Polymer Journal, Vol. 28, No. 8, pp 671—677 (1996)

Synthesis and Chemiluminescence of Polymers with a Releasable 10-Methyl-9-(*N*-butyl-*N*-*p*-toluenesulfonylcarbamoyl)acridinium Moiety

Motohiro MITANI,† Yasumi KOINUMA, Keigo FUGAMI,* and Masanori KOSUGI*

Tsukuba Research Laboratory, NOF Corporation, Tokodai, Tsukuba, Ibaraki 300–26, Japan
**Department of Chemistry, Faculty of Engineering, Gunma University, Kiryu, Gunma 376, Japan*

(Received December 20, 1995)

ABSTRACT: 2-[10-Methyl-9-(*N*-butyl-*N*-*p*-toluenesulfonylcarbamoyl)-9,10-dihydro-9-acridinyloxy]ethyl methacrylate (AOEM) was synthesized as a monomer having a chemiluminescent moiety. Radical polymerization of AOEM using 2,2′-azobis(isobutyronitrile) at 50°C gave a homopolymer ($M_n = 2.88 \times 10^4$, $M_w/M_n = 2.23$). Similarly, copolymers of AOEM with methyl methacrylate (MMA) were also obtained. The chemiluminescence spectrum of the homopolymer was consistent with that of AOEM or 10-methyl-9-(*N*-butyl-*N*-*p*-toluenesulfonylcarbamoyl)acridinium trifluoromethanesulfonate. One AOEM moiety of the polymer showed a light yield at 64% to 99% of the level of AOEM, and the total light yield was proportional to the release yield of 10-methyl-9-(*N*-butyl-*N*-*p*-toluenesulfonylcarbamoyl)acridinium salt from the polymer. Release of 10-methyl-9-(*N*-butyl-*N*-*p*-toluenesulfonylcarbamoyl)acridinium salts from both polymers and AOEM was observed under acidic conditions. Similar acidic release was also confirmed by measuring the time courses of light emission.

KEY WORDS Chemiluminescence / Polymer / Acridinium Salt / Release /

Chemiluminescent compounds such as luminol and acridinium salts are used as labels for antigens or antibodies.[1] Immunoassay using antibodies labeled with chemiluminescent compounds is well-known as a means of highly sensitive detection.[2-4] The sensitivity of the detection of analytes is dominated by the quantum yield of the chemiluminescent compound and the number of chemiluminescent compounds per molecule of antibody, because the analytes are generally detected as conjugates such as "(immobilized antibody)–(analyte)–(antibody labeled with chemiluminescent compounds)". Therefore, if the number of chemiluminescent compounds per molecule of antibody is constant, the strategies for enhancement of the sensitivity are classified into two categories. One is the development of a new chemiluminescent compound with a high quantum yield. The other is the development of a polymer that has many pendent chemiluminescene moieties. The former has extensively been investigated. However, a more efficient molecule than acridinium salts has not been developed. Moreover, the sensitivity is limited by the quantum yield of the molecule. On the other hand, in the latter, there is theoretically no limit in sensitivity, because it equals the quantum yield of each chemiluminescent moiety multiplied by the number of moieties per molecule of polymer.

Gundermann *et al.* synthesized a homopolymer containing luminol units. The light yield per luminol unit, however, was only 0.0005 times that free luminol.[5,6] We also previously synthesized a homopolymer of styrene attached to a chemiluminescent acridine derivative, the light yield of which was similarly low. The reason for the low light yields would be attributed to the self-quenching due to the close arrangement of the chemiluminescent moieties. To avoid this kind of loss, it is therefore essential to make the latter strategy useful. We have now designed a new polymer having chemiluminescent moieties that are easily released under the measurement conditions. The concept is shown in Figure 1. In the assay, the chemiluminescent polymer is labeled on the antibody at the end of the polymer. After the conjugate of the "(immobilized antibody)–(analyte)–(antibody labeled with chemiluminescent polymer)" is formed, the chemiluminescent moieties are then released. The released moieties should emit light without loss observed in the corresponding polymer-bound analogs. Because a large number of chemiluminescent moieties are liberated per analyte molecule, higher sensitivity of the assay can be realized. Here we report the synthesis of 2-[10-methyl-9-(*N*-butyl-*N*-*p*-toluenesulfonylcarbamoyl)-9,10-dihydro-9-acridinyloxy]ethyl methacrylate (AOEM) and the preparation of its homopolymer and copolymer with methyl methacrylate (MMA) as a model of chemiluminescene polymers having a release function for immunoassay. We also report the release abilities of chemiluminescent acridinium salts from the polymers and the chemiluminescent properties of those liberated moieties.

