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STRUCTURE OF CHEMICAL COMPOUNDS, METHODS OF ANALYSIS AND PROCESS CONTROL

DETERMINATION OF HYDROXYCHLOROQUINE BY CATHODIC ELECTROCHEMILUMINESCENCE

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Cathodic electrochemiluminescence (CECL) of hydroxychloroquine (HCQ) in aqueous solutions on an aluminum electrode is described. The analytical possibilities of the phenomenon are evaluated. The conditions for HCQ determination in slightly alkaline and acidic solutions are optimized. The possibility of analysis in the concentration range 0.1 – 300 mg/L is shown. CECL can be a supplement to well-known methods of HCQ determination in pharmaceuticals by a method based on a new type of luminescent response signal.

Keywords: hydroxychloroquine, cathodic electrochemiluminescence, luminescence analysis, aluminum electrode.

Chloroquine (**I**) and hydroxychloroquine (**II**) are currently indicated for treatment of malaria and rheumatoid diseases. Also, research results in which **I** demonstrated *in vitro* activity against 2019-nCoV were published [1, 2]. The antiviral activity of **II** for treating severe acute respiratory syndrome of coronavirus 2 (SARS-CoV-2) was found to be stronger than that of **I** [3].

Many chloroquine derivatives, including **II** (Fig. 1), are known to possess intense photoluminescence (PL) [4]. Cathodic electrochemiluminescence (CECL) on an aluminum (Al) electrode is a less common electrochemical method for exciting luminescence than PL [5]. The CECL phenomenon is related to tunneling of hot electrons through the surface oxide during cathodic current pulses. Many water-soluble aromatic compounds are capable of intrinsic CECL on an Al cathode [6]. However, the list of electroluminophores is still not as broad as that of compounds that luminesce upon excitation by UV light. The structure of **II** suggested that this compound was capable of CECL. However, CECL of **II** has not been reported to the best of our knowledge. The present work proposed a new CECL method for determination of **II** in pharmaceuticals. Preliminary experiments confirmed the hypothesis that **II** was capable of emission upon electrochemical excitation. The goal of the work was to characterize CECL of **II** and to optimize the determination conditions.

EXPERIMENTAL PART

The experimental setup for measuring CECL consisted of an electrochemical cell, PI-50-1.1 potentiostat, PMT-38 with a BNV2-95 power supply, and a two-channel recording system based on an L-154 data collection board in a personal computer. The electrodes were made of Al (99.99%) cylinders 4 mm in diameter that were treated with HF (48%), rinsed with distilled H_2O, and fixed in a Teflon body. ECL was measured in a flow-through cell constructed as shown in Fig. 2. In all instances, the electrode was held in a stream of acidic carrier that was flowing continuously through the apparatus before adding the activator. The sample was added using a manual syringe in which the needle was replaced by a fluoroplastic capillary of internal dimeter 0.6 mm. The sample volume was 1 mL; addition time, ~1 sec.

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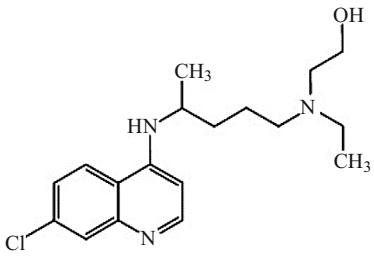

Fig. 1. Structure of hydroxychloroquine (**II**) [2-({4-[(7-chloro-4-quinolinyl)amino]pentyl}ethylamino)ethanol].

ECL was generated by rectangular pulses of variable polarity, alternating cathodic pulses of potential –8 V (during which the light intensity was recorded) and passivation periods of 1.7 V.

Two considerably different systems were investigated. System A used a sample containing **II** in weakly alkaline solution that was injected into a stream of H_2SO_4 (40 mM). System B used a dilute HNO_3 solution of **II** that was injected into a stream of HNO_3 (80 mM). The preliminary solution compositions and excitation conditions were selected based on experience with CECL of inorganic and organic luminophores in systems differing in sensitivity to aromatic compounds that were stable for long times. The analyte was an aqueous solution of **II** sulfate from the pharmaceutical 200 Plaquenil®. The composition of the pharmaceutical was **II**, lactose monohydrate, povidone (K25), corn starch, magnesium stearate, Opadry OY-L-28900 [hypromellose, macrogol 4000, titanium dioxide (E171), lactose monohydrate]. Immard tablets (Ipca Laboratories, India) also contained **II** (200 mg) and the excipients corn starch, potassium hydrogen phosphate, talc, colloidal silicon dioxide, polysorbate 80, and magnesium stearate.

