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Sensors and Actuators B 107 (2005) 372–378
SENSORS AND ACTUATORS B CHEMICAL
www.elsevier.com/locate/snb

Construction and analytical applications of a palm-sized microcontroller-based amperometric analyzer

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Received 19 June 2004; received in revised form 14 October 2004; accepted 28 October 2004
Available online 8 December 2004

Abstract

The design and development of a palm-sized (9 cm × 11 cm × 3 cm), cost-effective, microcontroller-operated analyzer for direct amperometric measurements is described. The low-power-consumption electronics used allow 8 h of autonomous operation with a 9 V battery (110 mAh), making thus this unit suitable for in-field measurements. Its operation is based mainly on the simple two-electrode potentiostatic mode, although the three-electrode mode is an option. The use of a microcontroller combined with analog and digital supporting circuits allows: (i) generation of the applied potential and the acquisition of analog signals with a gain auto-scaling capability; (ii) interaction with the operator for setting the measurement parameters; (iii) processing of data and displaying result on an LCD screen. Numerical data are automatically stored in memory and they can be retrieved by a personal computer through an RS232 port, either for creating measurements archives or for advanced processing. For this purpose, a program has been developed (on the LabVIEW platform) to provide a user-friendly graphical interface. The utility of this amperometric analyzer was assessed by performing experiments for the determination of ascorbic acid in standard solutions and pharmaceutical tablets.

Keywords: Amperometric analyzer; Potentiostat; Microcontrollers; Ascorbic acid assay

1. Introduction

Electrochemical detection has been widely used for the determination of a plethora of electro-active compounds of particular clinical, food and environmental importance [1,2]. Among the various electrochemical detection methods and transducers have been proposed so far, amperometric chemical sensors and biosensors have attracted a remarkable attention, since besides the analytical simplicity they offer, they can be potentially used for in-field measurements where other powerful techniques, that is chromatography, mass spectroscopy, etc., are impractical [2].

The key-unit of any biosensor-based amperometric analyzer is its potentiostat, an electronic circuit that precisely controls the potential of the working electrode, and therefore, its redox properties. Potentiostats have been routinely employed for both research and commercial type analytical devices and there are many examples of sensors that require miniature, low-cost and/or low-power electronics for use as blood glucose [3] and lactate meters [4], toxic gas sensors [5] or portable clinical analyzers [6]. Successful potentiostat-based designs have been proposed for applications in multi-channel flowing solution analysis [7], with low power consumption [8], or for low-power wireless remote environmental monitoring [9], or for a 'virtual' electro-analytical instrument for square wave voltammetry [10].

Using integrated circuit (IC) techniques a number of successful potentiostat designs have been proposed [11]. This approach allows the production of small size and low power

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doi:10.1016/j.snb.2004.10.029

consumption potentiostats, which are commercially available and allow even micro-electrode applications [12]. Subminiature sensor systems, as might be required for certain μTAS [13] and fully implanted medical applications [14] can be addressed using application specific standards products (ASSP) [15]. Despite the advantages of these integrated electrochemical potentiostat system-on-a-chip (PSoC) for certain applications as mentioned above, for the great plethora of conventional electrochemical sensor applications, in both market and research, the move to an ASSP based is probable unnecessary, and may be also be a prohibitively expensive step to undertake [8].

The aim of this work was to design, construct and test a simple, inexpensive, battery-operated palm-sized amperometric analyzer, capable of functioning either at a stand-alone mode, or to be connected with a PC for a more comprehensive data processing. The proposed analyzer has been constructed by using off-the-shelf electronic components and programmed under a philosophy to be suitable for analytical applications by using chemical sensors and biosensors.

2. Design and construction of the amperometric analyzer

The block diagram of the amperometric analyzer is shown in Fig. 1. The operational amplifiers (OA1-OA4) TLC27M4 (Texas Instruments Inc., USA), with an input impedance of 10^{12} Ω are used in the analog part (potentiostat, *i*/*V* converter, voltage followers) of analyzer. The automatic selection of the *i*/*V* converter gain is accomplished by the dual four-channel analog multiplexer (MUX) 74HC4052 (Texas Instruments Inc.).

