EFFECT OF CETYLTRIMETHYLAMMONIUM BROMIDE ON THE ELECTROCHEMILUMINESCENCE AND ELECTROOXIDATION OF LUMINOL

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The electrooxidation of luminol in alkaline solutions in the presence of cetyltrimethylammonium bromide (*I*) was studied by linear sweep voltammetry on fixed and vibrating platinum electrodes. The presence of *I* in low concentrations (below the critical micellar concentration) brings about aggregation of the luminol, which is manifested by an increase in the anodic peak height and its shift towards lower potentials. In micellar solutions the peak height decreases owing to the slower diffusion of the bulkier micelles, the shift to lower potentials being preserved. The light-voltage curves correspond with the voltammetric curves, exhibiting identical shifts of the peak potentials in dependence on the concentration of the surfactant.

Oxidation of luminol (3-aminophthalic hydrazide) in alkaline solutions is accompanied by light emission ($\lambda_{max} \approx 420$ nm). The chemiluminescence intensity depends on the concentration of luminol as well as on the concentrations of the oxidants and catalysts and also of inhibitors to the reaction[1,2]. In the analytical chemiluminescence methods, which experimentally are not too demanding and feature high sensitivity, the intensity of the emitted radiation can be increased by orders of magnitude by adding aliphatic cationoid tensides, together with or without some suitable fluorescence indicator[3]. The characteristic emission of luminol can be induced also by its anodic oxidation on a platinum anode[4-6] or by electrolysis of the solution by alternating positive and negative pulses[7].

The aim of the present work was to investigate the effect of cetyltrimethylammonium bromide (*I*) on the electrooxidation and electrochemiluminescence of luminol, with a mutual comparison of the voltammetric (*I*-*E*) and light-voltage (*L*-*E*) curves.

EXPERIMENTAL

Stock solutions of *I* were prepared in a concentration of 10 mmol l^{-1}; the content of the surfactant was determined by titration with sodium tetraphenylborate according to Cross and Uno[8]. Luminol (Lachema, Brno) was repeatedly recrystallized from aqueous solutions of sodium hydroxide[7]; its purity was checked spectrophotometrically by measuring the molar absorptivity of the first long-wavelength band[7] (λ_{max} 347·5 nm, ε 7 480). Solutions of luminol, containing

1 mmol l^{-1} of the substance in 10 mmol l^{-1} sodium hydroxide, were made up immediately before the measurements.

The voltammetric and electrochemiluminescence measurements were carried out on OH 102 (Radelkis, Budapest) and LP 7c (Laboratorní přístroje, Prague) polarographs in the three-electrode connexion. The working electrode was consituted by a platinum wire, 0·5 mm in diameter, 0·1 cm^2 area, sealed in a glass tube. For joined voltammetric and electrochemiluminescence measurements, a platinum coil (wire diameter 0·5 mm) of the area of 0·7 cm^2, or a foil of the same area were used as the electrodes; the latter served also as the vibrating electrode (frequency 50 Hz). The electrodes were cleaned by cyclic generation of hydrogen and oxygen, the last step being oxidation of the adsorbed hydrogen at a potential of 0·05 V. A large-area platinum foil was employed as the auxiliary electrode. All potential values are given relative to the saturated calomel electrode value. The electrochemiluminescence (ECL) intensity was measured by using an M 12 FQS 35 photomultiplier (Zeiss, Jena) exhibiting the maximum sensitivity at 400 nm. The electronic spectra were scanned on a Unicam SP 8 100 spectrophotometer (Cambridge, England), the pH values were measured on a PHM 64 instrument (Radiometer, Copenhagen) with a precision of ±0·02 pH unit.

