., Tokyo, Japan) connected in series across a 7 megohm resistor. The voltage produced at the anode was measured vs a saturated calomel electrode (SCE) with a pH meter model M-5, Horiba Instruments, Tokyo, Japan), and automatically recorded on a one-pen recorder model VP-653B, Matsushita Communication Ind. Co., Tokyo, Japan). The current was measured with a Shimadzu microammeter, model MK-120. All solutions were stirred with a magnetic stirrer model V-5, Toyo Kagakusangyo Co., Tokyo, Japan). The temperature of the solutions was regulated by the water temperature of a surrounding beaker whose water was circulated from a constant temperature bath, and the outer vessel was insulated further by a urethane pad.

Method

In a 50 ml beaker, there is placed 25 ml 1 x 10^{-3} M PrCOSChI solution, and a proper volume of surfactant solution is added. The mixture is stirred magnetically. The two platinum electrodes and SCE are immersed into the solution and a constant current of 19.3 µA is applied across the platinum electrodes. The resulting potential of the platinum anode vs SCE is recorded. To the solution, 0.5 ml ChE solution is added to bring about hydrolysis of the substrate. Ca. 1 min is needed to run an electric potential-time curve. The slope of the curve, $\Delta E/\Delta t$, is determined at the inflection point, and the amount of surfactant originally present may be determined by calibration plots of $\Delta E/\Delta t$ against surfactant concentration. After each measurement, the platinum electrodes are washed thoroughly with redistilled water, immersed in 1 N perchloric acid for 15 sec, and rinsed again with redistilled water.

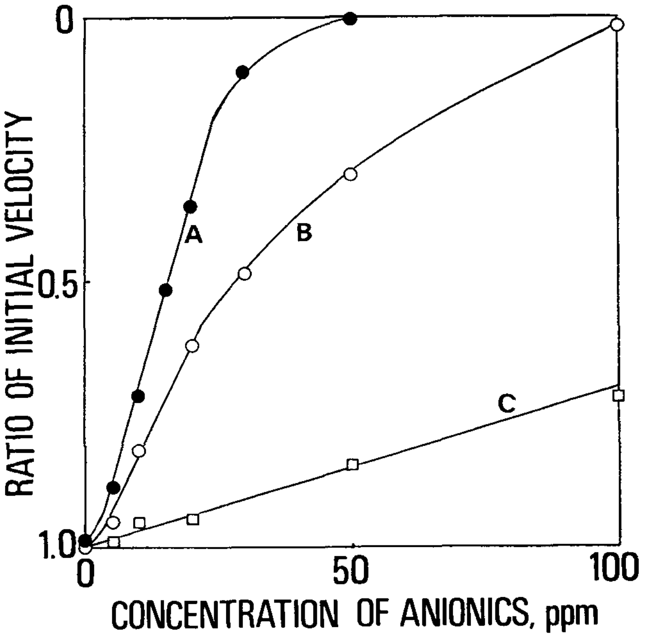

FIG. 3. Calibration plots of initial velocity vs anionic surfactant concentration. A. C_{12}-sodium linear alkylbenzenesulfonate. B. C_{12}-sodium branched alkylbenzenesulfonate. C. sodium dodecyl sulfate.

RESULTS

Factors Influencing the Enzyme Reaction Rate

Acyl groups of substrates: The electric potential-time curves for the enzymatic hydrolysis of 1 x 10^{-3}M MeCOSChI, EtCOSChI, and PrCOSChI, respectively, by 0.5 mg ChE were compared. The $\Delta E/\Delta t$ for PrCOSChI was the greatest, and, therefore, PrCOSChI was selected as a substrate material.

Concentration of substrate: PrCOSChI solution (25 ml) ranging in concentration from 0-1.5 x 10^{-3}M was allowed to react with 0.5 mg ChE. The initial rate of hydrolysis varies linearly with the concentration of PrCOSChI by low concentration range. At higher concentrations, 0.8 x 10^{-3}M or above, the rate becomes constant, regardless of the substrate concentration. The following experiments were all run at the fixed concentration of 1 x 10^{-3}M.

