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Enhancement of peroxyoxalate chemiluminescence intensity by surfactants and its application to detect detergent

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Abstract

Enhancement of peroxyoxalate chemiluminescence (PO-CL) intensity by a surfactant in the $\text{H}_2\text{O}_2/\text{bis}(2,4,6\text{-trichlorophenyl})\text{oxalate}$ (TCPO)/rhodamine B system was described. The effects of 15 surfactants were evaluated by comparing the ratio of a relative CL intensity (RCI) with surfactant to that of the blank in each system. In preliminary study, $\text{H}_2\text{O}_2/\text{imidazole-HNO}_3$ buffer/TCPO/rhodamine B system was used to study the effects of surfactants on PO-CL intensity. Fourteen surfactants reduced the CL intensity at the 2% concentration, where their relative CL intensities ranged from 0.6 to 93.5%. Some of these phenomena may be caused by a notable change of pH that was occurred by adding the surfactant.

Additionally, enhancement of PO-CL intensity was studied by using system 1) $\text{H}_2\text{O}_2/\text{TCPO}/\text{rhodamine B}$ and 2) $\text{H}_2\text{O}_2/\text{imidazole-HNO}_3$ buffer/TCPO/ systems. In the system 1, the favorable enhancement of CL intensity (ranged from 124 to 472%) was observed with 9 surfactants at the 0.5% concentration). This result suggested that several surfactants might play a role as a catalyst in the PO-CL reaction. There was no tendency to enhance CL intensity among the surfactant types. In the system 2, the enhancement of CL intensity was also observed by adding with 11 surfactants, which might be mainly caused by the fluorescent impurities of surfactants used.

Furthermore, detection of detergent commercially available was applied by using the system 1.

Keywords: peroxyoxalate chemiluminescence, synthetic surfactant, detergents, enhancement

1. Introduction

A peroxyoxalate chemiluminescence (PO-CL) method has been widely utilized in environmental, pharmaceutical and biomedical analyses owing to its high sensitivity and a need of simple instrumentation without a light source [1,2]. In the PO-CL system, H_2O_2 reacts with oxalates or oxamides under co-existence of a fluorophore to produce an emission of light. The reaction is achieved by a chemically initiated electron exchange luminescence (CIEEL) mechanism *via* a high energy intermediate which forms a charge transfer complex with the co-existing fluorophore. Then the electron is transferred to the fluorophore, which transits to an excited state and returns to a ground state by emitting a photon [3].

To improve the sensitivity of analytical method with PO-CL detection, various parameters such as pH [4], additives to reaction solvent [5] and catalysts [6-8] have been evaluated. Imidazole is one of the most useful catalysts in an aqueous PO-CL system [9]. Moreover, several studies on enhancement of PO-CL intensity in aqueous conditions by surfactants have also been reported [10-12]. Surfactants improved 1) excitation efficacy due to the solubilization of oxalate and 2) fluorescence yield of fluorophore. The mechanism of enhancement of CL intensity by surfactants seemed to be the combination of these improvements [10-12]. Cetyltrimethylammonium bromide was used to improve the reproducibility for determination method of glucose with a fiber-optic sensor immobilized with glucose oxidase [13]. Furthermore, sodium dodecyl sulfate (SDS) was added to carrier solution of a flow injection analysis system for determination of analytes derivatized with *o*-phthalaldehyde. These surfactants avoid rapid degradation of peroxyoxalate in water [14-16]. Utilizing this property of surfactant on PO-CL, a novel detection method for detergent might be developed. However, no

studies have been achieved for enhance effect of surfactant on PO-CL intensity with several surfactants under the same condition.

In this study, the effects of surfactants on PO-CL intensity were evaluated using the PO-CL system consisted of bis(2,4,6-trichlorophenyl)oxalate (TCPO), rhodamine B, H₂O₂ and imidazole. Fifteen surfactants such as cation-, anion-, zwitterionic- and non-ionic-types were evaluated. Furthermore, the commercially available detergents were determined by the method based on the enhancing effect of detergent on PO-CL intensity.

