

Electrochemiluminescent detection of labile radical intermediates of electrochemical reactions

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Abstract The article considers the basic principles of electrochemiluminescent spin traps diagnostic method which consists of efficient trapping of intermediate labile radical products (free radicals of complex organic molecules) by electrogenerated radical ions of electrochemiluminescers in different liquid-phase systems including bioobjects. The method permits qualitative and quantitative identification of such particles by measurement of electrolysis radical recombination luminescence (electrogenerated chemiluminescence) intensity which is emitted by electrolyzed solutions of organic electrochemiluminescers. The unique possibilities of the method are illustrated on examples of experimental study of systems with sodium tetraphenylborate and chloride ion.

Keywords Electrogenerated chemiluminescence · Free radicals · Mechanistic investigation · Assay

Introduction

Among the most promising application areas of electrogenerated chemiluminescence (ECL) that originates from recombination of radicals of organic substances in solutions during Faraday electrolysis is the assay, especially the biomedical one: simulation of processes in biosystems [1, 2], early diagnostics of diseases, including malignant neoplasms [3–5], detection of pharmaceuticals [6], as well as immune electrochemiluminescent assay that allows in vitro studies of antigen–antibody interactions [5, 7, 8]. Yet

another important area where ECL has considerable application potential is the study of mechanisms of chemical and electrochemical reactions. A fundamental role in this area was played by researches of F. Pragst and coworkers [9–15]. The purpose of this work is the study and discussion of applicability of electrochemiluminescent spin traps method that allows utilization of the ECL effect for detection of labile paramagnetic parts of complex organic molecules, possessing unpaired electronic spin—the free radicals (FR)—in systems of different nature, including bioobjects. The importance of the mentioned problem can be illustrated by the following examples.

First, it is a well-known fact that the emission of photons during certain chemical reactions in the tissues of humans and animals in vivo—the chemiluminescence (CL)—is of considerable interest for biological and medical research. This optical channel provides unique information about mechanisms of biophysical and biochemical processes that take place at the level of molecules and FR that are vitally important for activity of cells, tissues, and organs. The free radical peroxide oxidation of the membrane lipids and lipoproteins by molecular oxygen dissolved in the blood is an exoenergetic chemical process accompanied by formation of lipids peroxide FR. The recombination of the latter provides formation of CL emitters—excited molecules of cyclic hydroperoxides, aldehydes, ketones, and biogenic amines [3]. Thus, it is evident that reliable and simple identification of labile FR during the study of biological systems is rather important.

Second, in numerous electrode (*E*) reactions of organic compounds, including processes which initiate radical recombination luminescence, the participation of intermediate labile FR products plays an important role, thus their detection gives rather important information about mechanisms and kinetics of such reactions that are often

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complicated by chemical (*C*) and electrochemical stages—*EC* and *ECE* reactions.

There exists a number of methods for studying labile paramagnetic particles with unpaired spin that facilitate identification of the mechanism of the reaction—spin paramagnetic resonance, mass-spectroscopy, Raman spectroscopy, chemical spin traps, etc., that are too complex for routine research. Considering the specific character of ECL, it is applicable for detection of labile radicals including intermediates of electrode reactions of organic compounds, complicated by following electrochemical and/or chemical stages [16, 17]. The essence of the proposed method consists in proven efficient (i.e., having high probability) trapping of intermediate labile radical products (free radicals R^\bullet)—by electrochemiluminescent spin traps. The later are the electrogenerated electrophilic radical cations (RC) or nucleophilic radical anions (RA) of corresponding organic electrochemiluminescers. The detected ECL quanta γ_{ecl} that are emitted during such process allow both qualitative and quantitative (if the stoichiometry of the trapping reaction is known) determination of FR in the system under the investigation:



where ${}^1A^*$ is the excited singlet molecule of electrochemiluminescer (the emitter). The capabilities of the proposed method are shown below using electrode reactions and ECL in systems with sodium tetrphenylborate (NaTPB) $B(C_6H_5)_4Na$ and chloride as an example.