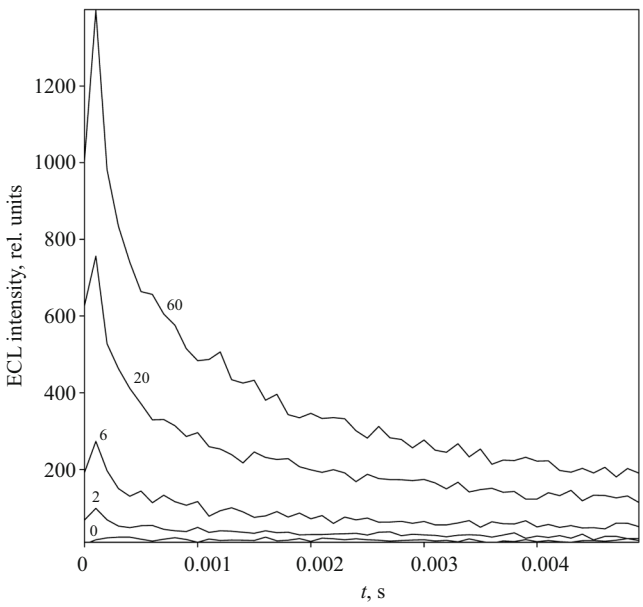

Fig. 3. Kinetic curves for CECL of solutions of **II** in the concentration range 2 – 60 mg / L (all four solutions contained 25 mM $Na_2B_4O_7$ + 0.1 M H_3BO_3 + 2 mM $KBrO_3$).

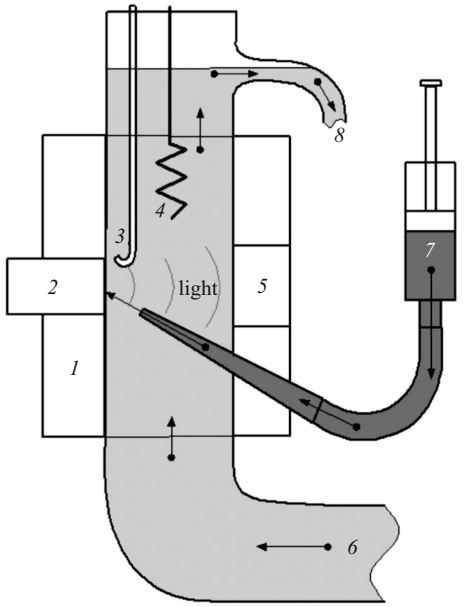

Fig. 2. Flow-through cell for CECL generation: Teflon body with inner diameter 8 mm (*1*); aluminum electrode (*2*); Luggin capillary for reference electrode (*3*); auxiliary electrode (*4*); optical window (*5*); carrier inlet (*6*); sample inlet (*7*); drain (*8*).

First, the tablet coating was mechanically removed. Then, a cleaned tablet was ground and the soluble part was leached with distilled H_2O. A stock solution of **II** was produced in this manner. Series of solutions of lower concentrations were prepared in borate buffer immediately before measurements. One of the oxidants potassium bromate, iodate, or periodate was added to the solution before the sample was added.

The CECL photoluminescence spectrum was measured at various wavelengths on a setup consisting of an MDR-3 monochromator and a Hamamatsu H101 – 8249 photomultiplier module using sequential addition of samples of constant composition.