2. Materials and Methods

2.1. Chemicals

The 15 surfactants used were alkylsulfate/triethanolamine (Monogen T-423S, 36%, A), sodium poly(oxyethylenetridecylether)laurylsulfate (Hitenol 330T, 33%, B), disodium poly(oxyethylene)laurylsulfosuccinate (Neo-Hitenol L-30, 27%, C), sodium dioctylsulfosuccinate (Neocol YSK, 70%, D), sodium poly(oxyethylene laurylether)acetate (Neo-Hitenol ECL-30S, 27%, E), poly(oxyethylene laurylether)phosphoric acid (Plysurf A208B, F), cetyltrimethylammonium chloride (Catiogen TMR, 30%, G), benzalkonium chloride (Catiogen BC-50, 50%, H), lauryl dimethylamino acetic betaine (Amogen S, 30%, I), lauryl dimethylamine oxide (Amogen AOL, 32%, J), mixture of poly(oxyalkylenealkylether) (Noigen CL-200, K), poly(oxyethylene)tridecylether (Noigen TDS-30, L), poly(oxyethylene)tridecylether (Noigen TDS-200D, M), fatty acid diethanolamide from palm (Dianol CDE, N) and poly(oxyethylene)sorbitane monooleic acid ether (Sorgen TW-80V, O), and were purchased from Daiichi Kogyo Seiyaku Co. (Kyoto, Japan).

Bis(2,4,6-trichlorophenyl)oxalate (TCPO), imidazole and SDS from Tokyo Chemical Industries Co. (Tokyo, Japan) were used. Rhodamine B, H₂O₂ and CH₃CN were purchased from Wako Pure Chemical Industries Co. (Osaka, Japan). Other reagents used were of analytical reagent grade. Water was deionized and distilled by an Aquarius GSR-500 automatic water distillation apparatus (Advantec Mfs, Inc., Tokyo, Japan).

Surfactants were diluted appropriately with water and used for evaluation of effect on PO-CL intensity. Measurement of pH values of surfactants was performed by a pH meter F-22 (Horiba, Kyoto).

2.2. Assay procedure of PO-CL

The surfactant solution (25 μ L) in a test tube was evaporated under N₂ gas, and to the residue, 25 μ L of 0.5 μ M rhodamine B in CH₃CN, 5 mM imidazole-HNO₃ (pH 6.5) and 120 mM H₂O₂ in CH₃CN were added and mixed for 10 sec (preliminary study). In the system 1 and 2, addition of imidazole-HNO₃ buffer and rhodamine B in the preliminary study was replaced by addition of water and CH₃CN, respectively. After adding 25 μ L of 0.25 mM TCPO in CH₃CN, the CL intensity was measured at room temperature for 120 sec with a Luminescencer PSN AB-2200 (Atto Co., Tokyo, Japan). The effects of 15 surfactants were shown as a relative CL intensity (RCI) to the CL intensity of the blank (=CL without surfactant was taken as 100) in each system. Decay curves of CL intensity of surfactants were prepared by using kinetic mode of the instrument with 1 sec of gate time.

2.3. Measurement for fluorescence spectra of surfactants solutions

Fluorescence spectra of surfactant solutions were measured by a 650-10S

fluorescence spectrophotometer (Hitachi Co., Tokyo). Ten times diluted surfactants with water or methanol (except for sample D) was used for measurement of fluorescence spectrum. Sample D was measured without dilution.

2.4. Determination of commercially available detergents

Four kinds of commercially available detergents (I-IV) were determined. The details of the products were unknown because it was not mentioned by manufacturers. In the system 1, the detergents were diluted with water. The limit of detection was defined as the content of detergent at a signal-to-blank (S/B) ratio of 2. Repeatability of the method was presented as %RSD of five-times replicate measurements with 0.25% (I), 5×10^{-4} % (II), 0.1% (III) and 1×10^{-4} % (IV) of sample.

