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Optical sensor with liquid crystal sensitive element for amino acids detection

Abstract. The sensor of amino acids has been investigated. Ranges of linearity have been established on dependences of the wavelength of minimum transmission of optical radiation on the concentration of amino acids.

Streszczenie. Badana jest możliwość wykorzystania materiałów ciekłokrystalicznych jako czułego elementu optycznego czujnika aminokwasów. Ustalono granice liniowości na zależności długości fali minimalnej transmitancji promieniowania optycznego od stężenia aminokwasów. Sensor optyczny z elementem ciekłokrystalicznym do wykrywania aminokwasów

Keywords: optical sensors, monitoring amino acids, biological objects.
Słowa kluczowe: sensory optyczne, monitorowanie aminokwasów, obiektów biologicznych.

Introduction

Recently, the problem of monitoring amino acids in various biological objects, for example, in blood serum and food became actual. Such an analysis is necessary for the study of the impact of medicines, control of biological processes, the production of pharmaceuticals such as biologically active additives, amino acid mixtures and vitamin complexes. Predominantly, biosensors [1-3] are used for the investigation of amino acids. However, existing methods for monitoring amino acids are complex, bulky and require skilled personnel [4-7].

The sensitive element for amino acids monitoring

One of the solutions of this problem is to use optical sensors with liquid crystal (LC) sensitive element for amino acids monitoring. Next, we consider the characteristics of research objects (asparagine, valine, methionine).

Asparagine ($C_4H_8N_2O_3$, 2-amino-3-carbamoyl-propanoic acid) is one of the twenty standard amino acids that appear in the proteins composition. Asparagine is not an essential amino acid for humans as it can be synthesized from glutamine using asparaginase as a catalyst. Asparaginase cleaves asparagine in blood serum to aspartic acid and ammonia. Predominantly, asparagine is found in protein products, it is an important factor in increasing the body resistance. Aspartic acid salts are used in athletes diet when they undergo high physical activity. A number of asparaginase-based biosensors make it possible to determine the concentration of amino acids in the range of 10^{-1}-10^{-10} M [3, 8, 9].

In articles [10, 11], in order to determine the concentration of asparagine in blood plasma in patients with acute lymphoblastic leukaemia, a biosensor with a sensitive element based on plant asparaginase has been used. This biosensor is able to determine the concentration of asparagine in the range of 10^{-1}-10^{-10} M.

In paper [12], a sensor with an ion-selective field-effect transistor as a sensing element was used for measuring the concentration of L-asparagine. The transistor control scheme was presented. The sensor allows measuring the concentration of L-asparagine within the rage of 10^{-2}-10^{-6} M.

Polyimide based amperometric biosensor was developed for the in vitro determination of L-asparagine in serum samples for monitoring L-asparagine levels in leukaemia. Polyimide was used as an immobilized enzyme membrane. The detection limit of the developed biosensor is 10^{-6}M. [13]

Methionine ($C_5H_{11}NO_2S$, (S)-2-amino-4-(methylsulfonyl)-butanoic acid) is a poorly soluble α-amino acid present in many proteins. Methionine promotes tissue growth and is also needed to maintain the required level of nitrogen in the body. It plays an important role in the metabolism due to the presence of a mobile methyl group H_3S in its molecule. Methionine is used to treat liver toxic lesions and prevent its diseases. It reduces the concentration of cholesterol in the blood and increases the phospholipid level.

In article [14] genetically-encoded FRET-based nanosensor for methionine detection was developed. Genetically-encoded fluorescence resonance energy transfer (FRET) sensors represent a promising technology for measuring metabolite levels and corresponding rate changes in live cells. Dynamic limit of the detection range of the proposed nanosensor and mutants is 7.0μM to 5.0mM.

In paper [15] fluorescence-based methionine biosensor was developed for detection of specific metabolites in single bacterial cells. The sensor allows measuring the concentration of methionine within the rage of 0.2-23.5 mM.

Valine ($C_5H_{11}NO_2$, (S)-2-amino-3-methylbutanoic acid) is soluble in water crystalline powder. It promotes the regeneration of muscle fibres and injured tissues, supports the exchange of nitrogen in the body, participates in the regulation of nerve processes, stabilizes the hormonal background, prevents serotonin level decreasing. Valine is one of the main ingredients in the growth and synthesis of human body tissues. Together with leucine and isoleucine, it is a source of energy in muscle cells that increases muscle coordination and alleviates the body's sensitivity to pain, cold, heat etc.

