ELECTROCHEMILUMINESCENT ANALYZER ELAN-3D FOR ASSAY AND RESEARCH

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The work is devoted to development of automated electrochemiluminescent analyzer ELAN-3d aimed at electrochemical/ electrochemiluminescent (ECL) assays including biomedical and environmental applications. The developed device fills the gap of inexpensive ECL equipment aimed at research work. ELAN-3d consists of external unit containing potentiostat and PMT module in a light-proof box and PC for control. The external unit is connected to PC via Advantech PCI-1711 data acquisition card. ELAN-3d is mainly intended for investigation of new ECL compositions, development of sensors and assay methods, study of new electrode materials.

Introduction

Luminescent and electrochemical methods of assay play the key role in medical, laboratory and clinical investigations. Chemiluminescent method of analysis is a widely used luminescent method due to its high sensitivity since the analytical signal can be detected at the level of several photons per second. At the same time chemiluminescent method possess a lack of control over the reaction course. In contrast to chemiluminescent methods the electrochemical (EC) methods allow precise control of the reaction course using potential program provided by operator [1]. But electrochemical methods often suffer from insufficient detection limit comparing to chemiluminescent ones. The electrochemiluminescent method of assay combines advantages of both mentioned methods and partially eliminates their lacks. It is based on light emission during chemical reaction of species that are generated electrochemically at the electrode when control electric signal is applied. Absence of spontaneous light emission, different nature of exciting (electric) and analytical (optical) signals eliminates background noises and allows detection limit as low as 10^{-10} M [2].

Despite of essential advantages of ECL assay over many other methods the list of available commercial equipment for ECL investigations is currently rather scarce. Most of available equipment is aimed at laboratory and clinical investigations using immune ECL assay and standard protocols and do not allow changing assay procedure. The purpose of present work is development of software-hardware apparatus for automated electrochemical and ECL research of liquid samples, ECL compositions, new electrode materials, assay methods etc. The developed system allows full control of potential program for ECL excitation, components of ECL composition and electrochemical cell elements.

As an example of application the system was used for investigation of ECL properties of flat transparent ITO (indium-tin oxide) electrodes that were modified by thin film of polymethylmethacrylate containing molecules of organic luminophors using Langmuir-Blodgett technique. Such choice of sample for investigation is caused by high analytical potential of ECL assay using immobilized luminophors [3].

Analyzer ELAN-3d for ECL investigations

The structure of ELAN-3d complex is given on fig. 1.The name “ELAN” stands for “**el**ectrochemiluminescent **an**alyzer” whereas “3d” indicates that it is the third digital generation of such equipment developed in our laboratory [4]. The complex contains 3 main units: unit for control of electrochemical and ECL measurements (control unit), PC and data acquisition board Advantech PCI-1711.

Control of ELAN-3d is done using PC with corresponding software and data acquisition board PCI-1711 (PC-LabCard series, Advantech Co., Ltd) connected to PCI bus of PC. The board is connected to control unit with a cable. Such data acquisition boards are widely used for automation of industrial and laboratory equipment, input and output of digital and analog signals.

The structure of ELAN-3d is determined by the need to provide the following functionality for EC and ECL research: potentiostating of three-electrode cell, simultaneous measurement of photons emission and current through the working electrode and their linkage to working electrode potential.

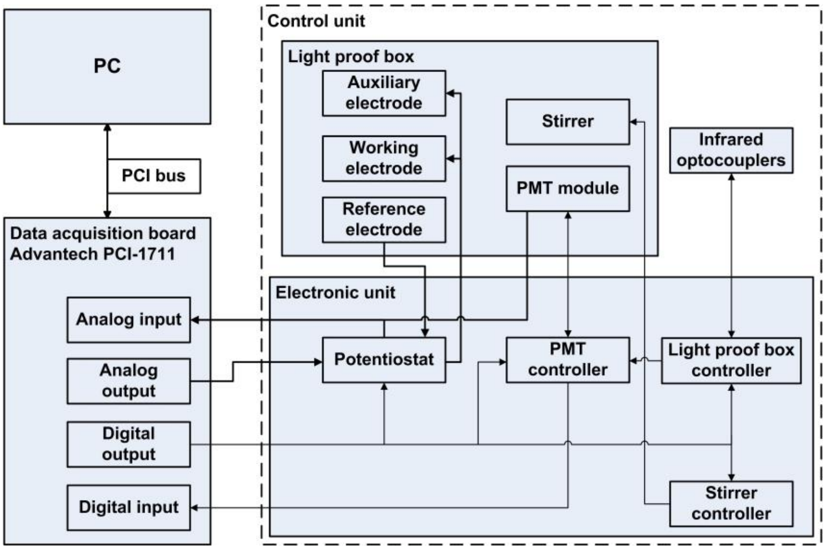

Figure 1. Structure of ECL analyzer ELAN-3d.