The heart of the analyzer is the eight-bit microcontroller MC68HC705C8A (Motorola Semiconductors, Inc., USA). This microcontroller provides a total of 32 I/O lines that can be individually programmed as input or output lines and it is internally equipped with 256 bytes of RAM (general purpose registers, pointers, counters) and 8 kb of EPROM (for storing the program code). An additional 8 kb serially accessed EEPROM AT24C64 (Atmel Corp., USA) is used for the temporal storage of analytical data making them available for other devices through an RS-232 port after the measurements.

The serial 16-bit digital-to-analog converter (DAC) MAX541 (Maxim Integrated Products) is used to generate the potential applied to the working electrode (E_{WE}). The serial 12-bit analog-to-digital converter (ADC) MAX1202 (Maxim Integrated Products) is used for the digitalization of the analytical signal. It can operate as either eight single-ended channels or as a four differential channel device and can be used either with unipolar or bipolar inputs. The logic level converter MAX232 (Maxim Integrated Products) is used for the conversion of the TTL level voltages to ±12 V levels for implementing the RS-232 serial protocol for the communication with external devices. The nominal maximum sampling rate of the MAX1212 ADC (given by Maxim Integrated Products) is 133 kHz. For the conversion, the ADC uses either its internal V_{ref} = 4.096 V or an external one. In practice we used an external V_{ref} = 2.048 V produced by the IC LM4040 (Maxim Integrated Products). In two-electrode mode, the ADC inputs are unipolar (both positive) while in three-electrode mode the inputs are bipolar. The maximum voltage value that can be converted with the MAX1202 is equal to V_{ref} in unipolar mode (2.048 V) and equal to $\pm V_{ref}/2$ in bipolar mode (±1.024 V).

The operator interacts with the amperometric analyzer through a three-key keypad and a 2 line × 16 characters liquid crystal display screen. The control program has been designed so that a limited number of keystrokes is required for selecting functions and setting the operational parameters.

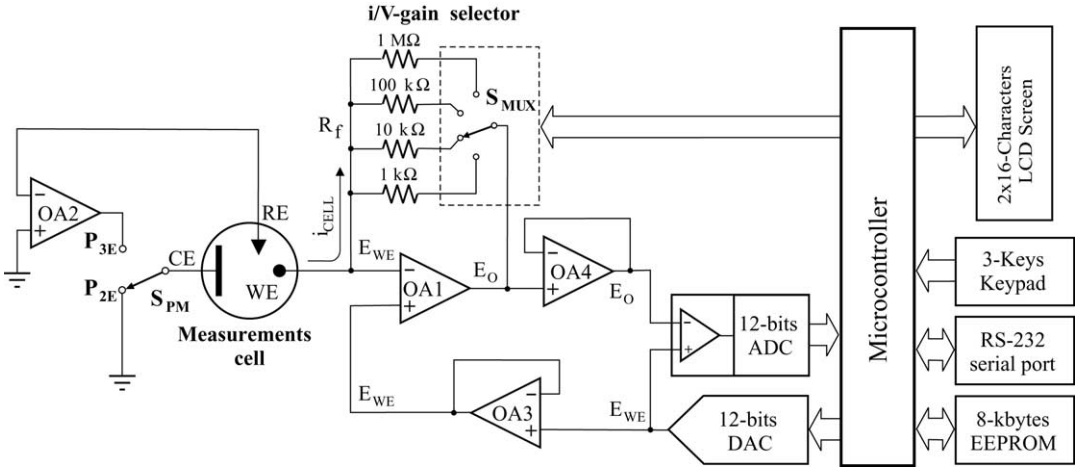

Fig. 1. Block diagram of the amperometric analyzer. WE: working electrode; RE: reference electrode; CE: counter/auxiliary electrode; OA1: operational amplifier used as the *i*/*V* converter; OA2: operational amplifier acting as potentiostat in the 'three-electrode' mode, OA3 and OA4: operational amplifiers used as voltage followers (buffers). S_{MUX}: analog multiplexer manipulated by the microcontroller for automatic *i*/*V* gain selection; S_{PM}: manually operated switch for selecting potentiostatic mode; P_{2E}: 'two-electrode mode'-position; P_{3E}: 'three-electrode mode'-position.