EXPERIMENTAL

Materials

p-Toluenesulfonyl chloride and butylamine were obtained from Tokyo Kasei Kogyo Co., Ltd. Triethylamine, 2-hydroxyethyl methacrylate, methyl methacrylate (MMA) and 2,2′-azobis(isobutyronitrile) (AIBN) were obtained from Wako Pure Chemical Ind., Ltd. Sodium hydride was obtained from Kishida Chemical Co., Ltd. Methyl trifluoromethanesulfonate was obtained from Aldrich Chemical Co., Inc. Dichloromethane, tetrahydrofuran (THF), *N*,*N*-dimethylformamide (DMF) and benzene were purified according to the usual methods. Synthesis of 9-(chlorocarbonyl)acridine hydrochloride is described in the synthesis section.

† To whom correspondence should be addressed. Present address: *New Business Development Division, NOF Corporation, Ebisu, Shibuya-ku, Tokyo 150, Japan.*

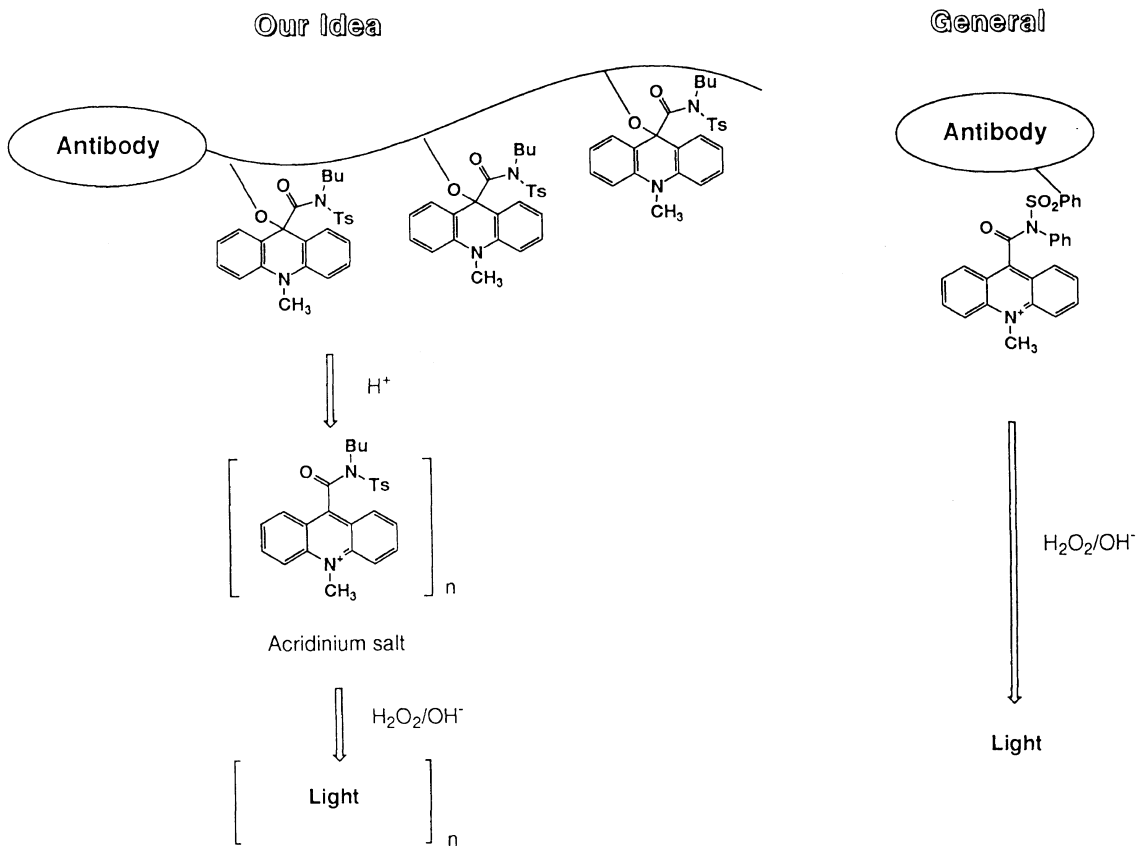


Figure 1. Antibodies labeled with chemiluminescence compounds.

Measurements

^{1}H NMR spectra were taken on a JEOL JNM-EX270 FT-NMR spectrometer. IR spectra were recorded on a JASCO FT-IR 7300 spectrophotometer. Mass spectra were taken on a JEOL JMS-DX303 spectrometer by a fast atom bombardment (FAB) method. Elemental analyses were performed by a PERKIN ELMER 2400II CHNS/O analyzer. Melting points were measured by a SEIKO SSC-5200 Tg/DTA analyzer. Gel permeation chromatography (GPC) was performed on a JASCO 880-PU high performance liquid chromatography system using a PL gel (Polymer Laboratories, 10 μm, MIXED-B) and THF as an eluent. A JASCO 830-RI detector was used. The GPC data were evaluated from the calibration curve using standard polystyrenes.

