Time-resolved detection of electrochemiluminescence of luminol

Johanna Suomi[a], Markus Håkansson[a], Qinghong Jiang[a], Miia Kotiranta[a],
Mika Helin[a], Antti J. Niskanen[b], Sakari Kulmala[a],*

[a] *Helsinki University of Technology, Laboratory of Inorganic and Analytical Chemistry, PO Box 6100, FIN-02015 HUT, Helsinki, Finland*
[b] *Microelectronic Center, Helsinki University of Technology, PO Box 3500, FIN-02015 HUT, Helsinki, Finland*

Abstract

The luminescence lifetime of the electrochemiluminescence of luminol was studied at oxide-coated silicon and aluminum electrodes. In an undivided cell, the ECL was found to be produced only by cathodic processes, and luminol could be detected down to picomolar levels with time-resolved measurements.
© 2004 Elsevier B.V. All rights reserved.

Keywords: Time-resolved electrochemiluminescence; Luminol; Insulating film-coated electrodes; Silicon electrodes; Aluminum electrodes

1. Introduction

Hot electrons can be tunnel-emitted into vacuum and insulating liquids [1], as well as into aqueous electrolyte solutions from metal/insulator/metal tunnel junctions (M/I/M) [2]. We have so far used another tunnel junction type, namely conductor/insulator/electrolyte solution (C/I/E) junctions to inject hot electrons into aqueous solutions and thus produce electrogenerated chemiluminescence (ECL) of different types of luminophores [3]. So far we have not had opportunity to fabricate M/I/M junctions and all the work has been carried out using more simple C/I/E junctions, mainly $Al/Al_2O_3/E$ and $n\text{-}Si/SiO_2/E$ junctions [3]. The tunnel emission of energetic hot electrons through insulating oxide film during cathodic pulse polarisation of the electrode offers a possibility to electrochemically excite a wide variety of luminophores, which can be used as labels in bioaffinity assays [4].

Time-resolved luminescence detection can offer excellent sensitivity in determinations, since the background luminescence can be efficiently separated from the long-lived luminescence by measuring the luminescence after a certain time has passed from the end of the excitation pulse. This technique can be used both with photoluminescence and with electrochemiluminescence (ECL) measurements [5].

Reactions, which are difficult or impossible to carry out at conventional active metal electrodes in aqueous solutions, can be carried out with cathodic pulse polarisation under conditions in which hot and/or hydrated electrons are generated.

The present studies concentrated on the ECL of luminol under different conditions, which revealed that our earlier assumption that ECL of luminol would always be short-lived [6], turned out quite interestingly to be a wrong concept. The behaviour of different electrode materials (aluminum, silicon, platinum) and electrolyte solutions on the lifetime of the luminescence was studied as well as the effect of some solution additives on the intensity and the decay of the ECL.

2. Experimental

ECL measurements were carried out using instrumentation previously described [7]. The counter electrode was a Pt wire (0.4 mm diameter) and the working electrode was Al [7] or Si. Al electrodes were cut from a nominally 99.9% pure Al band, 0.3 mm thick (Merck Art. 1057, batch 250 K18381957) and were covered with a 2–3 nm thick natural oxide layer. The Si electrodes were made of antimony-doped n-type

* Corresponding author. Tel.: +358 9 451 2601; fax: +358 9 462 373.
 E-mail address: sakari.kulmala@hut.fi (S. Kulmala).

silicon(1 1 1) with resistivity of 8–15 mΩ cm. The insulating oxide films on the silicon were fabricated by thermal oxidation. Clean silicon wafers (Okmetic Oy, Espoo, Finland) were loaded into the furnace at 700 °C and the temperature was increased by 10 °C/min until 850 °C, which was used as the oxidation temperature. The oxidation was performed in 10% O_2–90% N_2 atmosphere, and after the desired oxide layer thickness was achieved the furnace was cooled to 700 °C in 100% N_2 atmosphere. Thickness of the oxide films was determined by ellipsometry with refractive index of $n = 1.465$.

The effective electrode area in the cell was 38.5 mm^2 and the total size of the electrodes was 9.5 mm × 9.5 mm. The effective area of the wire counter electrode was about 0.19 mm^2.

The ECL measurements were made in 0.05 M sodium tetraborate ($Na_2B_4O_7$) buffer at pH 9.2. The coulostatic pulse generator was adjusted to yield 39 μC cathodic pulses with −50 V applied pulse voltage. Luminol (5-amino-2,3-dihydrophtalazine-1,4-dione) was purchased from Aldrich, sodium tetraborate decahydrate was pro analysis grade from Merck as was the NaN_3.

