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doi:10.15199/48.2018.06.07

Apparatus “Spark” for luminescent and electrochemiluminescent measurements

***Abstract**. The article is devoted to the development of the apparatus “Spark” for integration a photodetector with a current output in a measurement system. Developed apparatus “Spark” includes a current-to-voltage converter, an output filter, and a source of a high voltage, that are sufficient to combine a photomultiplier tube with a different analytical instrumentation. Use of apparatus “Spark” with electrochemical station CHI 800C CH Instrument and photomultiplier tube CR-105 by Hamamatsu Photonics is shown by over an electrochemiluminescent assay of a test solution.*

Streszczenie.** W artykule opisano urządzenie “Spark” przeznaczone do pomiarów luminescencji. Urządzenie wyposażone jest w fotodetektor przetwornik prąd-napię, filtr wyjściowy i źródło wysokiego napięcia do współpracy z fotopowielaczem. **Urządzenie “Spark” do pomiarów luminescencji i luminescencji elektrochemicznej

Keywords: transimpedance amplifier, photomultiplier tube, electrochemiluminescence.
Słowa kluczowe: pomiar luminescencji, fotopowielacz, wzmacniacz transimpedancyjny.

Introduction

Electrochemical methods are very attractive to solution content characterization as sensitive, selective, quick, and quite simple technics. A limiting factor of electrochemical technics is electrons amount transferred during the analytical reaction, which should be enough for detection by electronics. The last one is determined by the versatility of modern operational amplifiers they set up a base of a potentiostat, main instrumentation for voltammetric electroanalysis. A solving this problem in electroanalysis is possible to use an electrogenerated chemiluminescence phenomenon (ECL) [1-4].

The registration of the light emission is attractive method for analysis chemical and biochemical reactions [5-8]. ECL is a light emission that is a result of electrochemical reactions that appear during an electrode polarization in specific solutions. The difference in chemical mechanisms of a light generation determines riches of ECL analytical techniques [4, 9]. This powerful analytic method is widely used and foremost in immunochemistry [10]. Routine analyses by this method are characterized by a registration of a weak light during electrolysis of a sample solution so photomultiplier tubes (PMT) [11] frequently are used as a detector in ECL analyzers.

Commercial instrumentation for immunochemical assays is limited for a research work as expensive and inflexible devices. [12] Their high productivity is not too important in research as so mainly development works ask frequent changes in the functionality of measurement instrumentation. To conduct electrochemiluminescent analysis is needed combination of luminescent and electrochemical instrumentation. Different electrochemical potentiostats support a data collection in an additional channel, thus attractive possibility to use combination them with PMT as a base for ECL analyzer. Technician problem of a direct connection of PMT to a data acquisition system is a general problem of data integrity. As PMT has output current signal so current to voltage transformation is required. After this transformation, the output signal should match to input characteristics of the data acquisition system. One side of the problem is mainly potentiostats only propose a channel for an analog signal digitizing without preliminary data processing. Another side is PMT has a wide bandwidth signal. Thereby, intermediate data processing should harmonize PMT signal with a potentiostat opportunity.

Developed apparatus “Spark” is essential to integrate PMT with electrochemical analytical instrumentation, his view is at figure 1. Developed apparatus “Spark” is essential for the PMT integration with electrochemical analytical instrumentation, it is in figure 1.

Structure

The developed device contains next principal units: a current-to-voltage converter, a 2nd order Butterworth low pass filter, a high voltage source, two microcontrollers, a front control panel and a back panel with connectors, power unit. Interactions of units are exhibited on the block diagram of apparatus “Spark” (fig. 2). All elements are mounted in the small case. It also is possible an independent utilization as a high voltage source or a transimpedance amplifier with an output low pass filter. Specification of the apparatus is in Table 1 “Technical Characteristics”.

Fig.1. Front and back views on apparatus “Spark”

Table 1. Technical characteristics

Parameter	Value	Unit
Current-to-voltage convertor		
Input current ranges	0.1, 1, 10, 100	µA
Input bias current	(typical) 1.5	pA
Integration time constant	variable*	
Output filter		
Type	low pass, Butterworth	
Order	2nd	
Schematic	Salomon-Key	
Maximum output voltage	± 11.84	V
Maximum output current	50	mA
High voltage source		
Output voltage	Variable	
Output voltage range	0…-1500	V
Voltage setting absolute precision	0.1	%
Voltage setting repetition precision**	0.0015	%/°C
Time stability	0.1 (after 30 min preheating)	%/h
Temperature stability	0.1	%/°C
General		
Dimensions (w × l × h)	165 × 220 × 95	mm
Mass	1	kg
Power net parameters	190 … 250/50…60	V/Hz
Power	< 10	W

The apparatus operation is controlled by two included microcontrollers. Due to needing a big amount of input/output ports to control buttons, indicators, and units, two same low-cost microcontrollers ATMega16 (Atmel-Microchip Co.) were used in the schema.