Synthesis of Monomer

N-Butyl-p-toluenesulfonamide (**1**). Butylamine (3.84 g, 0.052 mol) and triethylamine were dissolved in dichloromethane (50 ml) and to the solution was added dropwise a dichloromethane (25 ml) solution of *p*-toluenesulfonyl chloride (10 g, 0.052 mol) at 0°C. The reaction mixture was stirred for three days at room temperature. The precipitates were filtered off, and the filtrate was washed with water and then dried over magnesium sulfate. After the evaporation of dichloromethane, the residue was purified by silica gel chromatography (eluent: ethyl acetate/hexane = 1 : 5). **1** (11.62 g, 0.051 mol) was obtained in 98% yield.

9-(N-Butyl-N-p-toluenesulfonylcarbamoyl)acridine (**2**). **1** (11.5 g, 0.051 mol) was dissolved in DMF (200 ml). Sodium hydride (60 wt% in oil, 4.41 g, 0.11 mol) was added to the above solution at 0°C. After the solution was stirred for 30 min at room temperature, it was again cooled to 0°C. 9-(Chlorocarbonyl)acridine hydrochloride (14.75 g, 0.053 mol) prepared according to the method of White *et al.*[7] was added portionwise to the above solution. The reaction mixture was stirred for one day at room temperature. The reaction mixture was then diluted with ethyl acetate (500 ml), washed with water several times, and dried over sodium sulfate. After evaporation of ethyl acetate, yellow crystals were obtained in 65% yield. The crystals were purified by recrystallization from ethanol.

10-Methyl-9-(N-butyl-N-p-toluenesulfonylcarbamoyl)-acridinium trifluoromethanesulfonate (**3**). **2** (1.0 g, 2.31 mmol) was dissolved in dichloromethane (50 ml), and methyl trifluoromethanesulfonate (10 g, 0.061 mol) was added to the solution dropwise. The reaction mixture was stirred for four days at room temperature under an argon atmosphere and then refluxed for one hour. Methyl trifluoromethanesulfonate was removed under reduced pressure and the residue was purified by silica gel chromatography (eluent: dichloromethane/methanol = 25 : 2). **3** was obtained as a yellow oil which was crystallized with methanol–diethyl ether as a yellow powder in 86% yield, mp 166°C.

2-(10-Methyl-9-(N-butyl-N-p-toluenesulfonylcarbamoyl)-9,10-dihydro-9-acridinyloxy)ethyl methacrylate (AOEM) (**4**). **3** (83 mg, 0.14 mmol) was dissolved into 2-hydroxyethyl methacrylate (8.0 g), and triethylamine (150 μl, 1.07 mmol) was added to the solution at room temperature. The reaction mixture was stirred for one day at room temperature. 2-Hydroxyethyl methacrylate was removed by distillation under reduced pressure. The residue was purified by basic alumina column chroma-

tography (eluent: ethyl acetate/hexane = 9 : 40). The purified compound [43 mg (0.075 mmol) 54% yield] was recrystallized from ethyl acetate/hexane (1 : 1) as a white powder, mp (decomposes) 153°C. ^{1}H NMR (C_6D_6): δ 8.07 (d, 2H, J = 8.25 Hz), 7.32 (d, 1H, J = 7.92 Hz), 7.32 (d, 1H, J = 7.92 Hz), 7.09—7.03 (m, 2H), 6.81 (d, 2H, J = 8.2 5Hz), 6.71—6.65 (m, 2H), 6.57 (d, 2H, J = 8.25 Hz), 5.93 (s, 1H), 5.08, 5.07 (s, 1H), 3.96 (t, 2H, J = 5.12 Hz), 3.33 (dd, 2H, J = 8.91, 7.59 Hz), 3.05 (t, 2H, J = 5.12 Hz), 2.76 (s, 3H), 1.89 (s, 3H), 1.68, 1.67 (s, 3H), 1.16—1.07 (m, 2H), 0.63—0.53 (m, 2H), 0.48—0.42 (m, 3H). ^{13}C NMR (C_6D_6); 169.84, 167.00, 144.25, 140.76, 138.30, 137.01, 130.63, 129.96, 129.41, 128.26, 125.36, 121.34, 119.45, 113.47, 79.61, 64.09, 61.47, 47.68, 33.16, 32.30, 21.61, 20.75, 18.66, 13.76. IR (cm^{-1}); 2950, 1725, 1699, 1598, 1482, 1350, 1170. UV-Vis spectrum (DMF) ε = 8630 (at 312 nm), (DMF–0.1 M HNO_3 = 1 : 1, v/v) ε = 4590 (at 426 nm). FAB-MS; 575 ($M^+ - 1$), 448. Elemental analysis; Calcd for $C_{32}H_{36}N_2O_6S$: C, 66.65%; H, 6.29%; N, 4.86%. Found: C, 66.36%; H, 6.29%; N, 4.69%.