3. Results and discussion

3.1. Enhancement of PO-CL intensity by surfactant

The examined fifteen surfactants and their pH values are listed in Table 1; A-F, G and H, I and J, and K-O were cation-, anion-, zwitterionic- and nonionic-type surfactants, respectively. The optimized concentration for preliminary study and system 1 and 2 were 2%, 0.5% and 2%, respectively. In the preliminary study consisted of H_2O_2 , imidazole- HNO_3 buffer, bis(2,4,6-trichlorophenyl)oxalate (TCPO) and rhodamine B, most surfactants quenched CL depending on the amount of surfactant. Figure 1 shows the quenching effect of 2% concentration of surfactants on the PO-CL intensity ranging from 0.6 to 93.5% of the blank. Samples F, J, I and N showed remarkable quenching effects on PO-CL intensity. One of the reasons for this quenching may due to notable change of pH that occurred based on adding the surfactant. The pH values of these

surfactants were extremely far from suitable pH (around 6.5) for this PO-CL reaction. The effect of pH on RCI was examined in preliminary study (data was not shown), and pH 6.5 was selected. Additionally, the decay-curves of CL intensity for blank (a) and sample F (b) and N (c) were monitored (Fig. 2). The surfactants with low pHs decreased RCI and prolonged luminescence time (Fig. 2(b)) compared to that of blank, while that with alkaline pHs also decreased RCI and produced flashed luminescence (Fig. 2(c)). However, the reason of quenching effect by samples having neutral pHs such as sample A and E was unclear. Next, effect of micelle formation on RCI was examined by SDS whose critical micelle concentration (CMC) in water is 3.5 mM [17]. The constant RCI was obtained using SDS ranging from 2 to 16 mM involving CMC, so no influence of micelle formation on RCI was observed (data was not shown). Although improvement of fluorescence yield of fluorophore by addition of surfactant has been known as one of the mechanisms of enhancement, the increase of RCI due to improvement of fluorescence yield of fluorophore was not ever exhibited in the preliminary study.

Table 1 Synthetic surfactants examined in this study and their pH

Type	Sample	Compound	pH
Anion-type	A	Alkylsulfate/triethanolamine	7.29
	B	Sodium poly(oxyethylenetricetyloxy)laurylsulfate	8.37
	C	Disodium poly(oxyethylene)laurylsulfosuccinate	5.24
	D	Sodium dioctylsulfosuccinate	6.02 ^a
	E	Sodium poly(oxyethylene lauryloxy)acetate	6.03
	F	Poly(oxyethylene lauryloxy)phosphoric acid	0.88
Cation-type	G	Cetyl trimethylammonium chloride	5.28
	H	Benzalkonium chloride	7.76
Zwitterionic-type	I	Lauryl dimethylamino acetic betaine	5.64
	J	Lauryl dimethylamine oxide	8.22
Non-ionic-type	K	Poly(oxyalkylenealkyloxy)	7.91
	L	Poly(oxyethylene)tridecylether	8.35
	M	Poly(oxyethylene)tridecylether aqueous solution	6.40
	N	Fatty acid diethanolamide from Palm	9.79
	O	Poly(oxyethylene)sorbitane monooleic acid ether	7.47

^apH of non-diluted solution.