The interest towards study of ECL systems with NaTPB is caused by wide application of sodium Ph_4B^- salt (Ph stands for C_6H_5) as an analytical reagent for quantitative detection of certain metal cations (e.g., K^+) using different methods considering the fact that the ECL emission with participation of NaTPB was observed earlier in our laboratory during electrolysis by pulsed unipolar or direct voltage that originated from anodic space [18]. The ECL assay method possess a number of advantages over traditional analytical methods [5] for NaTPB detection due to higher detection accuracy and lower detection limit whereas electrochemical detection of NaTPB is complicated due to irreversible reaction nature, essential electrode fouling, and rather contradicting data about electrooxidation of Ph_4B^- being an *EC* process with labile but not identified exactly intermediate radicals [19]. Thus, using the method of electrochemiluminescent spin traps, we expect to obtain new data about electrochemistry of Ph_4B^- anion that will be useful for its sensitive detection.

Experimental

Electrochemical measurements were performed in a three-electrode 16 ml cell using either glassy carbon disk ($\varnothing=2$ mm), Pt disk ($\varnothing=2$ mm) or Pt rod ($\varnothing=1$ mm, 30 mm in length) working electrodes, Pt foil auxiliary electrode (50 mm² area), and saturated Ag/AgCl reference electrode connected to the cell by Luggin capillary. The flat bottom of the cell is placed on top of photomultiplier tube (PMT) compartment. All electrochemical and ECL measurements were done with home-designed apparatus ELAN-2 m that was previously described [20] using cyclic voltammetry (CVAM) and rotating disk electrode (RDE) methods. Potential scan rate of 100 mV/s was used for CVAM and 20 mV/s for RDE measurements, with electrode rotation rate $\omega=2,000$ rpm. Supporting electrolytes used for electrochemical and ECL experiments were $LiClO_4$ and tetrabutylammonium perchlorate. ECL and fluorescence spectra were measured using Ocean Optics Inc. QE65000 fiber-optic spectrometer. Rubrene (>98%), NaTPB (>99.0%), tetrabutylammonium tetraphenylborate (>99.0%), and acetonitrile (anhydrous 99.8%) were all purchased from Sigma-Aldrich; $LiClO_4$ (98%) was purchased from Synex Pharma Technologies Co. and were used without further purification. 1,5-Diphenyl-3-(parachlorophenyl)-pyrazoline (1, 3) (DPSP) was synthesized in the Institute for Single Crystals of the National Academy of Science of Ukraine. Tetrabutylammonium perchlorate was synthesized from tetrabutylammonium hydroxide (>98%) and perchloric acid (>98%). *N,N'*-dimethylformamide (DMF) was purified using low-pressure rectification under argon atmosphere.

Results and discussion

Conducted electrochemical and ECL investigations of systems containing a number of electrochemiluminescers and NaTPB (using CVAM and RDE methods at positive potentials) showed that the oxidation of Ph_4B^- in DMF with $LiClO_4$ supporting electrolyte is electrochemically irreversible and is accompanied by fast chemical reaction with electrochemically reactive radical products (*ECE* mechanism). The apparent number of electrons n_{app} transferred to the electrode during oxidation reaction varies from $n_{\text{app}} \cong 2$ at low electrode polarization rates to $n_{\text{app}} \cong 1.4$ for $v=0.6$ V/s, Fig. 1. Presence of the *C* stage is indicated by the total absence of reverse cathodic peak on the current–voltage curve of Ph_4B^- system caused by the reduction of Ph_4B^- oxidation product to original form during reverse of electrode polarization program. Obtained experimental results together with published materials of other authors [18, 21–