RESULTS AND DISCUSSION

The voltammetric curves of luminol in alkaline solutions, obtained with the vibrating Pt anode, are shown in Fig. 1. In solutions free of surfactants, luminol only displays a shoulder on the curve of decomposition of the base electrolyte; in the presence of cetyltrimethylammonium bromide the potentials of the oxidation are shifted to lower values and a well-developed voltammetric wave appears. The threshold concentration of I requisite for obtaining a clear-cut wave is about 0·1 mmol l^{-1}. In micellar solutions of *I* ($c_{mc} = 0{\cdot}2 - 0{\cdot}4$ mmol l^{-1}) the wave position remains unchanged, but the limiting current decreases owing to the more reluctant diffusion of the bulky micelles. At a concentration of *I* of 9·2 mmol l^{-1} the limiting current attains as little as a half of its initial value. The same conclusions are drawn from experiments with the fixed Pt anode: an addition of *I* brings about a shift of the potentials of the E_p and $E_{p/2}$ peaks to lower values and an increase in the slope of the starting branches, and allows for a more accurate and precise reading of the peak heights and E_p values (Fig. 2). The highest changes in the potential values were observed at low tenside concentrations, $c_I = 0{\cdot}3$ mmol l^{-1} (Table I), at which the peak heights also grow. Additional increasing of the tenside concentration induces practically no changes in the potentials, while the peak heights decrease to indicate a micellization of the luminol. The maximum in the dependence of I_p on c_i corresponds to the critical micellar concentration of *I* ($c_{mc} = 0{\cdot}3$ mmol l^{-1}) (Fig. 3).

Other cationoid tensides – carbethoxypentadecyltrimethylammonium bromide and dimethyllaurylbenzylammonium bromide – exert an effect similar to that of *I*, only its extent is smaller. In the presence of sodium lauryl sulphate in low concentrations (0·64 mmol l^{-1}) the anodic peak of luminol is distorted and shifted to more positive potentials. In micellar solutions of sodium lauryl sulphate, the anodic peaks of luminol are virtually identical with those in solutions free of the agent.

The potentials of the peaks are shifted to lower values with increasing pH. In the presence of *I* ($c_I = 4$ mmol l^{-1}) the dependence of $E_{p(p/2)}$ on pH in the region of pH 11·5–13·0 is linear, with a slope of 0·059 V. The height of the anodic peaks, I_p, depends linearly on the luminol concentration ($c_L = 0{\cdot}01$ to 1 mmol l^{-1}) and on the square root of the potential rise rate ($v = 0{\cdot}5$ to 100 mV s^{-1}). As the latter variable increases, so do the E_p, $E_{p/2}$ values (Table I). It can be concluded that the electrooxidation of luminol is an irreversible, diffusion-controlled process; this is consistent with the published data of electrooxidation of luminol in pure solutions[9].

The favourable effect of cationoid tensides extends the analytical potential of the voltammetric technique. In the presence of *I* in low concentrations ($c_I = 0{\cdot}3$ to 0·6 mmol l^{-1}), as little as 2 μg of luminol in a volume of 2 ml (hence, 2 ppm) can be determined reliably applying the potential rise rate of 50 mV s^{-1}; in pure solutions the results are unreliable under such conditions.

The curves of the dependence of the ECL intensity on voltage (*L-E*) curves) correspond with the voltammetric *I-E* curves (Fig. 4), displaying identical shifts of the peak potentials in dependence on the tenside concentration. The height of the *L-E* peaks, I_p(ECL), exhibits a linear dependence on the luminol concentration: I_p(ECL) = = $(0{\cdot}47 \pm 0{\cdot}02)\, c_L$ (pH 10·65, $v = 250$ mV s^{-1}, c_L between 70 and 0·35 μmol l^{-1}; correlation coefficient $r = 0{\cdot}999$ for the level of significance $P = 99{\cdot}9\%$, number of

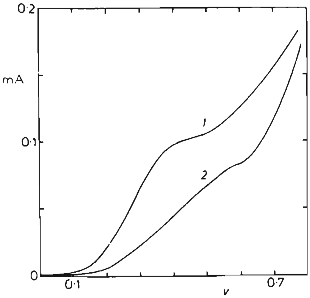

FIG. 1

Voltammetric curves of oxidation of luminol on a vibrating Pt electrode; $c_L = 0{\cdot}16$ mmol . . l^{-1}, pH 12·73, $v = 3{\cdot}3$ mV s^{-1}. *1* $c_I =$ = 0·64 mmol l^{-1}, *2* free of *I*