2.1. Analog circuit description

The operational amplifier (OA) is basically a differential amplifier having a large voltage gain, very high input impedance and low output impedance. The OA has an 'inverting' or (−) input, a 'non-inverting' or (+) input and a single output. The OA is usually powered by a dual polarity power supply in the range of ±5–15 V [16]. The following two fundamental rules apply to all circuits of any OA: (i) the current passing through the inverting (−) and non-inverting (+) inputs of the OA is practically zero, i.e. $i_{in} \approx 0$; (ii) at any closed loop configuration (connection of the output with the inverting input by any feedback element) the OA develops an output voltage v_o that practically equates the input voltages, i.e. $v_+ \approx v_-$. Rule (i) is a consequence of the extremely high input impedance Z_{in} of OAs (typically: $1\,M\Omega < Z_{in} < 10\,T\Omega$). Rule (ii) is associated with the extremely high open-loop gain A (typically: $10^5 < A < 10^6$ range) of OAs since under non-saturation conditions, it is always $v_o = A(v_+ - v_-)$.

The applied potentiostatic mode is selected through switch S_{PM} (Fig. 1). With S_{PM} in position P_{2E} the 'two-electrode' mode (main operating mode) is selected. In this mode no reference electrode (RE) is required and the counter electrode (CE) acts like a quasi-reference electrode, provided that only low-currents are measured and its surface is much larger than that of the working electrode. Under these conditions the electrochemical potential of CE remains practically constant. The potential of the polarizable working electrode can be easily adjusted against the CE potential.

The potential chosen for the working electrode E_{WE} is developed at the output of the DAC and it is applied at the non-inverting input of OA1, which acts as a current-to-voltage (i/V) converter. According to rule (ii), OA1 develops at its output a potential E_O that (through the feedback resistor R_f) makes the potential at its inverting input also equal to E_{WE}. Therefore, the potential applied to the working electrode versus the potential applied to counter electrode is also E_{WE}.

According to rule (i), the total current flowing through the cell (i_{CELL}) passes through the feedback resistor R_f of OA1, therefore

$$i_{CELL} = \frac{E_{WE} - E_O}{R_f} \quad \text{or} \quad E_O = E_{WE} - i_{CELL} R_f \tag{1}$$

Finally, due to the differential input mode used by the ADC, the potential digitized by the ADC is equal to the difference ΔE:

$$\Delta E = E_{WE} - E_O = E_{WE} - (E_{WE} - i_{CELL} R_f) = i_{CELL} R_f \tag{2}$$

Hence, the signal registered by the microcontroller is proportional to the cell current. According to the instructions given by the manufacturer, the presence of a 10 nF capacitor in the inputs of the ADC could be a factor of improving the stability of the latter; however, in the proposed analyzer only slow input signal changes are expected, so the use of filtering capacitors is not indispensable.

The i/V gain can be adjusted by selecting the feedback resistor R_f. In the present design, the following R_f values can be selected: 1.00, 10.0, 100 and 1000 kΩ, corresponding to i/V gains: 0.001, 0.01, 0.1 and 1 V/μA. For achieving the highest possible precision over a wide dynamic range of i_{CELL}-values the analyzer is equipped with the capability of auto-scaling, i.e. the software through the analog multiplexer S_{MUX} automatically selects the maximum R_f that does not overload OA1. Current ranges were evaluated by comparing the experimental current output (i_{exp}) for a specific resistor ($R_{standard}$) under a given voltage ($V_{applied}$), with the expected theoretical value ($i_{th} = V_{applied}/R_{standard}$). Over the proposed current ranges (50 nA–2 mA), relative error $RE = [(i_{exp} - i_{th})/i_{exp}] \times 100$ was lower than 0.05%. It is important to mention that the auto-scaling feature can be found only in a few, enhanced performance bench-type potentiostats [17]. Auto ranging prevents repetitive measurements on the same sample on trying to find the best current range by the 'trial-and-error' method, reducing thus the measurement time.

The primary potentiometric mode intended for the analyzer was the 'two-electrode' one, a mode that can be advantageously applied to miniature electrolytic cells. Still, a 'three-electrode' potentiostatic mode is often desirable, particularly when a well-defined working electrode potential is required and/or relatively high currents are measured over extended periods of time. Under these conditions the counter electrode is liable to polarization, which in turn can cause unwanted shifts of the actual electrochemical potential of the working electrode.