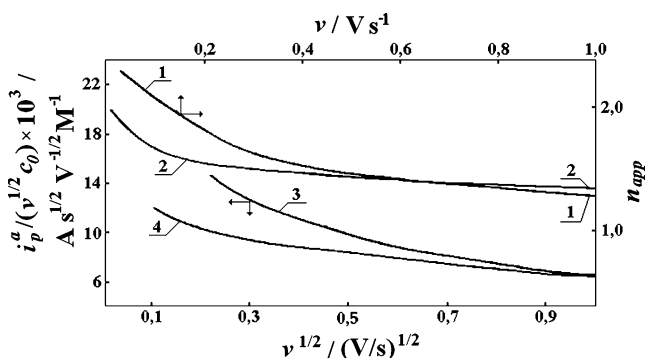
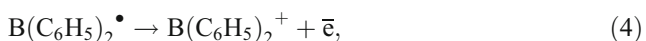
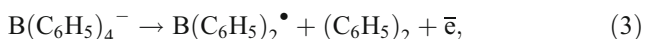


Fig. 1 Dependence of apparent number of electrons n_{app} of anodic reactions (1, 2) and oxidation current of the system (3, 4) vs. the rate of linear potential sweep in DMF, supporting electrolyte 0.1 M LiClO₄; containing 2 mM of NaTPB (2, 4) and 2 mM of DPSP

23] indicate that anodic reaction of Ph₄B⁻ occurs according to the following scheme:



with formation of labile radicals of B(C₆H₅)₂[•]. Further processes with participation of these species, i.e., the C mechanism were the subject of discussion of different authors [16, 18]. At the same time, in mentioned works, the radicals of B(C₆H₅)₂[•] were not detected directly.

The proposed method of identification of such labile intermediates using their trapping by electrochemiluminescent spin traps—the electrogenerated electrophilic FR of electrochemiluminescers (DPSP and other)—proved the presence of FR at the surface of anode at the C stage and participation of nucleophilic radicals of B(C₆H₅)₂[•] in light-emitting recombinations with electrochemiluminescer RC. The later was proved by registration of anodic ECL during the solution electrolysis. The emission of photons in such recombination process in the absence of electrogenerated RA of electrochemiluminescer indicates rather high rate of described electrochemiluminescent spin-trapping by RC of electrochemiluminescer that successfully competes with fast decay of the labile B(C₆H₅)₂[•] FR (Figs. 2 and 3). Comparison of fluorescence and ECL spectra of the mentioned system (Fig. 3) allows us to conclude that ECL is emitted by the singlet excited molecules of DPSP. This indicates a rather high energy released during such electrochemiluminescent spin-trapping of FR and characterizes mentioned system as the one occurring according to the S-path of electrolysis radical recombination luminescence (i.e., immediate formation of excited singlet emitters ¹A*) [16].

Study of rubrene ECL with TPB ion was conducted in acetonitrile using RDE method. In acetonitrile, the tetrabutylammonium tetraphenylborate was used instead of

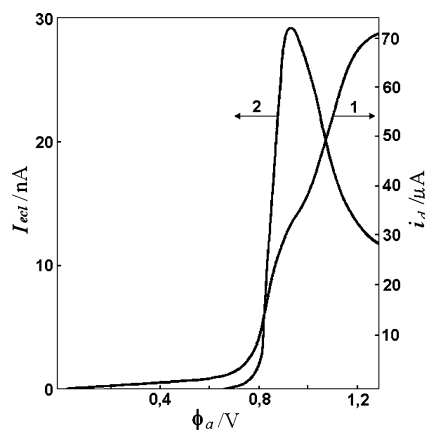


Fig. 2 Anodic current–voltage curve (1) and ECL–voltage curve (2) of 0.2 mM of DPSP in acetonitrile at the rotating disk electrode (Pt), 1 mM of NaTPB, supporting electrolyte 0.1 M LiClO₄. Working electrode polarization rate 20 mV/s, electrode rotation rate 209 s⁻¹

NaTPB salt. When TPB concentration was kept constant (5 × 10⁻⁴ M) and 10⁻⁶ M of rubrene was added to the solution, an ECL raise was observed at a potential of about 1.25 V. When rubrene content was increased up to 3 × 10⁻⁶ M, except ECL with rise at 1.25 V, there appeared a distinct plateau at 0.85 V. Further increase of rubrene concentration leads to the growth of first plateau corresponding to reaction starting at 0.85 V relative to the second one at 1.25 V. The first rise of ECL at about 0.85 V is attributed to oxidation of rubrene to RA and its further homogeneous reaction with TPB ion (the C stage) leading to light quanta emission. A rise of ECL at 1.25 V can be tentatively attributed to formation of rubrene dication and its fast reaction with TPB accompanied by ECL emission (see Table 1 for oxidation potentials).