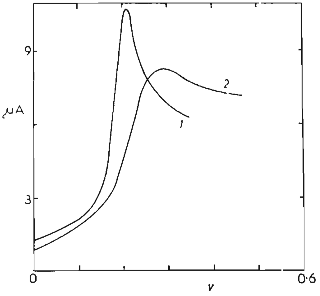

FIG. 2

Oxidation of luminol on a fixed Pt anode; $c_L = 0{\cdot}16$ mmol l^{-1}, pH 12·73, $v = 50$ mV . . s^{-1}; *1* $c_I = 0{\cdot}32$ mmol l^{-1}, *2* free of *I* determinations $n = 6$) and $I_p(\text{ECL}) = (0{\cdot}021 \pm 0{\cdot}002)\, c_L$ (pH 12·75, $v = 250$ mV s^{-1}, c_L between 100 and 2 μmol l^{-1}; $r = 0{\cdot}998$, $P = 99{\cdot}9\%$, $n = 6$), and a linear dependence on the square root of the potential rise rate: $I_p(\text{ECL}) = (3{\cdot}8 \pm 1)\, v^{1/2}$ (pH 13·20, $c_L = 0{\cdot}08$ mmol l^{-1}, v between 4·16 and 100 mV s^{-1}; $r = 0{\cdot}99$, $P = 99\%$, $n = 5$) and $I_p(\text{ECL}) = (1{\cdot}3 \pm 0{\cdot}6)\, v^{1/2}$ (pH 13·20, $c_I = 4$ mmol l^{-1}, $c_L = 0{\cdot}08$ mmol l^{-1}, v between 4·16 and 100 mV s^{-1}; $r = 0{\cdot}97$, $P = 99\%$, $n = 5$). Similarly as in the case of the voltammetric peaks, the E_p and $E_{p/2}$ potentials shift to higher values as the potencial rise rate is increased. This gives evidence that the *L-E* curves can be interpreted and utilized in the same manner as the voltammetric *I-E* curves, no interfering effect of the capacity current and the base electrolyte decomposition current appearing on the *L-E* curves; the peaks have a simpler shape preserved also at high rates of the potential rise, thus permitting the sensitivity of determination to be increased. At a rate of 250 mV s^{-1}, luminol can be determined in concentrations as low as 200 ppt.

In solutions freed from air by using nitrogen, the ECL intensity is suppressed: a five-minute bubbling with oxygen of pure solutions induces a 20% increase in the

TABLE I

Dependence of the potentials and heights of the anodic *I-E* peaks of luminol on the concentration of cetyltrimethylammonium bromide (c_I) and the potential rise rate; $c_L = 0{\cdot}16$ mmol l^{-1}, pH 12·73

c_I mmol l^{-1}	v mV s^{-1}	E_p V	$E_{p/2}$ V	I_p μA
0	4·16	0·24	0·20	2·1
	25	0·26	0·21	4·0
	50	0·28	0·22	6·2
	100	0·32	0·24	6·8
0·32	4·16	0·19	0·15	3·1
	25	0·21	0·17	5·6
	50	0·22	0·18	8·8
	100	0·25	0·20	10·3
0·80	4·16	0·18	0·15	2·2
	25	0·19	0·16	4·8
	50	0·21	0·17	7·4
8·2	4·16	0·20	0·16	1·7
	25	0·22	0·18	3·7
	50	0·23	0·19	5·4
	100	0·28	0·21	5·8

peak intensity, a peak broadening, and a shift of E_p to higher potentials; in the presence of I, the peak potentials are less susceptible to oxygen, but the peak height also grows by 20% on a 5 min saturation of the solution with oxygen.