The 'three-electrode' potentiostatic mode is achieved by shifting switch S_{PM} to position P_{3E}. In this case, the counter electrode (now acting as 'auxiliary electrode') is connected to the output of OA2. Now the electrolytic cell acts as a negative feedback element. The non-inverting input of OA2 is grounded and according to rule (ii), the output voltage of OA2 will acquire values that will keep the reference electrode at zero potential (virtual ground) at any moment. Since the potential of the working electrode is E_{WE} and the potential of the reference electrode is zero (both versus the circuit ground) the potential of the working electrode is maintained at E_{EW} versus the potential of the reference electrode, i.e. the potential of the working electrode is fully defined and controlled. According to rule (i), since the reference electrode is directly connected to the input of OA2 practically no current flows through it, therefore this electrode is not subjected to any polarization regardless of the value of i_{CELL}, which is now amply supplied by OA2 through the auxiliary electrode.

The operational amplifiers OA3 and OA4 have been wired as voltage followers and since their output voltage is equal to their input voltage they simply act as buffers. Although their presence is not strictly required, they are used to enhance the overall circuit stability.

2.2. Principles of operation

In an actual experiment, the user selects the applied potential (E_{WE}) along with the equilibration time (1–20 min). The user also enters the number (2–4) of the standard solutions and their concentrations. All inputs are made through the following three keys: (i) 'Up'/'Next' key, (ii) 'Down'/'Previous' key and (iii) 'Enter' key. The user can easily manipulate this keypad, and the possibility of erroneous entries is minimized.

The microcontroller sends the appropriate digital code to the DAC and the desired E_{WE} is applied it to the electrodes. After the selected time interval has been elapsed, a series of 64 equidistant ($\Delta t = 25$ ms) measurements of the i_{CELL} is made. The ADC converts this series with a sampling rate 40 samples/s, the mean value is calculated by the microcontroller, stored and finally associated with the corresponding concentration of the standard used. The whole procedure is repeated for each standard solution, plus once more for the unknown solution. The linear working curve is internally calculated by the least squares linear regression analysis, and then through the calculated and internally stored equation the concentration of each unknown is calculated and displayed on the LCD screen.

All the experimental parameters (E_{WE}, equilibration time, number of standards, the cell current values and the concentrations) are stored automatically in the EEPROM in order to be retrieved by a PC through the RS-232 if necessary. Once the calibration curve has been internally calculated, the analyzer allows the storage of up to 100 measurements, as long as the user does not choose to re-calibrate the system.

3. Software description

3.1. Stand-alone mode

The microcontroller has been programmed using a specially developed code written in assembly language. The flowcharts of the main program, as well as the flowcharts of the system calibration and of current measurements routines are shown in Fig. 2.

The basic steps of the main program (Fig. 2a) are the following:

- Steps 1–3: The system initializes the RAM to ensure that no errors will occur by existing data of a previous measurements session. The registers used in the assembly code and all hardware devices (ADC, DAC, EEPROM, multiplexer, LCD) are initialized.
- Step 4: The program asks the user whether the amperometric analyzer will operate as a stand-alone device or interfaced to a personal computer. In the latter case the computer program (in LabVIEW) acquires all stored measurements data for further processing.
- Steps 5–6: Provided that a stand-alone mode has been chosen, the user is asked to input the experiment parameters (potential, equilibration time). The system starts to measure the current value, derives pair of values (C, I) for each standard solution and the linear regression data are calculated and internally stored. More details of step 6 are given in the flowchart shown in Fig. 2b.
- Steps 7–11: The procedure is repeated for the unknown solution, and the current value is measured. The microcontroller calculates the unknown concentration, by means of the equation of the straight line, and displays its value on the LCD. All the data is stored to the EEPROM and appropriate warning messages are issued in case of invalid (out of range) current measurements and in case of exceeding the available EEPROM capacity (after 100 measurements). More details of step 7 are given in the flowchart shown in Fig. 2c.

3.2. Connected to a PC

The amperometric analyzer can be connected to a PC through an RS-232 port. Stored data in the internal EEPROM are sent to the PC in order to be processed thoroughly in LabVIEW (National Instruments, USA) using a graphical interface that was developed for this purpose.

The graphical interface allows the user to evaluate visually all the measurements of the experiment that has taken place. A typical view of the developed graphical interface (front panel of the virtual instrument) is shown in Fig. 3.