Described in article [14] biosensor allows measuring the concentration of intracellular valine within the rage of 1.5-23.4 mM. In paper [16] a novel optical sensor based on TiO_2 nanoparticles for Valine detection was developed. The sensitive layer was formed by a porphyrin coating on a TiO2 nanostructured surface. The photoluminescence spectra of this layer varied depending on the concentration of valine in the aqueous solution.

We investigated the spectral characteristics of the LC mixture with amino acid admixtures in the visible range in order to use these mixtures as a sensitive element of optical amino acid sensors. The basis of such sensitive elements is the selective light reflection, which is one of the properties of cholesteric-nematic mixtures of LC. Under the influence of various kinds of bio objects [17, 18], in particular, amino

acids [19], the wavelength of the selective reflection maximum of the LC changes.

The sensitive element consists of cholesteric liquid crystal BLO-62 and a nematic liquid crystal 5CB. The chosen cholesteric-nematic mixture has a minimum of optical transmittance at 520 nm at room temperature. The pure medical substances (asparagine, valine, methionine) or their standard distilled water solutions was added to this mixture. A cholesteric liquid crystal with an admixture of amino acids or their aqueous solutions was treated in an ultrasonic bath in order to obtain a homogeneous solution. The solution composed of CLC and the amino acid was deposited on the glass substrate without alignment layer and placed in the chamber for spectroscopic studies. The study was conducted at room temperature.

It should be noted that high temperatures of melting of amino acids, in particular, 220 °C for asparagine, 280 °C for methionine and 315 °C for valine prevent their proper dissolution in liquid crystal medium. Micro images obtained with optic microscope show that undissolved crystals of amino acids, distributed in a pattern, are distinctly seen in a transitional area of concentrations in solutions of amino acids in liquid crystal media.

Analysis of the obtained results. Fig.1 presents the dependence of wavelength of minimum transmission of optical radiation through liquid crystals on the concentration of crystalline powders of asparagine, valine and methionine. Two areas are observed on dependences: on the first (linear) area, the minimum band of selective transmittance is displaced into long wave area with an increase in the concentration of amino acids. On the second area, wavelength does not change with the increase in the concentration of amino acids.
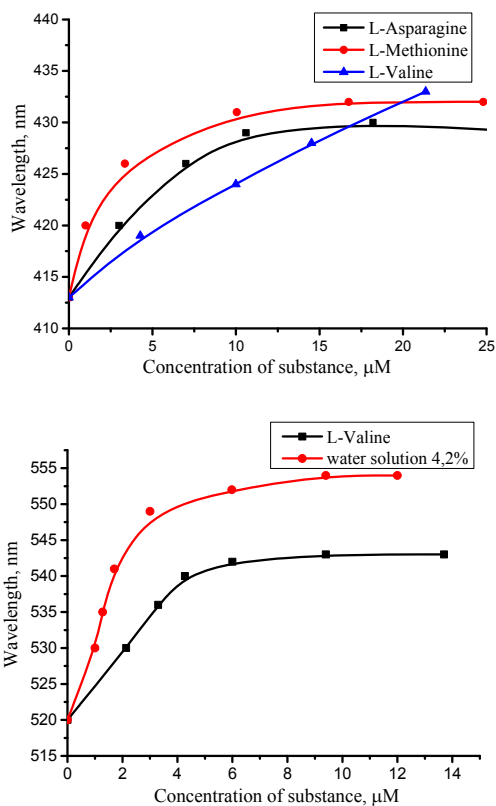

Fig.1. Dependence of wavelength of minimum transmission on the concentration of amino acids: a – valine, methionine, asparagine in matrix BLO-62; b – crystalline valine and its aqueous solution in matrix BLO-62+5CB
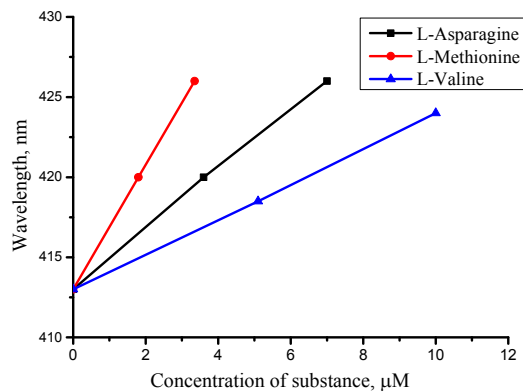

Fig.2. Range of linearity of changes in wavelength of minimum optic transmittance of liquid crystal matrix on concentration of amino acid

As a result of mathematical processing of experimental data, range of changes in concentration of amino acids has been established, in which the function of transformation has linear character. The range of measured concentrations can be somewhat extended considering transitional area of characteristic from linear area to saturation, however, the accuracy of measurement is diminished [20, 21].