Polymerization

The radical homopolymerization of AOEM and copolymerization with MMA of AOEM were carried out as follows; AOEM and AIBN were dissolved in benzene. The solution was placed in a glass ampoule and degassed by a freeze-thaw cycle. The ampoule was sealed and immersed in a water bath with shaking at 50°C. After polymerization, the reaction mixture was poured into a large amount of diethyl ether. The polymerization products were purified by three reprecipitations from diethyl ether and dried under reduced pressure. The absence of AOEM in the polymers was confirmed by the disappearance of the double bond peaks in the ^{1}H NMR spectra and by the GPC analysis. Elemental analysis for homopolymer; Calcd for $C_{32}H_{36}N_2O_6S$: C, 66.64%; H, 6.29%; N, 4.86%. Found: C, 65.95%; H, 6.31%; N, 4.55%.

Chemiluminescence Spectra

The chemiluminescence spectra of AOEM, the polymer of AOEM (run 1 in Table I), and acridinium trifluoromethanesulfonate (**3** in Scheme 1) were measured as follows; AOEM, the polymer of run 1, and **3** were dissolved in DMF at the concentrations of 0.033 g l^{-1}, 0.051 g l^{-1} and 0.036 g l^{-1} for each sample, respectively. Fifty μl of the DMF solution and 300 μl of 0.1 M HNO_3 aqueous solution including 0.5 wt% H_2O_2 were added into a quartz cell, which was then placed into the chemiluminescence spectrophotometer with a photodiode (Otsuka Electronics, IMUC-7000). Three hundred μl of 0.25 M NaOH aqueous solution was added to the cell by a syringe and the spectrum was measured at 25°C.

Measurement of Light Yield

The measurements of the light yield of AOEM or the polymers of AOEM were performed at three different concentrations. From the slopes of the measured plots, the light yields were calculated. A typical measurement was as follows; AOEM or the polymer of AOEM was dissolved in DMF at several concentrations. Twenty-five μl of the DMF solution and 300 μl of 0.1 M HNO_3 aqueous solution including 0.5 wt% H_2O_2 were added into a glass tube, and then the tube was placed in a chemiluminescence detector with photomultiplier (Aloka, Luminescence Reader BLR 201). After 20 s., 300 μl of 0.25 M NaOH aqueous solution was added to the tube by a syringe and the light emission was measured at 25 °C for 10 min.

Measurement of Ultraviolet-Visible Spectra

AOEM and the polymers of AOEM (runs 1 and 3 in Table I) were dissolved in DMF at the concentrations of 0.033 g l^{-1}, 0.051 g l^{-1} and 0.052 g l^{-1}. Five hundred μl of the DMF solution and 500 μl of 0.01 M NaOH–citric acid buffer solution or HNO_3 aqueous solution were added into a quartz cell, which was placed in the spectrophotometer (Hewlett Packard, HP 8452A) and then the spectra were measured.