As can be seen in Fig. 3, the front panel is separated in three sections: (i) the 'User Preferences' section, where the user can select the PC serial port that the potentiostat has been connected to and the measurements session for evaluation, (ii) the 'Experiment Conditions' section, where the potential selected, the concentrations of the standards and the corresponding cell current values are displayed and (iii) the 'Graph' section where the calibration curve, the straight-line equation and the correlation coefficient are presented. Given that the PC can handle more significant digits during mathematical calculations, some improvement on the precision of the unknown concentrations can be achieved.

4. Results and discussion

4.1. Analytical performance

The analytical performance of the amperometric analyzer was evaluated by using ascorbic acid as a model analyte. All measurements were made in the CV-2 voltammetric cell (BAS, USA) using a glassy carbon working electrode (BAS), polarized at +0.65 V, and a graphite electrode (6.5 mm diameter, Ringsdorf-Werke, Germany) as an auxiliary one. The supporting electrolyte was a 50 mM phosphate buffer pH 5, in 0.5 M KCl.

The accuracy of the current readings was evaluated by comparing the current measurements obtained by the proposed amperometric analyzer with those obtained by a com-

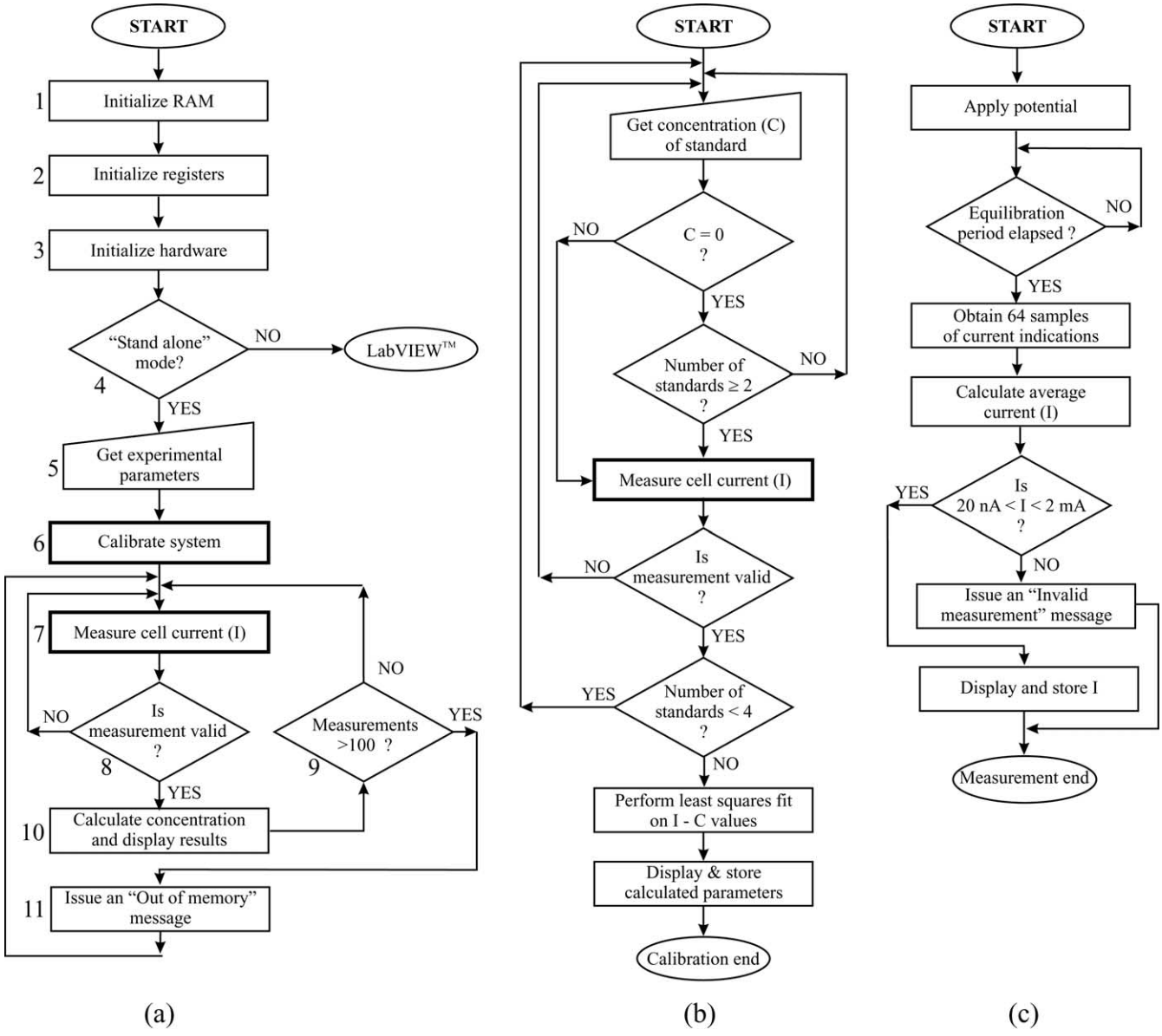

Fig. 2. Flowcharts of the control program of the amperometric analyzer: (a) main program; (b) system calibration routine; (c) measurement of cell current routine.

mercial electrochemical system (Autolab PSTAT10, Eco-Chemie, The Netherlands), using the same standards solutions of ascorbic acid. Excellent agreement was attained, with a mean relative discrepancy of only 0.4%.