3. Results and discussion

We observed in this study that the ECL luminescence lifetime of luminol is exceptionally long, of the order of several milliseconds (Table 1, Fig. 1). Many organic luminophores have very short luminescence lifetimes; e.g. platinum(II) coproporphyrin [9] has a 100 times shorter lifetime than luminol in the present study. The difference in the lifetimes allows the simultaneous excitation of other luminescent molecules and chelates having short luminescence lifetime together with luminol. In previous work [10] we have reported simultaneous excitation of $Ru(bpy)_3^{2+}$ with a Tb(III) chelate. Similar time-resolved CL detection of luminescent labels is possible for luminol and $Ru(bpy)_3^{2+}$. The inset of Fig. 2 shows the ECL spectrum of a mixture of luminol and $Ru(bpy)_3^{2+}$ which displays that the compounds can be
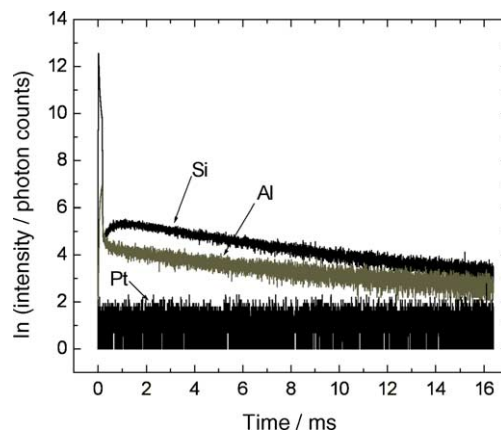

Fig. 1. ECL intensity of 0.1 μM luminol on three electrodes: Pt, Al with natural oxide film, and n-Si with 3.6 nm oxide film. The luminol solutions were made in 0.05 M $Na_2B_4O_7$ at pH 9.2. Analysis conditions: Pulse charge 39 μC, pulse voltage 50 V and pulse frequency 20 Hz. The intensity was measured from 5000 excitation pulses.
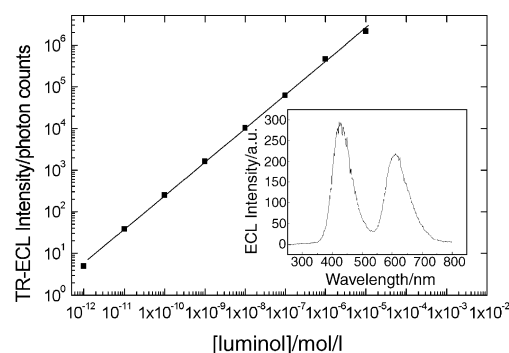

Fig. 2. Time-resolved ECL intensity of luminol as a function of concentration; inset: ECL spectrum of a mixture of luminol and $Ru(bpy)_3^{2+}$. Conditions: 420 nm interference filter with a band width of 40 nm. Pulse charge 120 μC, pulse voltage 40 V and pulse frequency 20 Hz, 0.05 M $Na_2B_4O_7$, pH 9.2, 0.01 M NaN_3, the intensity was integrated over 1000 excitation pulses. Inset conditions: otherwise as above, but 1.0×10^{-5} M luminol, 2×10^{-7} M $Ru(bpy)_3^{2+}$, 5.0×10^{-4} M $K_2S_2O_8$, and pulse frequency 100 Hz, measured with Perkin-Elmer LS 5 spectrometer.

Table 1
Comparison of luminol ECL lifetimes with previously measured lifetimes of other luminescent compounds

Compound	Electrode, solution	Lifetime (s)	Reference
Aromatic Tb(III) chelates	Al, oxide-covered 1.0 M Na_2SO_4 in 0.2 M H_3BO_3 at pH 9.2	$1.3 \times 10^{-3} - 2.2 \times 10^{-3}$	[8]
Pt(II) coproporphyrin	Al, oxide covered 1.0 mM $K_2S_2O_8$ in 0.05 M $Na_2B_4O_7$ at pH 9.2	14.5×10^{-6}; 31.5×10^{-6}; 44.8×10^{-6}	[9]
Luminol, 0.1 μM	Al, oxide-covered 0.05 M $Na_2B_4O_7$ at pH 9.2	6.5×10^{-3}; 11.7×10^{-3}	
Luminol, 0.45 μM	Al, oxide-covered 0.05 M $Na_2B_4O_7$ at pH 9.2 + 1.5 M ethanol	34.5×10^{-3} low intensity!	
Luminol, 0.1 μM	Al, oxide-covered 0.05 M $Na_2B_4O_7$ at pH 9.2 + 0.01 M NaN_3	2.7×10^{-3}; 14.2×10^{-3}	
Luminol, 0.1 μM	Al, oxide-covered 0.05 M $Na_2B_4O_7$ at pH 9.2 + 0.001 M $K_2S_2O_8$	3.0×10^{-3}; 7.2×10^{-3}	
Luminol, 0.1 μM	n-Si, 3.6 nm oxide film 0.05 M $Na_2B_4O_7$ at pH 9.2	7.3×10^{-3}	

individually detected also by using wavelength discrimination.

The ECL decay of luminol in air-saturated solution was close to a single exponential process at oxide-coated n-Si with luminescence lifetime close to 7 ms (Fig. 1, Table 1). On oxide-covered aluminum, however, the corresponding decay curve was somewhat more curving and could be fitted as two separate luminescent processes, in which the shorter luminescence life time was similar to that produced on n-Si and the longer life time was of the order of 10 ms (Table 1). The differences in the lifetimes shown in Table 1 are partly caused by additives affecting the primary radicals, and partly by so far undetermined processes. The dominating luminescence lifetime of luminol in borate buffer appears to be between 6 and 7 ms.