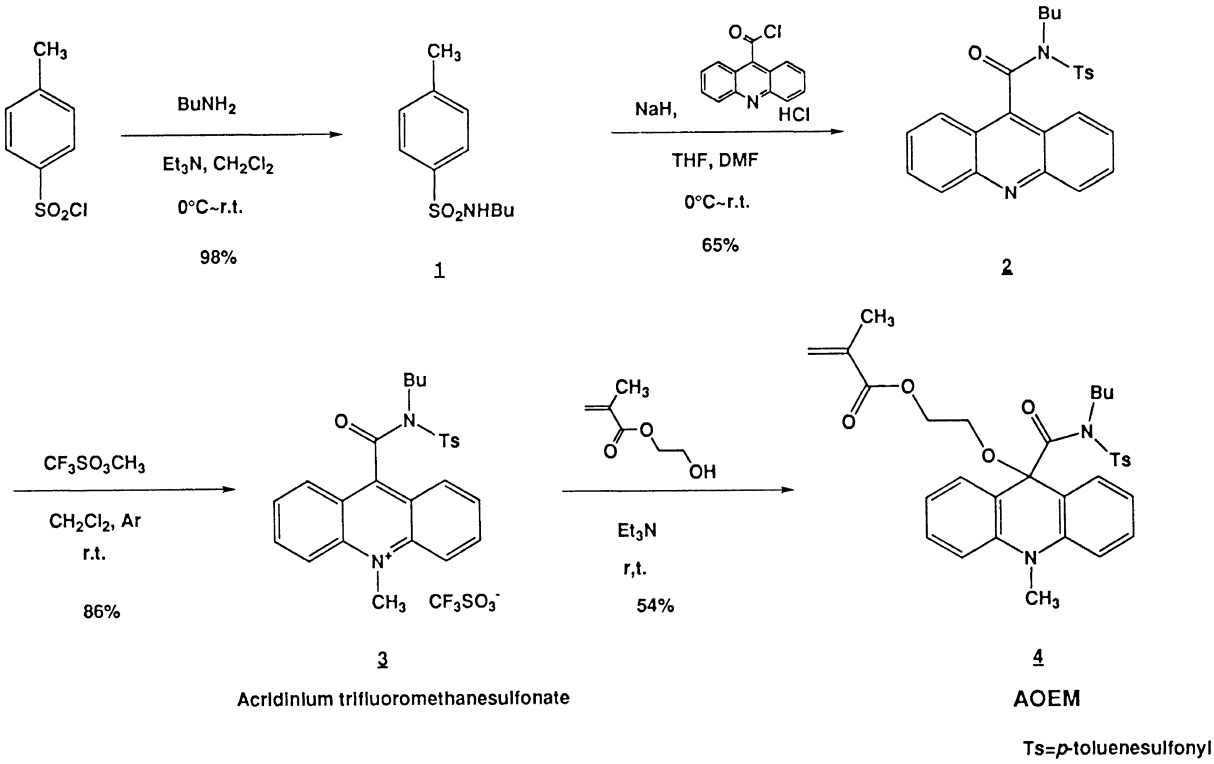
RESULTS AND DISCUSSION

Synthesis and Polymerization of AOEM

AOEM was synthesized according to Scheme 1. The step from **2** to **3** and the final step from **3** to **4** were modifications of the methods of Mattingly[8] and McCapra,[9,10] respectively. The total yield was about 30% based on *p*-toluenesulfonyl chloride. The AOEM was purified by basic alumina gel chromatography, followed by recrystallization, because it was unstable on silica gel. Based on the thermogravity analysis of AOEM, 20% of the weight of AOEM was lost at 153°C. The weight of the HEMA moiety corresponds to 22% of that of AOEM ; therefore, this might indicate cleavage of the HEMA moiety.

The homopolymerization of AOEM and copolymerization with MMA were performed in benzene at 50°C using AIBN as an initiator. The results are summarized in Table I. In spite of the bulkiness of AOEM, homopolymerization proceeded efficiently to yield the homopolymer ($M_n = 2.88 \times 10^4$, $M_w/M_n = 2.23$) as a pale yellow powder (run 1). The M_n corresponds to 46 units of the AOEM moiety. AOEM could also be easily copolymerized with MMA (runs 2 to 6).

Table II shows the molar concentrations of AOEM in the monomer mixtures, the molar content of the AOEM moiety in the polymers calculated from the nitrogen contents obtained by elemental analyses, and the average numbers of the AOEM moiety in the polymers estimated from the latter values and the M_n values (Table I) of the polymers. The molar concentrations of the AOEM moiety in the polymers were approximately parallel to the corresponding molar concentrations in the monomer mixtures. Although the molar concentration of AOEM in the monomer mixtures of run 3 was higher than that of run 4, the average number of the AOEM moieties in the polymer of run 3 was fewer than that of run 4. This might arise from the higher M_n of the polymer of run 4 compared with that of run 3 (Table I). Even if the molar concentrations of AOEM in the monomer mixtures are the same, a higher molecular weight polymer would contain a larger average number of AOEM moieties.



Scheme 1. Synthesis of AOEM.

Table I. Radical polymerization of AOEM with MMA in Benzene at 50°C

Run	AOEM	MMA	AIBN	Conc. of monomers	Time	Yield	$M_n \times 10^{-4}$	M_w/M_n
	mmol	mmol	mmol dm^{-3}	mol dm^{-3}	h	wt%		
1	0.26	0	5.7	0.15	40	36	2.88	2.23
2	0.22	0.260	5.7	0.27	40	37	3.12	1.99
3	0.087	1.03	5.7	0.64	40	55	3.65	1.85
4	0.070	3.11	26	4.1	20	56	8.58	1.90
5	0.035	3.04	31	4.1	20	50	7.75	1.88
6	0.018	3.05	26	4.2	20	50	8.11	1.82

Table II. The mole concentration and the average number of AOEM in the polymer

Run	[AOEM] in monomer	[AOEM] in polymer	Average number of AOEM moiety[a]
	mol%	mol%	
1	100	100	46
2	46	38	42
3	7.8	6.5	18
4	2.2	2.8	21
5	1.1	1.7	12
6	0.59	0.82	6

[a] The number of AOEM moieties per one molecule of the polymer. The average number of AOEM moieties = (M_n) × ([AOEM] in polymer)/(molecular weight of AOEM)

Chemiluminescence Spectra

Chemiluminescence was observed when the DMF solution of homopolymer (run 1 in Table II) or AOEM was mixed with 0.1 M HNO_3 aqueous solution including 0.5 wt% hydrogen peroxide, followed by the addition of 0.25 M NaOH aqueous solution. The spectra of chemiluminescence were measured by a spectrophotometer equipped with a photodiode array. Figure 2 shows the results including that of **3** in Scheme 1 for comparison. The thin line, the thick line and the dotted line indicate the spectrum of run 1, that of AOEM and that of **3**, respectively. All three spectra were in agreement. These spectra were observed widely from 380 to 580 nm having two peaks at 430 and 460 nm and were consistent with the fluorescence spectrum of *N*-methylacridone.[11] In addition, the presence of *N*-methylacridone in the residue after the light emission from run 1 and AOEM was confirmed by UV-Vis spectrophotometry. Because acridinium salts such as **3** are reported to be sources of emitters, excited *N*-methylacridone, the polymer of run 1 or AOEM would have played roles similar to that of the acridinium salts.