The philosophy of the construction was to develop an analytical device able to perform in-field measurements. Such kind of assays often demands fast measurements, even at the cost of a lower accuracy. For example, if we wish to have a rough estimate of the concentration level of a marker within less than 5 min, we can choose the two-standard solution calibration mode and an equilibration time of 1 min. On the other hand, if a better accuracy is needed, a four-standard solution calibration mode is preferable. The equilibration time ranges between 1 and 20 min, since the optimum time interval is strictly dependent on the experimental conditions (size of the working electrode, stirred or quiescent solutions, concentration level of the analyte of interest) of each particular assay.

Using the two- and four-standard solutions calibration mode and a 2 min equilibration time, four different concentrations of ascorbic acid over the wide concentration range 0.025–25 mM were determined. As can be seen in the results shown in Table 1, using a two-standard solution calibration mode is possible to measure ascorbic acid with a relative error of $6 \pm 1\%$, which is acceptable for many industrial applications. Performing the same set of experiments with the four-standard solutions calibration mode, the corresponding relative error was 2.5%. The relative standard deviation of the measurements, for a standard solution of 0.25 mM ascorbic acid was found 0.85% ($n = 15$). The analytical applicability of the amperometric analyzer was also tested in combination

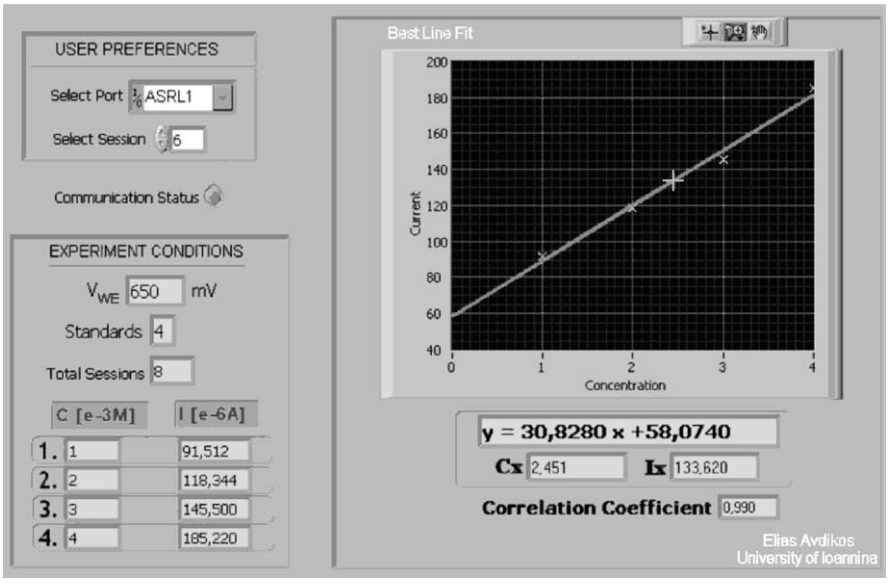

Fig. 3. The graphical interface (front panel).

with a previously reported electrochemical sensor developed in our lab for the determination of ascorbic acid in pharmaceutical tablets [18]. Prior to the measurement step, one tablet was directly dissolved in the supporting electrolyte solution and diluted to 1 L. The analytical results are included in Table 1. Comparing the results received with the proposed potentiostat with those obtained by the official titrimetric method with DCPI, a good agreement was achieved. The mean relative error was 2.2%.