Comparison of peak ECL of first and second waves versus rubrene concentration is shown on Fig. 4. Both dependencies show rather linear behavior with the slopes of linear fit being 1.8 ± 0.1 for the first plateau (correlation coefficient $r=0.996$) and 0.96 ± 0.037 for the second one ($r=0.998$). This allows

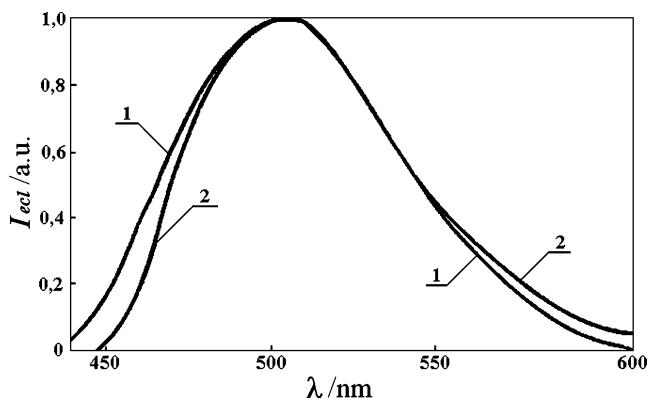


Fig. 3 Spectra of ECL (1) and fluorescence (2). Solution contains 5 mM of DPSP+50 mM of NaTPB in DMF. ECL is excited by DC electrolysis at 0.8 V vs. SCE, Pt rod working electrode, Ø 0.8 mm

Table 1 First oxidation potential of investigated substances in acetonitrile

Substance	TPB ⁻	DPSP	Rubrene	Cl ⁻
$E_{1/2}^{ox}$, V	0.91 ^a	0.80	0.87	1.15

^a TPB oxidation potential at a rotating glassy carbon disk electrode at scan rate of 20 mV/s. The same results observed by Geske, 0.46 V vs. SCE at scan rate 1.18 mV/s (rotating Pt disk electrode) [21]; Pal et al., $E_p=0.78$ V vs. SCE at scan rate 50 mV/s (cyclic voltammetry, glassy carbon electrode) [23]

concluding that ECL reaction of TPB with rubrene RC occurs in two steps whereas dication leads to rubrene excitation in one step. This comes from the ratio of slopes of both curves that is very close to 2.

Mentioned experimental results together with known data on mechanisms of NaTPB oxidation [18] allow proposing the following scheme of ECL reaction in systems with TPB ion and electrochemiluminescer A [24]:

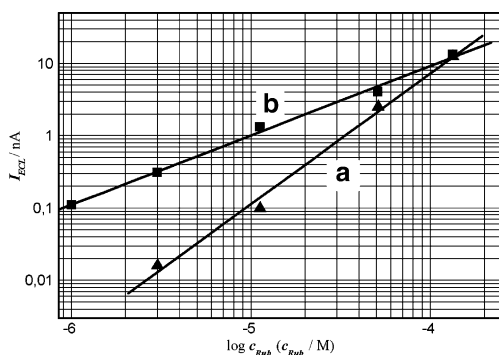
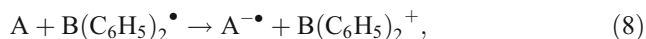
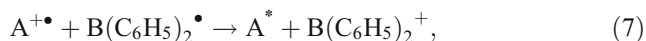
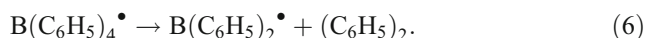
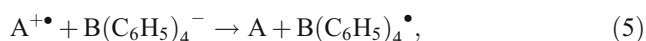


Fig. 4 Dependence of peak ECL intensity of first (a) and second (b) plateaus vs. rubrene concentration in acetonitrile in the presence of 5×10^{-4} M of tetrabutylammonium tetraphenylborate on glassy carbon rotating disk electrode. Supporting electrolyte 0.05 M tetrabutylammonium perchlorate, working electrode polarization rate 20 mV/s, electrode rotation rate 209 s^{-1}

The ECL emission γ_{ecl} detected by PMT photodetector present in the analytical complex “ELAN-2 M” is the source of analytical signal that proves the presence of FR as the intermediates of the EC type electrode reaction.