Fig. 1

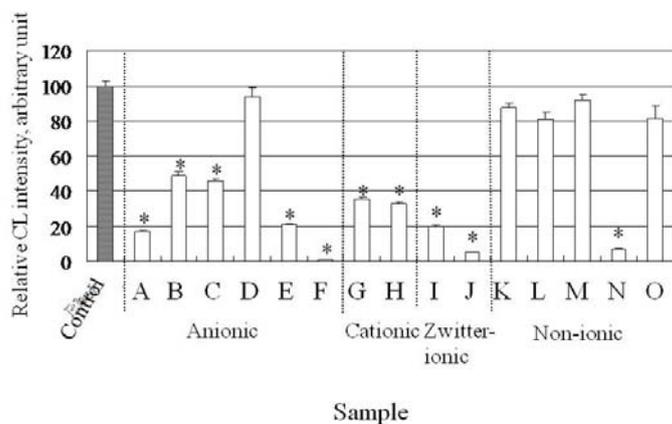
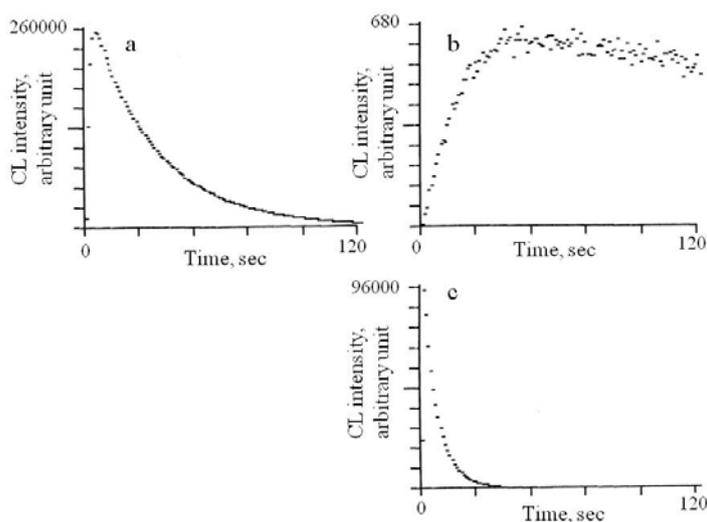


Fig. 2



The effect of surfactants on RCI was examined in the system 1 which consisted of H_2O_2 , TCPO and rhodamine B (Fig. 3). The significant enhancement of CL intensity in the range of 1.7-4.7 times was observed compared to the blank by addition of several surfactants at 0.5% concentration. Sample B, D, H and L showed highly enhancement of RCI. This result suggested that several surfactants spiked might play a role as a catalyst in the system 1. However, no tendency to enhance CL intensity among surfactant types could be observed. In Fig. 3, RCI by the H_2O_2 /TCPO/rhodamine B/imidazole- HNO_3

buffer (=preliminary study) without surfactants is used as a control, where imidazole catalyzed the PO-CL reaction. No surfactants showed similar enhancing effect on RCI as imidazole which is often used as a catalyst in aqueous PO-CL reaction. And quenching of CL in system 1 due to notable change of pH was also occurred harder than those in preliminary study. Because there is no buffer in the system 1. So enhancement of CL in sample F, J and N could not be observed.

The linearity study with system 1 using sample B was demonstrated. The calibration curve of sample B in the range of 0.025-0.5% showed a good linearity ($r=0.985$). The repeatability of the proposed method with five-times replicate measurements at 0.025, 0.05, 0.1, 0.2 and 0.5% of sample concentration were in the range of 2.3-7.1 (%RSD).

The combinations of surfactants and CL reagents to evaluate enhancement of aqueous PO-CL in previous papers are listed in Table 2. Any studies were demonstrated in the absence of catalyst in PO-CL system and did not describe catalytic effect of surfactant as an additional effect. The potential mechanism of enhancement of CL intensity might be concluded as the combination of the improvement of 1) excitation efficacy due to the solubilization of oxalate and 2) fluorescence yield of fluorophore in previous papers [10-12].

Jonsson *et al.* reported a study on the catalytic effect of substituted imidazole derivatives on PO-CL using H_2O , TCPO and 3-aminofluoranthene as CL reagents [7]. Formation of an intermediate with the imidazole derivatives proved by spectrophotometric measurement of decomposition of TCPO was a proof of their catalytic effect. Sodium azide enhanced RCI of the H_2O_2 /TCPO/pyrene system, whose effect was higher than triethylamine, another active catalyst in PO-CL reaction [6]. Furthermore, aliphatic amines such as polyamines, and therapeutic drugs (*e.g.* FK-506,

cyclosporin A, rifampicine and angiotensin I) also enhanced PO-CL reaction consisted of the $\text{H}_2\text{O}_2/\text{bis}[4\text{-nitro-2-(3,6,9-trioxadecyloxycarbonyl)phenyl]oxalate/}$ sulphorhodamine 101 system [8,18]. These drugs with molecular weights between 800 and 1500 accelerated the formation of reactive intermediate, hydroperoxy oxalate, which appeared as the most reactive intermediate for CL detection. However, the required condition of catalyst to yield enhancing effect on PO-CL was not clear.