Similar dependences for aqueous solutions of amino acids are presented in fig. 1b taking valine as an example. The character of dependences does not change; however, application of aqueous solutions enables detection of amino acids in lesser concentrations than in pharmaceutical drugs. Besides, the application of mixtures BLO-62 and 5CB significantly increases the sensitivity of measurement. Application of cholesteric liquid crystal increases the value of maximum measured concentration (within linearity of characteristic).

The obtained spectral dependences are viewed from the point of relationship of molecules of cholesteric liquid crystals with molecules of amino acids and cholesteric liquid crystals with aqueous solutions of amino acids.

A chiral atom of carbon is present in the structure of amino acids, thus, they can counteract with cholesteric liquid crystal. Thus, amino acids are optically active admixture and, being added to the structure of a cholesteric liquid crystal, resulting in an increase in helix pitch distance. The structure of amino acid molecules is predominantly linear, planar and resembles spiral structure of cholesteric. Amino acids in aqueous solution exist in the form of zwitterion. In such form, proton is displaced from carboxyl group to nitrogen, which receives partially positive charge, whereas carboxyl group is partially negative. Thus, amino acids in solution in zwitterion form counteract better with liquid crystal than amino acids in the form of crystals. However, when pH is within the range 2.2-9.4, amino acid usually contains both negative carboxylate and positive group of α-ammonium and has zero charge.

Fig. 2 shows the dependence of wavelength of minimum optic transmittance on the concentration of amino acids (valine, methionine, and asparagine) within the linearity range. Given dependences were used during the development of an optical sensor of amino acids.

Schematic realization of an optical sensor of amino acids

According to the results of the research, an optical micropower sensor of amino acids based on the PSoC microcontroller was developed. This microcontroller has significant hardware resources that allow realizing an optical sensor with minimal additional peripheral elements.

Flexible internal microcontroller architecture makes it possible to create high-sensitivity optical sensors using synchronous detection methods [20]. Much of the digital analysis of an informative sensor signal is made using the developed application software. Mathematical methods of linear approximation of dependence of spectral characteristics on the concentration of investigated amino acids are used for statistical analysis.

The practical realization was performed using the developed optical unit, which consisted of an optical radiation source and a photoelectric converter [17]. The basic set PSoC 5LP and an optical unit were used for accomplishing preliminary research.
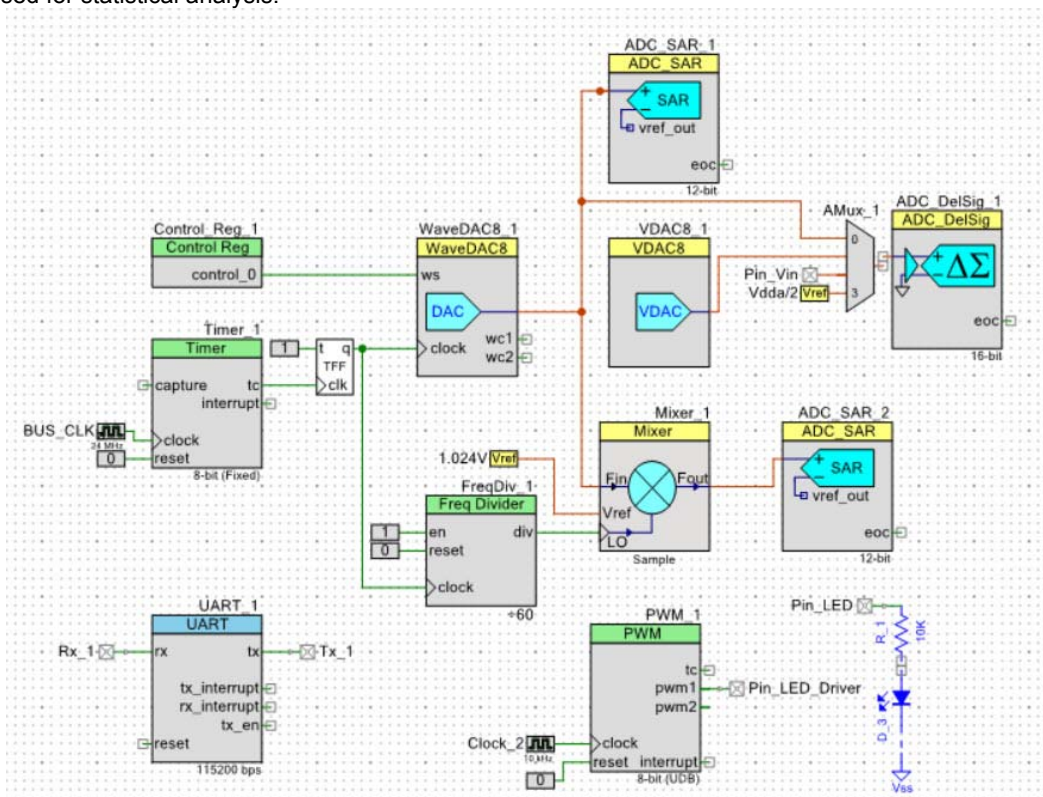