TABLE 1. Optimized Conditions for HCQ Determination

System	Carrier composition	Injected solution	Cathode pulse length
A	40 mM H_2SO_4	25 mM $Na_2B_4O_7$, 0.1 M H_3BO_3, 2 mM $KBrO_3$	5 ms
B	80 mM HNO_3	80 mM HNO_3, sat'd (at 25°C) H_3BO_3 solution	180 ms

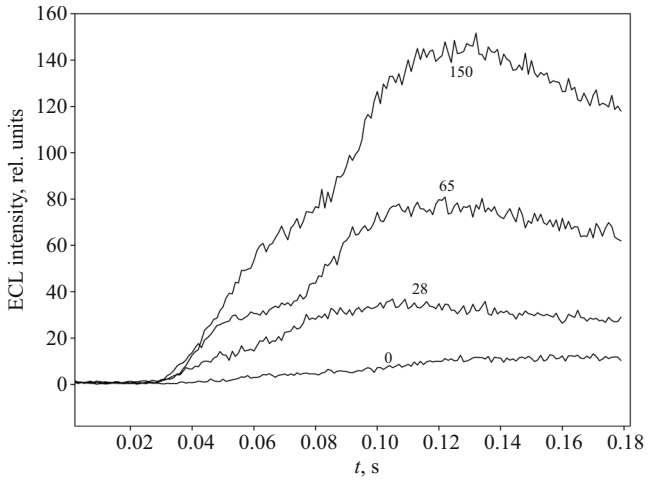

Fig. 4. Kinetic curves for CECL of solutions of **II** in the concentration range 0 – 150 mg / L (all four solutions contained 80 mM HNO_3 and saturated H_3BO_3).

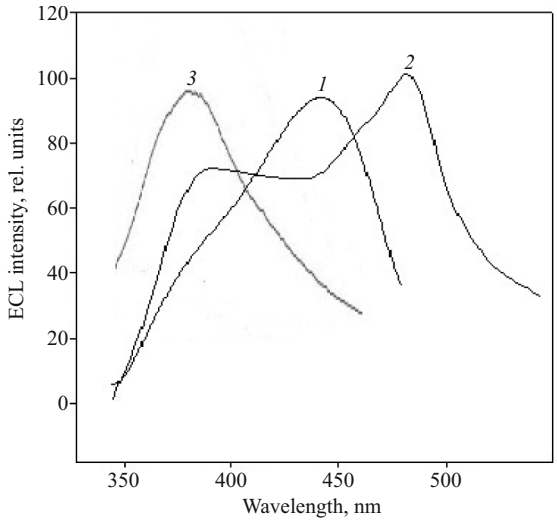

Fig. 5. CECL spectra of **II** in system A (curve *1*) and system B (curve *2*) as compared to PL spectrum of **I** (curve *3*) (data from the literature [7]).

RESULTS AND DISCUSSION

Figure 3 shows kinetic curves in weakly alkaline solution; Fig. 4, in acidic solution. The solution composition was chosen based on experience with other organic luminophores [6]. The results demonstrated that a CECL response occurred upon adding **II** in both systems.

The kinetic curves showed the primary signal in the CECL method. Each of the curves was the PMT response as a function of time from the start of the cathodic pulse following injection of a sample into the carrier. The number in the figures show the concentration of **II** in mg/L. The kinetic curves of solutions of **II** in Fig. 4 in the concentration range 0 – 60 mg/L (all four solutions contained 25 mM $Na_2B_4O_7$, 0.1 M H_3BO_3, 2 mM $KBrO_3$) and in Fig. 5 in the concentration range 0 – 150 mg/L (all four solutions contained 80 mM HNO_3 saturated with H_3BO_3) showed that the signal intensity increased with increasing concentration. Figure 3 shows that the light signal in this solution was quickly quenched. The duration of the cathodic pulse did not need to be increased to 5 ms. Figure 4 shows that CECL decayed slower in system B. The pulse length was 180 ms.

The optimal conditions for determination of **II** were selected by varying the contents of the supporting solution components.

Preliminary experiments with system A studied the effect of Na_2CO_3 concentration on the signal. The greatest signal intensity was observed for 20 mM Na_2CO_3. Then, the effectivenesses of oxidants HIO_4 and $KBrO_3$ on the signal in

TABLE 2. Normalized CECL Signal for Solutions Prepared from Plaquenil and Immard Drugs. Solutions Contained Nominal 50 mg / L of **II** in System B, $P = 0.95$

Sample	Normalized signal, %	s_r	n
Plaquenil, No. 1	100 ± 4	0.04	6
Plaquenil, No. 2	94 ± 6	0.06	6
Plaquenil, No. 3	101 ± 5	0.06	9
Immard, No. 1	101 ± 6	0.05	5
Immard, No. 2	103 ± 4	0.04	6
Immard, No. 3	112 ± 5	0.04	5