Microcontroller MC 1 is responsible for a reading of a buttons condition, an indication of an operation mode by LEDs. Accompany with these functions it switches feedback loops in the filter and the current-to-voltage converter to set operation parameters.

Microcontroller MC 2 drives the high voltage source according to settings obtained by attached control buttons. The operation state of the high voltage source is indicated on 7-segment indicator and LED.

Sequential switch on, firstly, activation control loop based on digital potentiometer and lately connection power unit to the high voltage source by CMOS relay, prevents the uncontrollable appearance of a high voltage at output of the apparatus. So PMT will be guaranteed switch off during period of "Spark" switching on.

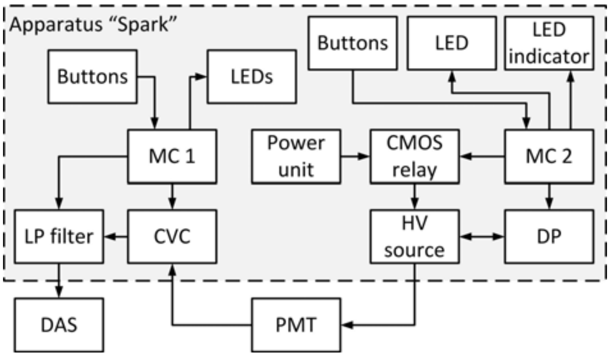

Fig.2. Block diagram of apparatus "Spark": LEDs – light emitted diodes, LP Filter – low pass filter, MC 1 and MC 2 – microcontrollers, HV source – high voltage source, DP – digital potentiometer, PMT – photomultiplier tube, CVC – current-to-voltage convertor, DAS – data acquisition system (potentiostat).

Schematics

The light registration sensitivity of PMT operated in a current mode is controlled by two factors. First one is a current-to-voltage conversion coefficient and the second is a magnitude of a high voltage of PMT powering.

The developed device has a four-decade variation range of a conversion coefficient that expands a dynamic operating range. The current-to-voltage converter is based on a current follower that is inverting amplifier with a resistor in a negative feedback loop (fig. 3). The conversion is according to the equation:

$$U_{CVC} = -\frac{I_{PMT}}{R_{feedback}} \tag{1}$$

where: U_{CVC} – an output voltage of the converter, I_{PMT} – a photocurrent produced by PMT, $R_{feedback}$ – a resistor value in the negative feedback loop. Because PMT is negative polarity powered, an initially negative photocurrent gives a positive voltage after the transformation according to equation 1. Then it is directed to the low pass filter.

Precision, very low noise, low input bias current, wide bandwidth JFET operational amplifiers AD8610 (Analog Device Co.), U1, is a base of the converter. Feedback resistors, R1…R4, are switched by reed relays, S1…S4, to minimize loose of a weak current signal. A technician trick is used to minimize degradation of reed relays, prevent excitation of the amplifier by switching a feedback. A branch with two relays is connected parallel to feedback loops, each of them contains a reed relay and a feedback resistor. In new branch, one relay is reed relay, S5, other is optical CMOS relay, S6. Each of them adds properties of other. CMOS relay is insensitive to amount and conditions at a switching moment. When a reed relay is open, it is characterized by a very good insulation. It is essential to prevent a leakage of a signal into a feedback loop.

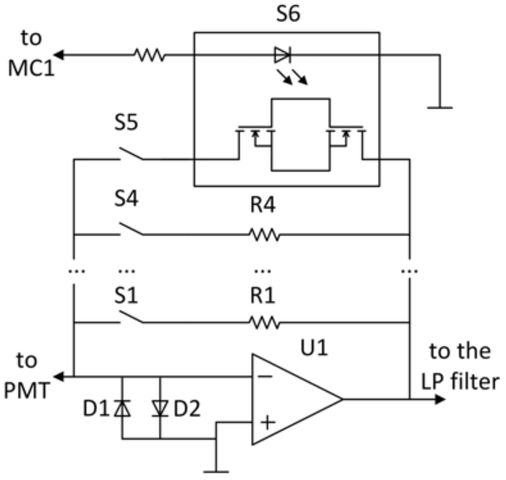

Fig.3. Programmable current-to-voltage convertor.