Light Yields of the Polymers and Release Yield of Acridinium Salts from the Polymers

The measurements of the light yield of the polymers of AOEM or AOEM were performed at three different concentrations. From the slopes of the measured plots, the light yields were calculated. Results are summarized in Table III. The light yields are shown as the light yield

per AOEM moiety in the polymers, and the value for AOEM is also given for comparison. The relative light yields were calculated from the light yield per AOEM moiety and the average number of AOEM moieties in the polymer. The release yields of the acridinium salts from the polymers in runs 1, 3, and 4 were measured by spectrophotometry at pH 1 and were compared with that of AOEM.

The light yield per AOEM moiety in run 1 was 0.99 times that of AOEM itself. So the relative light yield of run 1 was about 45 times that of AOEM, because the polymer contains about 46 units of the AOEM moiety. In the case of copolymers, however, the light yield per AOEM moiety decreased with the decrease in the content of the AOEM moiety in the polymers. This tendency was observed in the release yields. After treatment with acid, the copolymer that contains more MMA moiety would be less soluble and less efficient in releasing acridinium salts compared with the homopolymer decomposed by acid that possesses more hydroxyl groups.

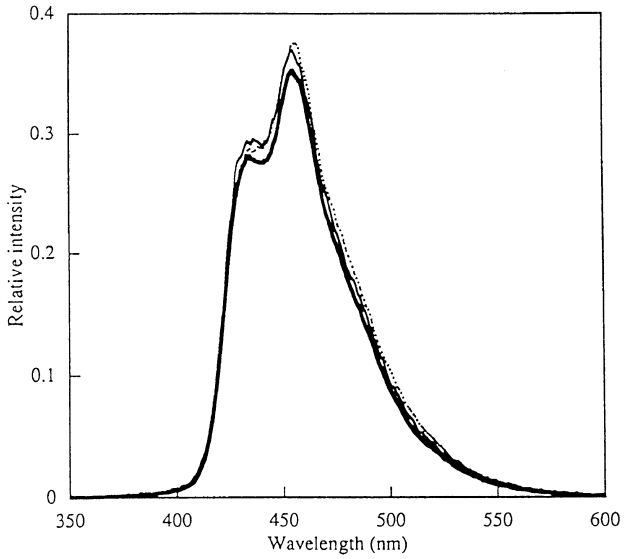


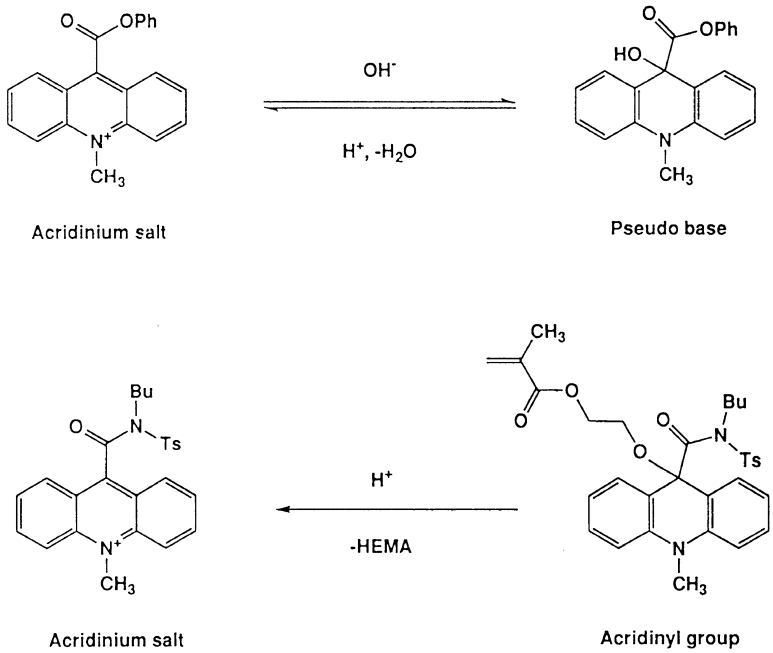
Figure 2. Chemiluminescence spectra of AOEM and the polymer of AOEM: Thick line, AOEM: thin line, run 1; dots line, compound **3**.