Concentration of enzyme: Varying amounts of ChE, in the range of 0-1.0 mg, were made to react with 1 x 10^{-3}M solution of PrCOSChI at 37 C. A linear relationship between the amount of ChE and $\Delta E/\Delta t$ appears up to 0.5 mg, but the slope gradually reaches a maximum at higher concentrations. The amount of ChE treated with 25 ml 1 x 10^{-3}M PrCOSChI was fixed at 0.5 mg.

pH: The effect of pH upon the initial rate of hydrolysis of substrate was studied at the various pHs in the range of 7.0-9.4. The rate increased gradually with pH, but spontaneous hydrolysis is liable to arise at high pH. Consequently, the following experiments were carried out at pH 7.40.

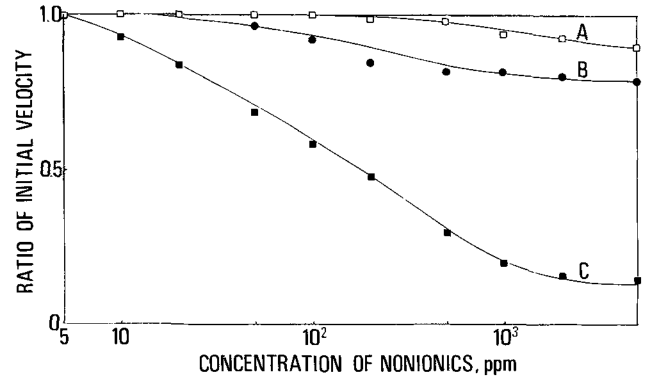

FIG. 4. Effect of nonionic surfactants upon initial velocity. A. Polyoxyethylene dodecyl ether. B. Polyxyethylene sorbitan monododecanoate. C. Polyoxyethylene octyl phenyl ether.

Temperature: The variation of $\Delta E/\Delta t$ with temperature was investigated in the range of 15-50 C. The initial rate of the enzymatic hydrolysis is proportional to the temperature up to 30 C. Then, it reaches a maximum and, at ca. 45 C or above, rapidly decreases owing to the inactivation of the enzyme. Therefore, the reaction temperature was controlled at 37 C.

Inorganic compounds: The effects of various inorganic salts, such as calcium chloride, magnesium chloride, sodium chloride, sodium sulfate, trisodium orthophosphate, sodium pyrophosphate, sodium tripolyphosphate, ferric chloride, potassium dichromate, and mercuric chloride, upon initial velocity of the enzyme reaction were studied. The results are given in Table I. The $\Delta E/\Delta t$ for the substrate solution free from inorganic compound is taken as unity. Calcium chloride acts as a strong activator and magnesium chloride is effective at enhancing ChE activity only at high concentrations. Five kinds of sodium salts scarecely affect the initial rate of the enzymatic hydrolysis. Ferric chloride, potassium dichromate, and mercuric chloride strongly inhibit the enzyme reaction.

Determination of Anionic Surfactants

The electric potential-time curves for the enzymatic hydrolysis of 1 x 10^{-3}M PrCOSChI by 0.5 mg ChE inhibited by C_{12}-LAS are shown in Figure 1. The slopes decrease with increasing the C_{12}-LAS concentrations, and the reaction is inhibited perfectly by 50 ppm C_{12}-LAS. For TBS, SDS, C_{12}-AOS, C_{12}-ES, and C_{12}-soap similar curves were obtained, and the plots of the initial velocity of the enzyme reaction against surfactant concentration are given in Figure 2. The $\Delta E/\Delta t$ for the substrate solution free from surfactant is taken as unity. The calibration curves for C_{12}-LAS, TBS, and SDS were prepared by using scales of uniform intervals (Fig. 3). By employing these curves, 5-25 ppm C_{12}-LAS and TBS and 5-100 ppm SDS can be determined.

Determination of Nonionic Surfactants

The inhibition curves for three kinds of nonionics, such as POE dodecyl ether, POE sorbitan monododecanoate, and POE octyl-phenyl ether are shown in Figure 4. The inhibitory action of these was not very strong at low concentrations in contrast to anionic surfactants. Consequently, it is difficult to determine trace amounts of nonionic surfactant directly by using these curves.