The application of the described method of electrochemiluminescent spin traps for study of electrochemical reactions in the systems containing chlorides of alkylammonium compounds, and different electrochemiluminescers has proved the presence of electrophilic FR intermediates that cannot be detected electrochemically. These FR (ACl^{\bullet}) are the products of fast nucleophilic addition of Cl^- to unstable electrogenerated RC of electrochemiluminescer A that possess rather high activity in solution as against to electrode processes.

Formation of electrochemically inactive complex was verified by using luminophor that has reversible oxidation in acetonitrile–rubrene. After addition of Cl^- ion, reverse wave of $A^{+\bullet}$ reduction totally disappears. Experiments show that the reduction of ACl^{\bullet} radicals at the electrode is not observed up to cathodic potential of A reduction to $A^{-\bullet}$. At the same time, the ACl^{\bullet} radicals can be efficiently trapped and detected by electrochemiluminescer RA species using the same exoenergetic light-emitting recombinations:



When $A^{-\bullet}$ RA are produced at the electrode after generating ACl^{\bullet} radicals a strong ECL is observed even for those luminophors that do not give recombinational ECL because of extremely low stability and lifetime of $A^{+\bullet}$ RC in acetonitrile (e.g., DPSP).

In all cases that were studied, the emitter of ECL is the singlet excited molecule of electrochemiluminescer $^1A^*$.

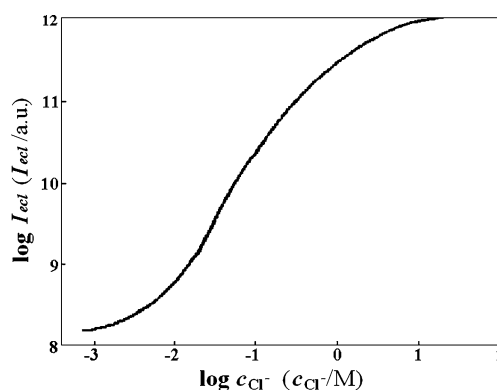


Fig. 5 Calibration curve for Cl^- anion detection. Cathodic ECL in solution of 10^{-3} M of rubrene in DMF, supporting electrolyte 0.1 M tetrabutylammonium perchlorate. Potential scan rate $\nu=0.1$ V/s. Photocurrent I_{ecl} in nanoamperes, c_{Cl^-} in millimolars

Unfortunately, obtained results do not allow revealing the mechanism of direct emitter formation. The latter, depending on ratio between change of Gibbs free energy of biradical recombination and the energy of singlet excited molecules, can be produced immediately (the *S*-route) or as a result of adding the energy of two triplet (*T*) excited molecules in *T-T* annihilation (the *T*-route). Considering rather high intensity of ECL emission in solutions containing Cl^- (up to 10^{12} photon/cm² s) the more probable is the *S*-route or the mixed *ST*-route of emitters' formation [25].

The presented method of ACl^- FR detection by electrochemiluminescent spin traps allowed development of titration ECL method of Cl^- ions detection. Comparing to other methods of Cl^- ion detection, e.g., potentiometric titration by Ag^+ cations, it has the advantage of lower detection limit (Fig. 5) [5].

Conclusion

The basic principles of electrochemiluminescent spin traps analytical method are considered. The proposed method is based on efficient trapping of intermediate labile radical products (free radicals of complex organic molecules generated either by electrolysis or other means) by electrogenerated radical ions of electrochemiluminescers in liquid media. The method permits qualitative and quantitative high precision identification of free radical particles by measurement of radical recombination luminescence intensity during electrolysis in solutions of organic electrochemiluminescers. The unique possibilities of the method are illustrated on examples of experimental study of systems with sodium tetraphenylborate and chloride ion.

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