4.2. Features and specifications

Main features and characteristics of the proposed analyzer are as follows:

- current ranges: 50 nA to 2 mA in four ranges;
- current accuracy: ±0.05% of current range;
- electrode connections: two or three;
- potential ranges: two-electrode mode, 0–2 V (step 1 mV);
- three-electrode mode, ±1.024 V (step 1 mV);
- calibration using 2, 3 or 4 standard solutions;
- data storing in EEPROM for further later analysis;
- calculation and storage of up to 100 samples;
- display: 2 × 16 characters LCD;
- keyboard (user's interface): three-key keypad;
- power supply: 220 V AC or 9 V rechargeable battery;
- operation lifetime: 8 h (using a 110 mA h battery);
- interfacing: RS-232 (including LabView-based software);
- dimensions: 9.1 cm × 11 cm × 3 cm.

Table 1
Analytical results for the determination of ascorbic acid in standard solutions and pharmaceuticals tablets

Calibration mode	Added ascorbic acid (mM)	Found[a] ascorbic acid (mM)	Relative error (%)
Two-standard	0.0250	0.0233	−6.8
Four-standard	0.0250	0.0241	−3.6
Two-standard	0.250	0.234	−6.4
Four-standard	0.250	0.246	−1.6
Two-standard	2.50	2.32	−7.2
Four-standard	2.50	2.45	−2.0
Two-standard	25.0	23.7	−5.2
Four-standard	25.0	24.3	−2.8
Four-standard	2.32[b]	2.27[c]	−2.2

[a] Average of three runs.
[b] Cal-C-Vita tablets.
[c] Titrimetric method with DCPI [18].

4.3. Future improvements

The analyzer could potentially be updated, as follows:

(i) By updating the *i*/*V* converter: This could happen by using a different multiplexer in order to select the appropriate resistor during the *i*/*V* conversion. This multiplexer would have better specifications meting thus the characteristics of an ideal switch ($R_{off} = \infty$, $R_{on} = 0$). Also, instead of a multiplexer, some field effect transistors (FETs) could be used as electronic switches. A third solution could be the replacement of the four-resistor autoscale with a digitally controlled potentiometer connected as a feedback resistor in the *i*/*V* converter.

(ii) By updating the analog to digital conversion stage: The use of an ADC with better specifications would minimize any errors to occur during the conversion of an analog voltage value to a digital one. In addition, the incorporation of a Programmable Gain Amplification Unit between the *i*/*V* converter and the ADC, would assist the amplification of the voltage value going to be converted in a digital value (most of the commercial ADCs introduce some errors when the input voltage value is extremely low).

5. Conclusions

The contemporary technology of electronic components allows the miniaturization and the construction of low-cost battery operated analytical instruments with embedded calculations capability. Such instruments can effectively substitute their more versatile and costly bench-type equivalents, especially for routine analytical applications. The amperometric analyser presented here can be used in place of other research-grade electrochemical instruments and is capable of producing the same analytical results despite its hundred-fold lower cost.

The analyser described here can be further improved by utilizing the new generation of microcontrollers and their associated components that allow more versatile programming, e.g. by adding the capability for obtaining measurements fulfilling predefined stability criteria, and for performing floating point calculations. Also, modern coloured miniature LCDs can be used as screens for displaying high-resolution graphics, making unnecessary the transfer of data to PCs for a more elaborate treatment.

Acknowledgements

Authors wish to thank Emeritus Prof. M.I. Karayannis, Dr. C.D. Stalikas (LAC) and Prof. F.A. Triantis (HEP-LAB) for their valuable contribution to the completion of this work.

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Biographies

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Constantinos E. Efstathiou received his BSc and PhD degrees in chemistry from the University of Athens, Greece in 1971 and 1976, respectively. He is a Professor of chemistry and he is currently the director of the Analytical Chemistry Laboratory of the Department of Chemistry in the University of Athens. He has 80 scientific publications on topics including electroanalytical techniques (ion-selective electrodes, stripping voltammetric techniques, modified electrodes), kinetic methods of analysis, chemometrics and applications of microcomputers in the automation of various analytical techniques. He is author and co-author of books on general Analytical Chemistry, Electroanalytical Techniques, Chemical Instrumentation and Microcomputers, and Chemometrics.