We will properly examine the mechanism behind the luminescence decay by using various free radical scavengers, but the limits of short communication do not allow us to present our theories in this paper.

The luminescence lifetime can be significantly affected if the excitation conditions at the working electrode are transformed purely reducing by adding oxidising radical scavengers such as ethanol, which produce reducing secondary radicals. The ECL lifetime increased from about 6 ms up to 35 ms as a result of the ethanol addition (Table 1) while the intensity of luminol TR-ECL in 0.05 M $Na_2B_4O_7$ solution (pH 9.2) decreased to 3% of the original intensity in the presence of 1.5 M ethanol in the solution. Thus, in solely reducing conditions much longer-lived but much weaker ECL is observed as in the normal situation where both superoxide radicals and hydroxylradicals are present in significant concentrations [6].

When azide ion (hydroxyl radical scavenger producing $N_3^\bullet$) was added into the system instead of ethanol, the decay curve could be fitted with two exponential parts, the longer luminescence lifetime being ca. 14 ms. When peroxodisulfate (necessary for efficient cathodic excitation of, e.g. $Ru(bpy)_3^{2+}$) was added instead of azide ions, the TR-ECL intensity was increased and the decay curve showed a fast decay process similar to the one seen in azide experiments, and a slower decay with luminescence lifetime similar to luminol in additive-free borate buffer (Table 1).

We also studied if the ECL of luminol is significantly produced at the Pt-wire serving as the counter electrode in the cell. To test the system we compared the results obtained with insulating film-coated electrodes with the results from a similar experiment but with a platinum working electrode. Another test was performed at a stainless steel electrode. Measurements were made on these electrodes with 0.1 μM luminol in $Na_2B_4O_7$ solution, first with cathodic polarity of the working electrode and then with anodic polarity, and the luminescence was detected (the electrode area ratio was ca. 200:1). No long-lived luminescence was detected either

with platinum or with stainless steel electrodes at neither of the polarities of the larger working electrode having area of 38.5 mm^2 (see Fig. 1); therefore we can safely say that the ECL observed with time-resolved measurements is entirely produced by cathodic reactions and the anodic ECL at the very small wire counter electrode has an insignificant contribution to the total ECL-signal also during the cathodic excitation pulse (Fig. 1).

Very low concentrations of luminol could be detected by TR-ECL even using very cheap disposable natural oxide-covered Al-electrodes, and the linear range of detection is very wide, from 1 pM to 5 μM (Fig. 2).

The relative standard deviation of the method at 10 pM concentration was 8.4% (N = 23).

4. Conclusions

Hot-electron induced ECL lifetime of luminol is relatively long and additives like ethanol and peroxodisulphate have an effect on both the luminescence intensity and on the lifetime. ECL of luminol is almost entirely induced by cathodic reactions on the oxide-coated aluminum or silicon electrodes. Luminol could be detected even at picomolar concentration levels with time-resolved detection. Further studies of this novel type of luminol ECL are in progress in our laboratory.

References

[1] C. Mead, J. Appl. Phys. 32 (1961) 646.
[2] D. Diesing, S. Russe, A. Otto, M. Lohrengel, Ber. Bunsenges. Phys. Chem. 99 (1995) 1402.
[3] S. Kulmala, T. Ala-Kleme, L. Heikkilä, L. Väre, J. Chem. Soc., Faraday Trans. 93 (1997) 3107;
    T. Ala-Kleme, S. Kulmala, M. Latva, Acta Chem. Scand. 256 (1997) 17;
    S. Kulmala, T. Ala-Kleme, H. Joela, A. Kulmala, J. Radioanal. Nucl. Chem. 232 (1998) 91;
    M. Helin, L. Väre, M. Håkansson, P. Canty, H.-P. Hedman, L. Heikkilä, T. Ala-Kleme, J. Kankare, S. Kulmala, J. Electroanal. Chem. 524–525 (2002) 176.
[4] S. Kulmala, M. Helin, T. Ala-Kleme, L. Väre, D. Papkovsky, T. Korpela, A. Kulmala, Anal. Chim. Acta 386 (1999) 1.
[5] S. Kulmala, S. Suomi, Anal. Chim. Acta 500 (2003) 21.
[6] S. Kulmala, T. Ala-Kleme, A. Kulmala, D. Papkovsky, K. Loikas, Anal. Chem. 70 (1998) 1112.
[7] J. Kankare, K. Fäldén, S. Kulmala, K. Haapakka, Anal. Chim. Acta 256 (1992) 17.
[8] S. Kulmala, A. Kulmala, T. Ala-Kleme, J. Pihlaja, Anal. Chim. Acta 367 (1998) 17.
[9] P. Canty, L. Väre, M. Håkansson, A.-M. Spehar, D. Papkovsky, T. Ala-Kleme, J. Kankare, S. Kulmala, Anal. Chim. Acta 453 (2002) 269.
[10] T. Ala-Kleme, S. Kulmala, L. Väre, M. Helin, Anal. Chem. 71 (1999) 5538.