Potentiostat of ELAN-3d is based on traditional one channel scheme that can be found in a number of books (see e.g. [1], the scheme of adder potentiostat) and input signal is synthesized (added) from two unipolar 12-bit DAC outputs one of which is inverted. Additionally the system allows electronic disconnection of reference and auxiliary electrodes from the circuitry for measurement of open circuit potential of working electrode and protection of circuitry against breakdown by static charge during manipulations with the cell.

Optical signal is detected using PMT module Hamamatsu H5784-20 (wavelength range 300 - 920 nm). It possesses high sensitivity, small dimensions, presence of embedded high voltage power supply and current-voltage conversion. Electronics of control unit protects PMT against external illumination in case of open light-proof box (control of light-proof box cover is done using infrared slit optocouplers).

The main technical parameters of the developed complex are: control of three electrode EC or ECL cell, polarization of auxiliary electrode in the ±10 V range with current up to 10 mA, potential resolution 1 mV, and accuracy of current measurement down to 1 pA. All this provides precise potentiostating of the cell that is important for analytical EC and ECL methods. The PMT module of ELAN-3d allows measurement of optical with the dynamic range of 70 dB and is suitable for investigation of a broad range of analytical reactions with different levels of optical emission.

Full control of ELAN-3d complex is done using specially written software. Communication between software and control unit is done via Advantech PCI-1711 board and its high level driver. The main functions of ELAN-3d software include: setting of experiment’s parameters and synthesis of corresponding potential program for polarization of working electrode of electrochemical cell; feeding of synthesized potential program to potentiostat during experiment and collection of response data (electrochemical current, optical ECL signal and some auxiliary ones); processing and display of collected data, navigation among collected data and their storage at hard disk of PC, control of some other elements of ELAN-3d operation (connection/disconnection of electrodes; control of PMT module operation; detection of potentiostat overload, ADC overflow and disconnection of reference electrode from the circuitry; measurement of equilibrium potential of redox system under investigation etc.).

Depending on the operation mode the display tab will contain one (only electrochemical) or two (electrochemical and electrochemiluminescent) data sets. Displayed data can be filtered (smoothed) using several simple algorithms. As an example fig. 2 shows display tab containing electrochemical response of aqueous tripropylamine (TPA) solution (7 consecutive scans) that is bubbled with argon leading to TPA removal due to its volatility.

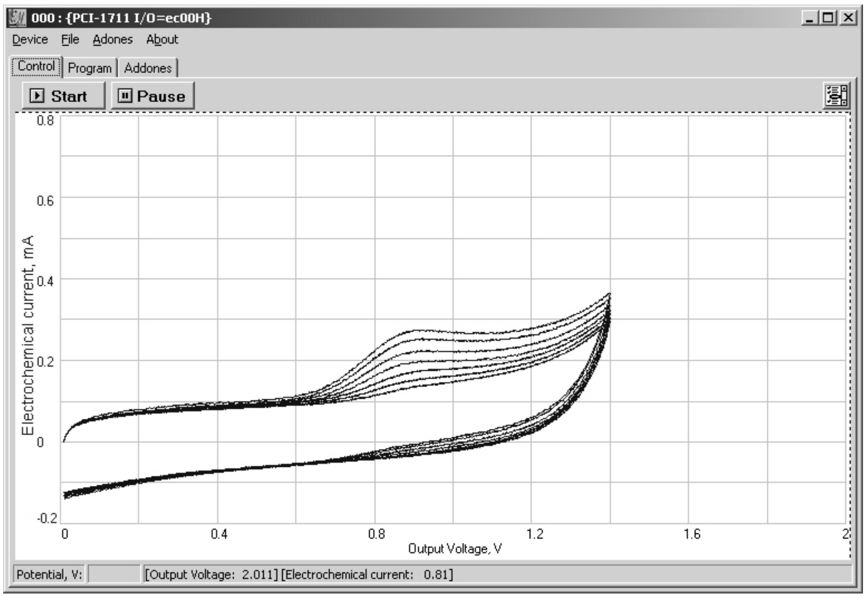

Figure 2. Display tab containing results of electrochemical investigation.