The operational principle of the additional loop is the next.

The first step, the reed relay in the additional loop is connected then the CMOS relay is connected. The appeared path is preferable for a signal than the connected feedback loop with a resistor is more resistive. So a current mainly flows through the new branch and the amplifier is inactivated by a shortcut at this moment.

The second step, a new feedback resistor is selected by a connection of a corresponded reed relay. And a previous resistor is deactivated by opening its reed relay.

The last step, after the feedback reconnection, the CMOS relay firstly is opened and, the reed relay lately is opened in the additional branch. A current does not flow through the reed relay on its opening because the additional branch had broken by the CMOS relay.

The procedure of reconnection of a feedback is finished.

A complicated reconnection procedure is compensated by an automatic microcontroller control. A profit of enlarged schematic is that any reed relay never is switched in a load state.

Two alternative directed diodes that parallel connected are used to protect the input of the operational amplifier with high impedance in a negative feedback. This chain prevents signal rise higher than a level of voltage drop on open p-n-junction independently to a current direction. Because current follower drives inverting inputs to the level of non-inverting input that grounded, an input current signal is not able to rise to a dangerous level by this chain it is a trusted protection solution [11].

Another way to control sensitivity a photodetector based on PMT is a voltage magnitude vary to power PMT. The last one has nonlinear dependence sensitivity to applied high voltage nonetheless support possibility PMT to work with different light intensity as weak as bright.

Voltage programmable high voltage source DW-N152-10k (TianJin Dongwen High Voltage Power supply Co., Ltd., China) is used in the apparatus. Its control was realized by digital potentiometer AD5231BRUZ10 (Analog Device, Inc.) and two solid-state relays KAQY212 (Cosmo Electronics Corp.). Optorelays connect ±15V source to the high voltage source, it safe sequence to turn it on. The reference voltage produced by the high voltage source is 10-bit precision divided with an excellent reproducibility. This performance that natural for a digital control is important to restore a PMT operation mode for analytical applications. The high voltage source only is activated (powered) then an operator press control button and MC2 fixes a position of the potentiometer wiper. All-time uncontrollable powering is excluded to prevent PMT damage obligatory that MC2 uses built-in a brown-out detector and a watch-dog timer for an operation control.

Signal integrity

The main task to perform signal integrity this is blocking high-frequency components of an input signal. High-frequency variation of a signal can be multiple reproduced in a digitized spectrum with a frequency of a data acquisition that known as an aliasing. In developed apparatus, an anti-aliasing filter is an active low pass filter.

The signal integrity in the developed apparatus that couples PMT and an external data acquisition device is processed by 2nd order Butterworth low pass filter (fig. 4). Butterworth filter has chosen because its response is the most flatness in a bandpass region [13]. To realize 2nd order Butterworth filter one operational amplifier is enough that is high-speed JFET amplifier AD825 , U2, in the apparatus.

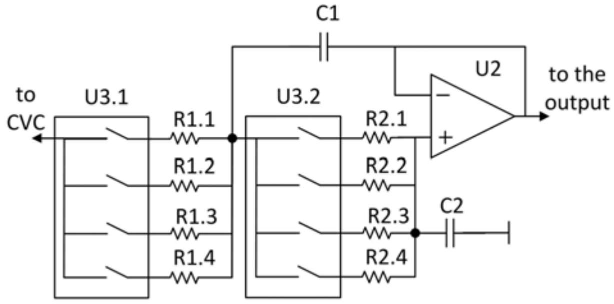

Fig.4. Programmable low-pass filter based on a Sallen–Key topology

Sallen–Key topology has selected because it is simple and is insensitive to components. A disadvantage is a price that a unity-gain and a flatness of response determine a slow decay of this filter response in a bandstop region.

Maximally flat passband frequency response of a second-order Butterworth filter is observed when Q of 1/√2 is. This is done by the next component selection C1 = 2 C2 = 1 µF and R1.x = R2.x = 750 Ω, 1.5 kΩ, 7.5 kΩ, and 15 kΩ, that gives 300, 150, 30, or 15 Hz cutoff frequency correspondently. The variation of the feedback is doing by double 4-channel analog multiplexer ADG 1409 (Analog Device, Inc.), U3, it only has maximum 5Ω on-state resistance.