Release of Acridinium Salts

It was reported that acridinium salts reversibly form the corresponding pseudo bases depending on the acidity of the media (Scheme 2).[11-13] It was also reported that an acridinyl compound affords the corresponding acridinium salt under acidic conditions.[9,10] The latter is quite similar to the reverse of the former. The UV-Vis spectra of AOEM at various pHs were obtained as shown in Figure 3. The colorless solution of AOEM turns yellow, and the absorbances at 368 and 426nm increased along with the generation of the acridinium salt in the UV-Vis spectrum. Similar changes were also observed in the cases of the polymers of AOEM. The spectral change in AOEM was similar to that of the pseudo base or that of the acridinyl compound which was reported by Kano *et al.*[14] Releases of acridinium salts from AOEM or polymers were monitored ranging from pH 1 to 6 by measuring the absorbances at 426 nm. The results are summarized in Figure 4. The absorbance above pH 5 was negligible in all cases, suggesting that AOEM and the AOEM moiety in the polymers are stable under these conditions. On the other hand, the absorbance increased with a decrease in pH in all cases. The results indicate

Table III. Light yield of the polymer and release yield of acridinium salt

Run	Light yield/AOEM moiety / $\times 10^{-17}$ counts mol^{-1}	Relative light yield/polymer	Relative release yield of acridinium salt
1	4.41	45	0.98
2	4.31	41	
3	4.14	17	0.96
4	3.31	16	0.77
5	2.93	8	
6	2.86	4	
AOEM	4.46	1	1



Scheme 2. Pseudo base formation of acridinium salt and decomposition of acridinyl group under acidic conditions.

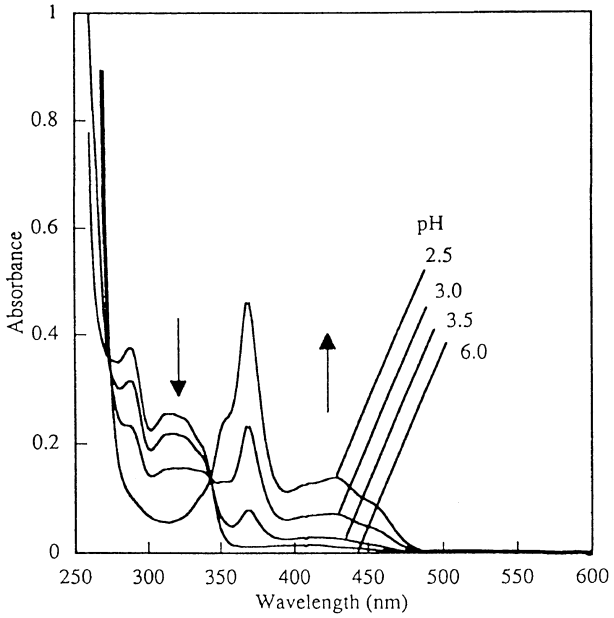


Figure 3. Absorption spectra of AOEM: AOEM, 63 μM; solvent, DMF/buffer (1 : 1, v/v).

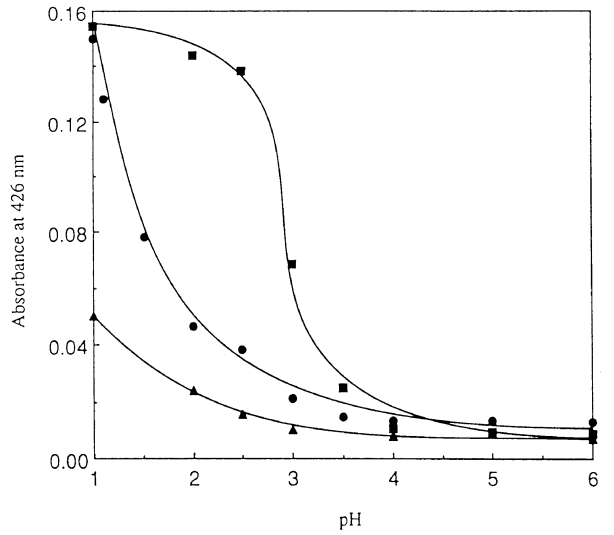


Figure 4. Effect of pH on release of acridinium salt: Square, AOEM; circle, run 1; triangle, run 3.

that more acridinium salts were released at lower pH. Only the curve of AOEM showed an inflection point at pH 2.9 and reaches a plateau at about pH 1. It seems that the difference between the curve of AOEM and that of the polymers comes from the difference in the reactivities toward protons.

Dependence of Chemiluminescence on pH

DMF solutions of AOEM or polymers (runs 1 and 3 in Table I) were treated with buffers of various pH values and the light yields were measured. The results are shown in Figure 5. The curves were similar to those in Figure 4. Neither AOEM nor the polymer of AOEM showed chemiluminescence without acid treatment. The results indicate that only the released acridinium salts participate in the light emission.

Time Course

The time course of the copolymer chemiluminescence

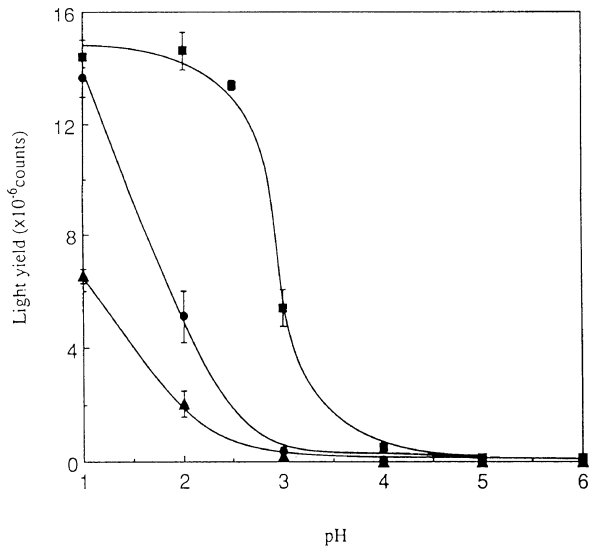


Figure 5. Effect of pH on the light yield: Square, AOEM; circle, run 1; triangle, run 3.