Experimental section

All chemicals used for in this work had “chemically pure” qualification. Preparation of solutions for electrochemical and ECL measurements was done using bidistilled water. Transparent ITO electrodes were modified with polymethylmethacrylate (PMMA) films using Langmuir-Blodgett technique (LB). PMMA was used as LB film forming agent in which organic luminescent molecules of rubrene were incorporated.

EC and ECL measurements were conducted in aqueous solution. ECL excitation of modified ITO electrodes was done using TPA coreactant. Modification of ITO electrodes’ surface was done using Langmuir-Blodgett trough LT-102, Microtestmachines, Co. For the transfer of film on the surface of aqueous subphase a solution of luminophor and PMMA in volatile and water immiscible solvent (chloroform) was prepared. The mixture was transferred on the water surface in LB trough. After solvent evaporation the film was compressed to the required surface pressure and was transferred onto the substrate using vertical dipping.

Investigation of EC and ECL properties of ITO electrodes modified by Langmuir-Blodgett films containing luminophor molecules was done using cyclic voltammetry method at 100 mV/s scan rate. TPA was used as a well known efficient ECL coreactant which works according to oxidative reduction mechanism [5]. In the present work EC and ECL properties of electrodes modified by PMMA/rubrene were studied. Considering limited range of available electrode potentials in water one can predict that electrochemical excitation of rubrene will encounter difficulties. But experiments indicated that immobilization of water insoluble electrochemiluminophors in the polymer matrix on the electrode surface permit simple excitation of their ECL in contact with aqueous solution containing coreactant. This fact is itself an important achievement in the field of aqueous ECL since before the range of luminophors that were used in aqueous medium (including immobilization at the electrode) was mostly limited to ruthenium bipyridine complex and some of its derivatives.

In order to verify analytical capabilities of developed complex ELAN-3d the investigation of ECL from LB films with rubrene versus TPA coreactant concentration was conducted. Fig. 3 shows corresponding dependence of ECL intensity for 10 layer LB film versus TPA concentration in aqueous solution. Obtained response has linear dependence of ECL in the range of TPA concentrations $10^{-5} \div 5\times10^{-2}$ M with detection limit 2.5×10^{-5} M (for signal to noise ratio equal to 3). The nonlinear ECL response at TPA concentrations above 5×10^{-2} M is associated with increase of solution alkalinity due to TPA itself [5].

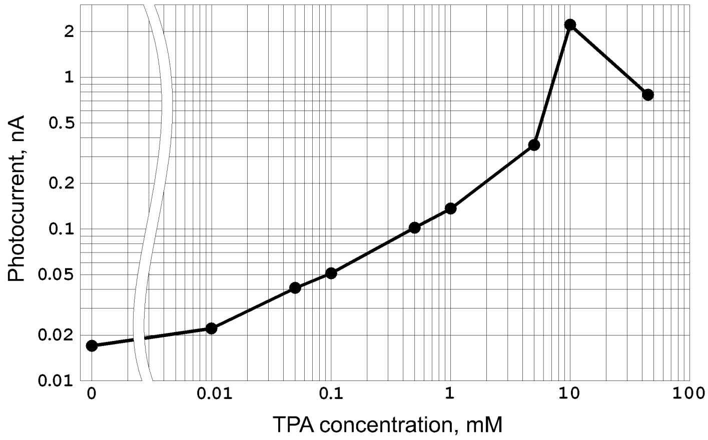

Figure 3. Dependence of ECL intensity of PMMA/rubrene LB film (10 layers) versus TPA concentration.

Conclusions

The automated complex ELAN-3d was developed to fill the gap in the commercial instrumentation for investigation of ECL reactions, development of analytical methods and investigation of new electrode materials. ELAN-3d ECL analyzer was designed and constructed from commercial components and certain home machined mechanical parts. A suitable software program was developed for control, data acquisition and visualization. Developed analyzer is manly oriented on investigation of new electrode materials, in particular flat electrode substrates modified by organic and inorganic films including LB films, conductive diamond coatings etc. and development of corresponding analytical method using those electrodes.

Using developed analyzer we have shown high analytical potential of aqueous ECL reactions at transparent ITO electrodes modified by Langmuir-Blodgett films containing water insoluble organic luminophor rubrene.

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References

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