The output current 50mA of the amplifier satisfy a next stage load so the output signal is directly kept to output connector to a combined data acquisition system.

Selection of operation mode can be done according the next approach. Minimal suppress should be in blocking bandpass [14]:

(2) $$A_{\min} = 20\lg\left(\sqrt{1{,}5} \times 2^{B}\right);$$

where: $A_{\min}$ – signal suppression, dB , B – ADC resolution, bits. Because a blocking filter is a second-order Butterworth, its amplitude response characteristics:

(3) $$H(f) = \frac{1}{\left(1 + \left(\frac{f}{f_c}\right)^{4}\right)^{\frac{1}{2}}};$$

where: f_c – the frequency, Hz, dB , f_c – the filter cutoff frequency, Hz.

The combination of equations (2) and (3) gives equation for the minimum sampling rate, f_s, for an acquisition system:

(3) $$f_s = f_c \sqrt[4]{1.5 \times 2^{2B} - 1}.$$

For example, if potentiostat sample rate is 10 ksps and ADC resolution precision in additional channel 14 bit, the filter cutoff frequency is recommended to select below:

(4) $$f_c = \frac{f_s}{\sqrt[4]{1.5 \times 2^{2B} - 1}} = \frac{10 \times 10^{3}}{\sqrt[4]{1.5 \times 2^{2 \times 14} - 1}} = 70{,}59\,Hz \cdot$$

In the apparatus, filter operation with cutoff frequency 15 and 30 Hz satisfies this criterion.

Functionality

All buttons are located on the front panel. Their functions are following.

"Power" switch turns the device on/off. The lightning of the button indicates a device operation. Current-to-voltage converter controls are at the left part of the front panel.

"Sensitivity" buttons control a conversion factor for a signal as 100, 10, 1, 0.1 µA/V correspondently.

"Response" buttons control an integration constant for a current-to-voltage conversion by a set of an appropriate capacitor in a feedback loop it can be 0.01, 0.1, 1, or 10 nF. The parallel combination of a resistor with a capacitor in a negative feedback loop of operational amplifier organizes an active low pass filter (or integrator) with a breakoff frequency at 1/(2πRC), where R and C are correspondent values of elements in the feedback.

"Filter" buttons drive a cutoff frequency of the output low pass filter to 300, 150, 30, or 15 Hz. Its proper value is fixed accordingly to data acquisition features of a connected device. Recommendations for a chosen value were discussed above.

High voltage source controls are at the right part of the front panel. An indication of settings directly is shown on a LED display. To change a high voltage setting red and green rectangle buttons are used. Both buttons are inactive when a high voltage is switched on. The high voltage source should be switched off state by "HV on/off" button to change the value. Lightning of this button indicates high voltage at the output. When the high voltage is switched on, its value is saved to EEPROM memory of the device. At the next turn on the device recalls this setting but a default state of the high voltage source is switched off in any way.

All connectors are on the back panel. There is one SHV connector for the negative high voltage output and two BNC connectors for an input signal from PMT and an output signal to a potentiostat or to other signal acquisition devices here. Additionally “Power net” socket and a fuse have placed a backside. The grounding of the device is sufficient for noise suppression and electrical safety.

Testing

Integration of PMT CR-105 (Hamamatsu Photonics, Japan) with potentiostat CHI 800C (CH Instrument, China) was done to test the device performances. The electrochemical experiment was accomplished in a light-proof box with a registration of a light that was electrogenerated chemiluminescence such ECL measurement was conducted. Tris(2,2'-bipyridyl) ruthenium(II) organometallic complex and its derivatives are extensively used as ECL reagents [1, 15-16].

A test solution for ECL experiment was 10 uM tris(2,2'-bipyridyl) dichlororuthenium(II) hexahydrate (purchased from Merck) in 2 ml of phosphate buffer solution (0.1 M concentration, pH = 7.0) and 1 mM tripropylamine co-reactant (purchased from Merck). Measurements were done in a three-electrode cell. It is a cylindrical 6 ml cell from borosilicate glass with a tetrafluoroethylene cap. Last one had holes to fix electrode system. The working electrode was glassy carbon disc electrode in a cylindrical tetrafluoroethylene insulation shield (opened working disk diameter is 3 mm), the counter electrode was cylindrical foil platinum electrode (with a surface area is 150 mm^2), the reference electrode was silver/silver chloride electrode. Linear potential sweep from 0 V to 1.4V and backward to 0 V (vs. Ag/AgCl reference electrode) was a polarization program for a cell electrolysis. The potential sweep rate was 100 mV/s. This electroanalytical technique is called a linear cyclic voltammetry method. Obtained experimental data are shown in Figure 5, it registered at “Sensitivity” 0.1 μA/V, “Response” 10 nF, “Filter” to 300 Hz, PMT powering voltage -700 V. At the graph ECL intensity arbitrary unit equal to volts that registered by CHI 800C potentiostat.