Fig. 3

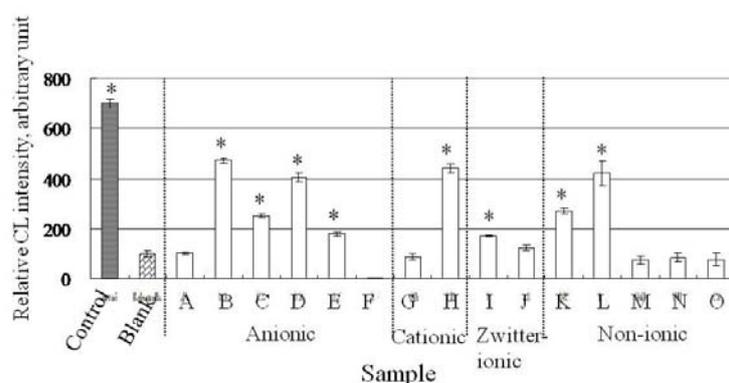


Table 2 The combination of surfactants and CL reagents in previous reports.

Surfactants	CL reagents	Reference
Hexadecyltrimethylammonium chloride, polyoxyethylene, dodecanol, 3-(<i>N</i> -dodecyl- <i>N,N</i> -dimethylammonio)-1-propanesulfonic acid	H_2O_2 , TCPO, 8-anilino-1-naphthalenesulfonic acid	10
Hexadecyltrimethylammonium chloride, sodium bis(2-ethylhexyl)sulfosuccinate	H_2O_2 , bis[<i>N</i> -[2-(<i>N'</i> -methyl-2'-pyridiniumyl)- <i>N</i> -[(trifluoromethyl)sulfonyl]]oxamide, 8-anilino-1-naphthalenesulfonic acid or rhodamine B	11
Polyoxyethylene dodecyl ether, polyoxyethylene hexylphenol ether	H_2O_2 , bis(2-butoxycarbonyl-3,4,6-trichlorophenyl)oxalate and 9,10-diphenylanthracene	12
Cetyltrimethylammonium bromide	H_2O_2 , TCPO, perylene	13

The reason why the surfactant quenched PO-CL in the preliminary study was not clear, whereas it enhanced RCI in the system 1 based on their catalytic effect. When the effect of imidazole concentration on RCI was examined in the preliminary study, 20 mM of imidazole gave the highest RCI, while excess imidazole (up to 20 mM) quenched PO-CL (data was not shown). And thus, the addition of surfactants at the optimum imidazole concentration in the preliminary study might lead to oversupply of catalyst.

In the system 2 including H₂O₂, imidazole-HNO₃ buffer and TCPO, CL intensity was enhanced 2.3-77.1 times compared to that of blank by adding with some surfactants (Fig. 4). Sample O showed the highest enhancement in all surfactants examined. But the CL intensity level was very weak compared to that of control (=412 times higher than that of blank). The reason of these enhancements in the system 2 might be due to the fluorescent impurities in these surfactants. Fluorescence spectra of surfactant solutions were measured by a fluorescence spectrophotometer. In Table 3, fluorescence spectral data are listed (except for sample I, J and O). Fluorescence spectrum of sample H having an aromatic ring might be by itself. However, all impurities could not be identified. As these results the acts of surfactant in PO-CL were 1) notable pH changer, 2) catalyst and 3) supplier of fluorophore. These actions occurred simultaneously in all systems, but the most influential one among them for each system might be appeared.