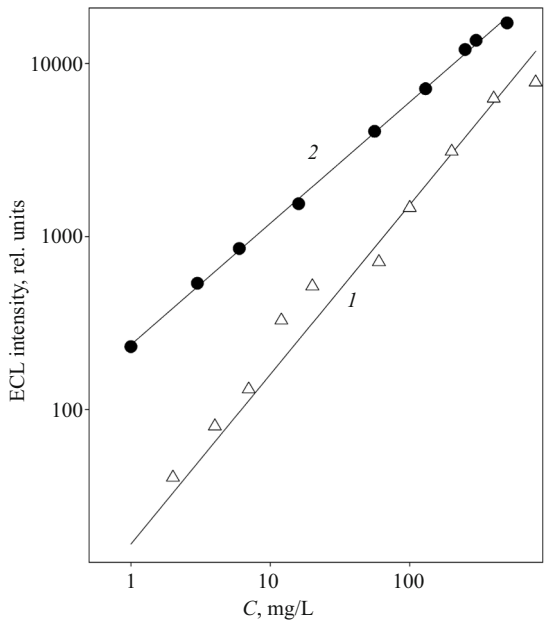

Fig. 6. Calibration curves for systems A and B.

solutions containing 40 mg/L of **II** in 20 mM Na_2CO_3 + 0.1 M H_3BO_3 were compared. The greatest intensity was observed for 12 mM $KBrO_3$. Thus, a supporting electrolyte containing 12 mM $KBrO_3$, 20 mM Na_2CO_3, and 0.1 M H_3BO_3 was optimal for determining **II** in weakly alkaline borate buffer solution. Table 1 presents the optimized conditions for determination of HCQ.

Solutions of this composition were used to measure CECL spectra. Figure 5 shows that spectra in systems A and B differed considerably from each other and from the PL spectrum in the literature [7]. (It is noteworthy that the PL spectrum was given not for **II** but for **I**. However, the system of conjugated bonds in these two molecules is identical.) Two components could be identified in the spectrum in system B, i.e., a violet band with a maximum at 380 nm and a blue band with a maximum at ~480 nm. The PL spectrum showed only the violet component. The spectrum in system A was intermediate in nature.

The difference was probably related not so much to the excitation method as to the pH range and the oxidant. Previously, the researchers added **I** phosphate (i.e., the protonated form) in distilled H_2O [7] while we used system A with borate buffer at pH > 8, where **II** existed as the free base.

Figure 6 shows calibration curves for systems A and B.

The integrated intensity, i.e., the areas under the curves in Figs. 3 and 4, was used as the analytical signal to construct the calibration curves. The signal was observed to be linearly dependent on the concentration in logarithmic coordinates. A comparison of Figs. 3 and 4 showed that the instantaneous CECL intensity in system A was almost an order of magnitude greater than that characteristic of system B. However, the integrated intensity of CECL during the pulse for system B was slightly greater because of the longer duration of the light pulse. However, the main advantage of system B was the good reproducibility (sr 0.03 for 20 mg/L of **II**, $n = 5$) while the sr was ~0.15 for system A. The potentially large gain of instantaneous intensity made system A more promising. However, its analytical possibilities are not yet fully realized, probably because of limitations of the used cell that are exacerbated upon injection of the alkaline solution into the acidic carrier. System B is currently preferred for analytical purposes. The detection limit was 0.1 mg/L; range of determined concentrations, 0.3 – 300 mg/L.

The method was checked using Plaquenil and Immard pharmaceuticals (Table 2). Three tablets from a package of Plaquenil and three Immard tablets were dissolved as described above to produce 1% solutions. Then, each of the six solutions was diluted 200 times. CECL was measured in system B from five to nine times. The dilution factor was chosen so that the signal/noise ratio was high enough and remained in the linear region of the curve. The CECL intensity at 50 mg/L of **II** was proportional to concentration with good accuracy. The average signal from the solution from the first Plaquenil tablet was considered the standard (100%). Table 2 shows that the relative values for solutions obtained from the five remaining tablets were close enough to 100% to indicate the procedure was robust.

Thus, electrochemical excitation of luminescence of **II** on an Al electrode was shown to be possible. A new method for determination of HCQ in pharmaceuticals was proposed based on this phenomenon.

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