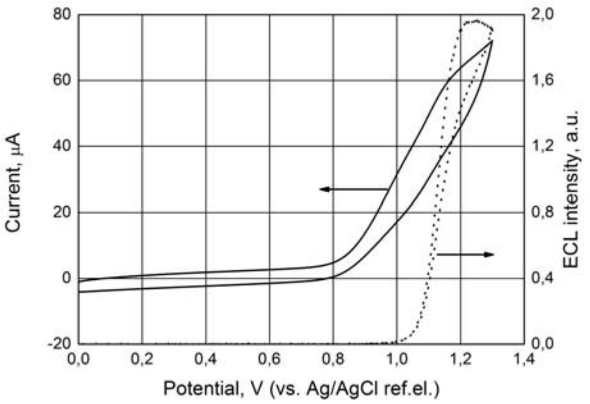

Fig.5. Electrochemical (solid line) and optical, ECL (dash line) responses of the test solution

The observed electrochemical current represents the behavior of an electroactive co-reactant presented in the test solution it masks electrochemical current associated with oxidation of tris(2,2'-bipyridyl) ruthenium(II)+2 ion. The last one has the sufficiently small concentration to electrochemical measurement. However, an observed light production is quite strong after 0.95V (vs. Ag/AgCl electrode) its using is possible for a chemical compound detection. Because light emission product is directly connected with following reaction with oxidized co-reactant thus a light response during electrolysis is directly dependent on tris(2,2'-bipyridyl) ruthenium(II)$^{+2}$ ion concentration.

Observed maximum of the emission at 1.25V is a result of a mass transfer of electroactive species at the working electrode. The intensity of ECL emission is a registry with a fine precision by PMT so a concentration of electroactive species can be determined very attentively by a control of a light emission. The calibration curve was determined as a linear fitting of experimental data (fig.6). Fitted curve with intercept 8,20884 and slope 27,94 has the coefficient of determination r^2 = 0,99186. The limit of detection for the selected analytical reaction and used instrumentation is clarified at 14×10^{-9} mol/l (signal to noise ratio = 3, probability level 95 %).

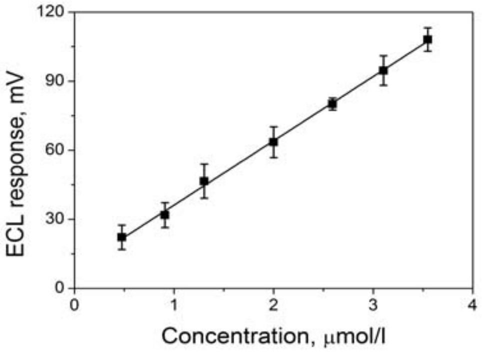

Fig.6. Calibration curve for the selected analytical reaction.

Obtained result of the developed apparatus testing for tris(2,2'-bipyridyl) ruthenium with TPA co-reactant ECL reaction is corresponded to data obtained by other authors [1, 20].

Conclusion

Developed apparatus ”Spark” assists integration light registration devices with an electrochemical instrumentation as different potentiostats. A shown example demonstrates a combining electrochemical station CHI 800C CH Instrument and photomultiplier tube CR-105 by Hamamatsu Photonics in an analytical system that can be able to perform electrogenerated chemiluminescent assays. ECL method sufficiently improves electroanalytical techniques possibility as methods that combines advantages of electrochemical and chemiluminescent analyses.

The realized signal processing satisfies needs of signal integrity. The operational control simplicity makes easy-in-use it. Its application with many electrochemical stations is possible as result of a variety of a data processing.

"Spark" utilization transforms analytical instrumentation for electrochemical assays to instrumentation that supports a powerful analytical method as ECL. Obtained results prove the high potential applicability of the apparatus in ECL analysis.

***Acknowledgments**:*

The work was done in the framework of Chinese Academy of Sciences President’s International Fellowship Initiative for visiting scientists and National Key Research and Development Program of China (Project No. 2016YFA0201300).

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