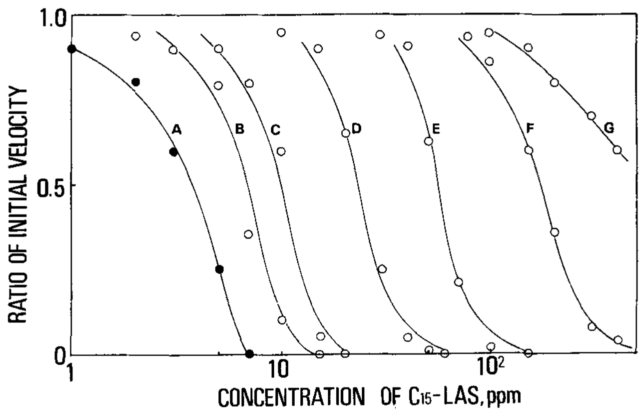

FIG. 5. Effect of polyoxyethylene dodecyl ether upon inhibition by C_{12}-sodium linear alkylbenzenesulfonate (LAS). A. No nonion. B. 5 ppm. C. 10 ppm. D. 20 ppm. E. 50 ppm. F. 100 ppm. G. 1000 ppm.

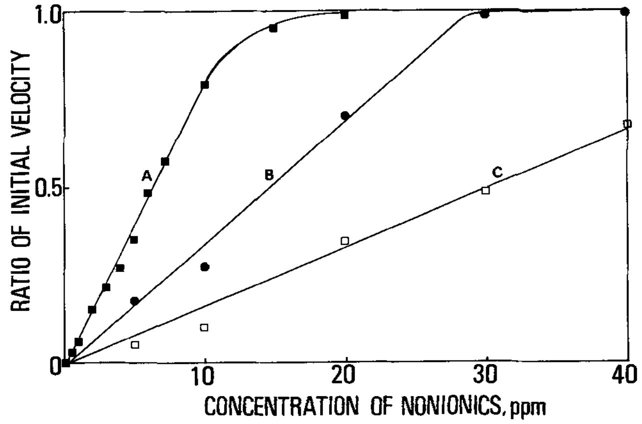

FIG. 6. Calibration plots of initial velocity vs. surfactant concentration. A. Polyoxyethylene dodecyl ether. B. Polyoxyethylene sorbitan monododecanoate. C. Polyoxyethylene octyl phenyl ether.

It has become apparent that the curve for C_{12}-LAS, shown in Figure 2A, is shifted to the high concentration region when a certain amount of POE dodecyl ether is added first, In other words, the inhibitory action of C_{12}-LAS seemingly is weakened by the addition of such nonionic surfactant. A similar result was obtained for C_{15}-LAS which is a stronger inhibitor than C_{12}-LAS and shown in Figure 5. The curves are shifted to the right with increase in additional amount of POE dodecyl ether.

Proper amounts of POE dodecyl ether, POE sorbitan monododecanoate, or POE octyl phenyl ether were added to the substrate solution containing 7 ppm C_{15}-LAS, and the effect of nonionic surfactants upon the initial rate of the enzymatic hydrolysis was investigated. In certain regions, the plots of the initial velocity vs concentration of nonionic surfactants give straight lines as shown in Figure 6. With the aid of these plots, 1-10 ppm POE dodecyl ether, 5-30 ppm POE sorbitan monododecanoate, 5-40 ppm, POE octylphenyl ether can be determined.

DISCUSSION

A potentiometric method has been described for the determination of microamounts of anionic and nonionic surfactants with the ChE-PrCOSChI system.

Both apparatus and procedure of this technique are relatively simple, and each measurement requires only a few min. Therefore, this method may be concluded to be a rapid assay system of trace amounts of surfactant. It is especially significant that the determination of microamounts of nonionic surfactant has become feasible. However, the selective determination of individual components of the surfactant mixture of the same ionic type or the different ionic types is not possible. Moreover, it is presumed that the length of alkyl or polyoxyethylene chains of surfactants influences the inhibitory or suppressive effects. Therefore, the selective determination of the components of a surfactant mixture is one of the most important subjects left to be done.

In analysis of the surfactant in waste water, river water, etc., various kinds of inorganic or organic substances containing the other type of surfactants often interfere with the measurement of initial velocity. However, inorganic compounds, such as sodium salts of tripolyphosphoric and sulfuric acids, which often are present in high percentages in the great majority of commercially manufactured detergents, hardly affect the rate of the enzymatic hydrolysis. This is very favorable for this method of analysis of these samples.

The clear difference in the inhibitory actions of anionic and nonionic surfactants is assumed to result from the intensity of interaction between surfactant and enzyme. The mode of inactivation has not become apparent. The mechanism of the protective effect of nonionics upon the inhibitory action of anionics is assumed to be based upon the suppression of the function of anionics by the formation of mixed micelle or complex. The cationic surfactants probably can be determined by the same procedure as described in the determination of nonionics, because the cationic and anionic surfactants are liable to form an insoluble complex.

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