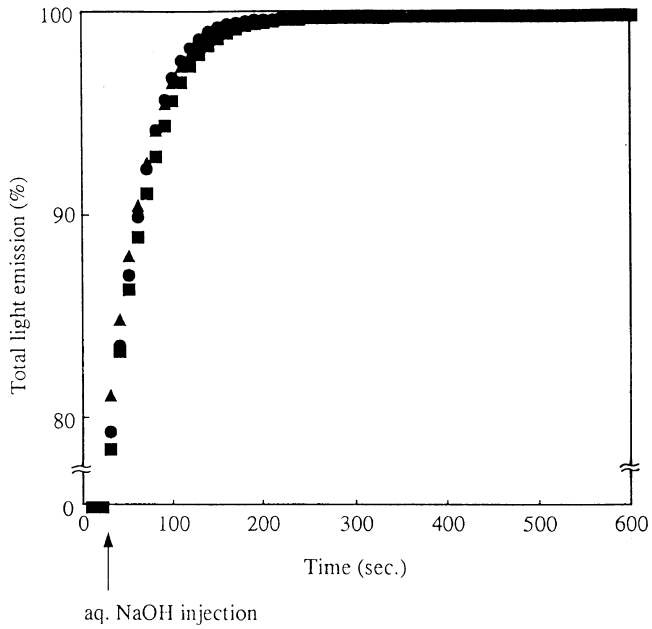


Figure 6. Time courses of light emission: Square, AOEM; circle, run 4; triangle, compound **3**.

(run 4 in Table I) is shown in Figure 6 with those of AOEM and **3** for comparison. The light emission was 80% of the total amount in 10 s and was almost finished within 2 min. This was consistent with that of AOEM or **3** and was not affected by the polymer chains. If the polymer does not release acridinium salts, the light emission from the polymers would be slower than that of AOEM or **3**. The time course of the homopolymer (run 1 in Table I) was similar to that of run 4. These results support our idea that acridinium salts were released from the polymers with AOEM moiety, followed by the light emission.

CONCLUSIONS

The radical polymerization of AOEM could be attained in spite of the large steric hindrance around the methacryloyl group. The light yield per molecule of the

polymer was proportional to the number of AOEM moieties in one molecule of the polymer and was dependent on the release yield of acridinium salts from the polymer. The polymers of AOEM release acridinium salts under acidic conditions, and then the light was emitted by the subsequent reaction with hydrogen peroxide under basic conditions. We have demonstrated the design and preparation of chemiluminescent polymers possessing a high light yield by radical polymerization of AOEM.

REFERENCES

1. K. -D. Gundermann and F. McCapra, in "Chemiluminescence in Organic Cheimistry," Springer-Verlag, Berlin, 1987.
2. A. Tsuji, M. Maeda, and H. Arakawa, *Anal. Sci.*, **5**, 497 (1989).
3. L. J. Kricka, *Clin, Chem.*, **37**, 1472 (1991).
4. L. S. Lancaster, *Endeavor*, **16**, 184 (1992).
5. K. -D. Gundermann, D. Lieske, B. Haase, and B. H. -A. Baca, *J. Biolumin. Chemilumin.*, **1**, 201 (1987).
6. K. -D. Gundermann and H. Giesecke, *Liebigs Ann. Chem.*, 1085 (1979).
7. E. H. White, D. F. Rosewell, A. C. Dupont, and A. A. Wilson, *J. Am. Chem. Soc.*, **109**, 5189 (1987).
8. P. G. Mattingly, *J. Biolumin. Chemilumin.*, **6**, 107 (1991).
9. F. McCapra, Jpn. Kohyo Tokkyo Koho, Japanese Patent, 3-501772 (1991).
10. F. McCapra, United States Patent, 5338847 (1994).
11. M. W. Cass, E. Rapaport, and E. H. White, *J. Am. Chem. Soc.*, **94**, 3168 (1972).
12. P. W. Hammond, W. A. Wiese, A. A. Waldrop, III, N. C. Nelson, and L. J. Arnold, Jr., *J. Biolumin. Chemilumin.*, **6**, 35 (1991).
13. J. S. Littig and T. A. Nieman, *J. Biolumin. Chemilumin.*, **8**, 25 (1993).
14. K. Kano, B. Zhou, and S. Hashimoto, *Bull. Chem. Soc. Jpn.*, **60**, 1041 (1987).