Fig. 4

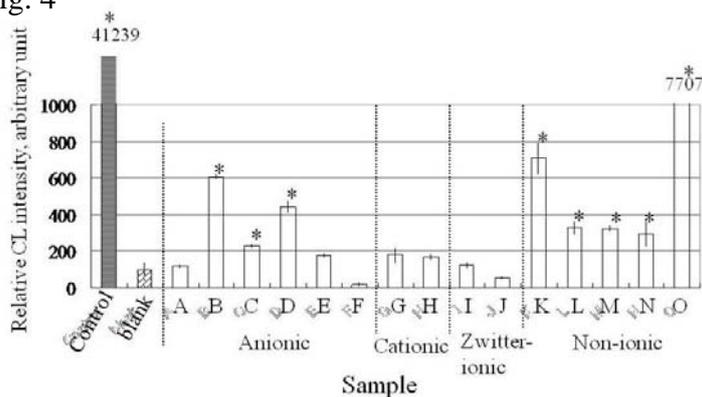


Table 3 Fluorescence spectral data of surfactant solutions.

Sample	λ_{ex}	λ_{em}	Sample	λ_{ex}	λ_{em}
A ^a	283	316	I ^a	ND ^c	ND ^c
B ^a	356	453	J ^a	ND ^c	ND ^c
C ^a	289	312	K ^a	297 ^a	343
D ^b	290	316	L ^b	304	346
E ^a	335	460	M ^b	304	346
F ^b	284	306	N ^b	ND ^c	ND ^c
G ^b	301	337	O ^b	358	451
H ^a	301	358			

Solvent:^awater; ^bMeOH.

^cND: Not detected.

3.2. Application to detect commercially available detergents

The system 1 was found to be suitable to detect surfactant, and thus it was applied to detect commercially available detergents. The assay procedure used was same as that described in the section 2.2. All four detergents (I-IV) examined could be successfully detected. The observed RCI was depended on the amount of detergent in the range of 0.05-0.5% (for I), 5×10^{-5} - 1×10^{-3} % (for II), 0.05-0.25% (for III) and 1×10^{-5} - 1×10^{-4} % (for IV). The limits of detection for the detergents I, II, III and IV at an S/B ratio of 2 were 0.16, 4.8×10^{-4} , 0.24 and 1.0×10^{-4} %, respectively. The detergent II and IV were detected highly sensitive. The reason of this difference in sensitivity might be due to species of surfactant included in detergents. Repeatability of the method for I-IV was 3.0, 5.3, 7.7 and 6.7%, respectively.

4. Conclusions

The effects of 15 surfactants on PO-CL were examined. Surfactants examined acted as notable pH changer, catalyst and supplier of fluorophore on PO-CL system. Among

them, sodium poly(oxyethylenetridecylether)laurylsulfate, sodium dioctylsulfosuccinate, benzalkonium chloride and poly(oxyethylene)tridecylether showed highly enhancement of RCI. Furthermore, the PO-CL system including H₂O₂ /TCPO/rhodamine B was found to be suitable for evaluation of catalytic effects of surfactants. As the proposed system could be successfully applied to detect commercially available detergents, it might be useful to check the residue of detergents on the vessels so on.

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Figure Captions

Fig. 1 Effects of surfactants on CL intensities in the preliminary study.

Sample: 2% detergents.

Other CL conditions were shown in the Experimental section.

* $P < 0.05$ vs Control (Sheffett's F -test).

CL intensity of control was taken as 100.

Fig. 2 Decay curve of CL intensity with/without addition of surfactants in the preliminary study.

Sample: a, blank; b, 2% sample F; c, 2% sample N.

Other CL conditions were shown in the Experimental section.

Fig. 3 Effects of surfactants on CL intensities in the system 1.

Sample: 0.5% detergents.

Other CL conditions were shown in the Experimental section.

* $P < 0.05$ vs blank (Sheffett's F -test).

CL intensity of blank was taken as 100.

Fig. 4 Effects of surfactants on CL intensities in the system 2.

Sample: 2% detergents. Other CL conditions were shown in the Experimental section.

* $P < 0.05$ vs blank (Sheffett's F -test).

CL intensity of blank was taken as 100.