A substantial difference between the *L-E* and *I-E* curves appears in the dependence of the peak heights on the concentration of tenside. At low concentrations of I, $c_I \leqq c_{mc}$, the *L-E* peak heights drop dramatically, while after surpassing the c_{mc} value they increase proportionally to the tenside concentration (Fig. 3). A similar dependence – with a minimum in the c_{mc} range – is typical of the fluorescence intensity for organic anions and cationoid tensides[10,11]. For fluorescein and cetyltrimethylammonium bromide this dependence is shown in Fig. 3. The drop in the fluorescence intensity is largely accompanied by a shift and splitting of the first long-wavelength band in the electronic absorption spectrum. Generally the same changes – quenching of fluorescence, metachromatic effect – can be observed with pure concentrated solutions; they are regarded as an evidence of aggregation of the molecules[12]. We suppose that at concentrations of I below c_{mc}, ion aggregation occurs,

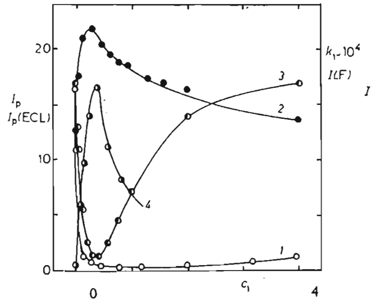

FIG. 3

Dependences of the *L-E* peak height (*1*; I_p(ECL) in arbitrary units), *I-E* peak height (*2*; I_p in μA), fluorescence intensity of fluorescein (*3*; I(F) in arbitrary units), and oxidation rate constant of pyrocatechol violet (*4*; $k_1 \cdot 10^4$ in s^{-1}) on the concentration of cetyltrimethylammonium bromide (mmol . . l^{-1})

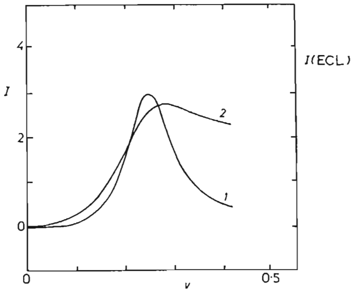

FIG. 4

Light-voltage (*1*) and voltammetric (*2*) curves for the oxidation of luminol on a fixed Pt anode; $c_L = 0{\cdot}08$ mmol l^{-1}, pH 13·20, $v = 25$ mV s^{-1}, $c_I = 4$ mmol l^{-1}; I in μA, I(ECL) in arbitrary units owing to hydrophobic interactions and a lowered electrostatic repulsion, even in dilute aqueous solutions. When the tenside concentration is raised above c_{mc}, micellization takes place, accompanied by a rise in the fluorescence intensity and other changes in the electronic spectra[10]. During the oxidation of luminol, the anion of 3-aminophthalic acid is excited[2]. Variations in the concentration of the cationoid tenside bring about the same changes in the intensity of the emitted radiation as with any other fluorescence indicator. Luminol in neutral and alkaline solutions occurs in the anion form (pK_a 6·74, ref.[13]). Low concentrations of *I* stimulate aggregation of luminol, leading to a rise in the *I-E* peak height (Fig. 3). The aggregation and micellization of substrates under the influence of tensides appears similarly in the oxidation with hydrogen peroxide catalyzed by trace amounts of Co^{2+} ions. Fig. 3 shows the dependence of the oxidation rate contants of pyrocatechol violet (PCV) on the concentration of *I* (pH 8·00, $c_{Co} = 0{\cdot}2\ \mu mol\ l^{-1}$, $c_{H_2O_2} = 84\ mmol\ l^{-1}$, $c_{PCV} = 39\ \mu mol\ l^{-1}$, $t = 25°C$), which again has the characteristic shape showing the maximum in the c_{mc} range (ref.[14]). In all dependences depicted in Fig. 3 the positions of the maxima and minima are only slightly influenced by the properties measured and the concentration of substrate, but depend on the tenside nature, indicating reliably its critical micellar concentration.

ECL is not restricted to electrooxidation of the known chemiluminescence indicators; an intense radiation can also be observed, *e.g.*, during the electrolysis of benzenoid hydrocarbons in polar solvents[15]. The dependence between the intensity of the emitted radiation and the concentration of the substrate or of inhibitors to the reaction is usually linear, and thus can form a basis for sensitive analytical procedures[15]. Since radiation can only result from exoergic reactions, chemiluminescence reactions are largely oxidation reactions, in which radicals take frequently part. Thus the electrochemical excitation may be a phenomenon of a very general nature, and the study of ECL during the oxidation of various substrates could raise the potential of the physico-chemical methods.

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