Fig.3. Configuration of the internal elements of the controller

The working principle of sensor based on analyzing the informative signal of the photoelectric converter. A microcontroller, whose digital ports use pulse-width modulation (PWM), controls the light source. The spectral characteristics of light are changed after passing through the sensitive element of the optical unit. These changes are recorded by a semiconductor photodiode.

Fig. 4. The external interface of the user during calibration of the sensor

The process of synchronous detection of an informative signal is performed using internal elements of the microcontroller. Configuration of the internal elements of the controller is fulfilled using the software PSoC Creator. The developed general structure of the sensor is presented in Fig. 3. For optical sensor implementation the following elements are used: 16-bit timer (timer_1) with TFF register, input control register, DAC (DAC8), successive approximation register (ADC_SAR), multiplexer (AMux), delta-sigma ADC (ADC_DelSig), serial interface (UART), frequency divider (FeqDiv) and pulse width modulator (PWM). The timer provides synchronization of the operation of all device nodes and is used to implement a synchronous detector based on the analog switch.

Application software was developed for informative signal analysis. Application software provides calibration of the sensor and input signal processing according to the selected mode. Initially, depending on the type of measured amino acid and the level of external light, calibrations are performed. In accordance with the calibration results, the recalculation and verification of the obtained signal values with the corresponding values of the control data arrays are fulfilled [18]. As a result of comparisons, the level of concentration is determined. The external interface of the user during the calibration of the sensor is shown in Fig. 4.

This approach makes it possible to develop a number of micropower optical sensors for other biomaterials concentrations determination.
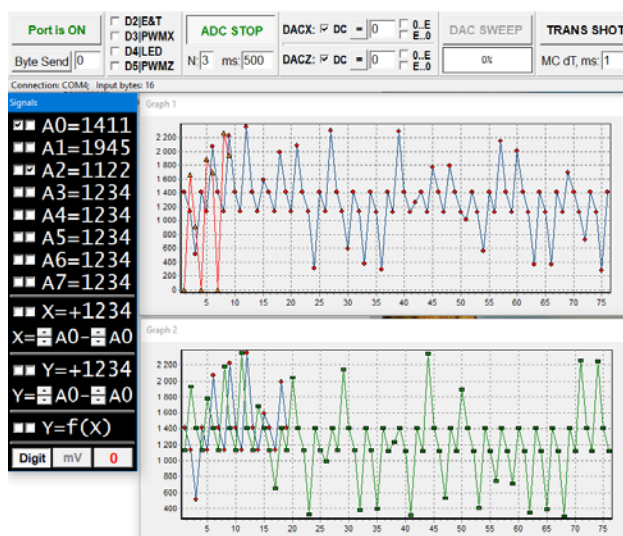
Conclusions

The sensor of amino acids has been investigated. Ranges of linearity have been established on dependences of the wavelength of minimum transmission of optical radiation on the concentration of amino acids.

It has been shown that the use of aqueous solutions enables detection of amino acids in lesser concentrations than in pharmaceutical drugs. It has been established that the sensitivity of measurement significantly increases in case of application of mixtures of Possibility of using liquid crystal substances as a sensitive element of optical cholesteric liquid crystal and high-polarity component. However, elimination of high-polarity component from this mixture increases the value of maximum measured concentration (within linear characteristics).

The approach of analysis and processing of the information signal of an optical sensor is proposed. This approach provides the high accuracy of measurement of the concentration of amino acids and allows sensor calibration in accordance with external interference.

The implementation of synchronous detection methods in the informative signal processing provides high accuracy and resistance to external interference.

The proposed schematic solutions for an optical sensor development based on the PSoC microcontroller allowed the implementation of a compact micropower device with a wide range of applications.

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