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Algorithm of microchip operation for controlling parameters of a latex agglutination reaction

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Abstract. In the given work the algorithm of work and structure of microchip construction, intended for kinetics researches of formation of modular complexes is considered during the reaction of latex agglutination. The physical principles of registration of enzyme adsorption isotherms on latex by method of light scattering are considered. The results of the reaction of adsorption of protacrin enzyme on hydroperoxide latex's are shown.

Keywords: latex, adsorbed protein, antigen-antibody reaction, light scattering methods, microchip.

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1. Introduction

A significant recent interest to development of new methods of quantitative determination of contents of fibers in a solution is noted. For this purpose, a broad possibility is given by devices with the operation principle based on the surface plasmon resonance. This method of deriving information about the amount of adsorbed protein on a surface of metal consists in registration of angular dependence of light reflection ability for metal surface on the boundary with protein adsorbed on it.The given method is applied for the development of immune analyzers during researches of antigen-antibody reaction kinetics. This method allows to selectively determine availability of a given type protein in a solution. As marker selective molecules - antibodies that specifically interact with molecules of protein of the certain type are used. Construction and principle of operation of biochip of the indicated type and outcomes of a research of fibrinogen adsorption kinetics on a surface of polycrystalline gold by a method on the surface plasmon resonance are described in [1,2].

In our work results of researches on a possibility of biochip creation for monitoring an immune reaction antigen-antibody by a method of latex agglutination are considered. The reaction of latex agglutination can be examined as process of protein adsorption on monodispersed polymeric latex.

Adsorption process of polymers on a surface is characterized by parameter adsorption isotherms, that is isotherms of structure modification of solution [3]. For the two-component systems the process of adsorption functionally can be described by two parameters: the quantity of moles of initial substance adsorbed by the quantity of moles of adsorbent. Depending on their ratio, a character of curves, which characterize functional dependence of adsorption process, is determined as adsorption isotherms. The parameter for a quantitative evaluation of the kinetics of latex agglutination reaction can bo measured by a change of intensity of a light beam in solution. For the practical application of a latex agglutination method for the selective analysis of proteins in a solution it is necessary to decide a number of problems:

- development of a technique of the quantitative analysis of protein molecules of the certain kind in a solution;
- development of a technique of the simultaneous quantitative analysis of protein contents in a mixture of two or more components in a solution;
- development of a technique of the selective protein analysis of a specific type from a mixture of components of unknown structure.

The given work is directed on a practical solution of the delivered problems. There will be represented results on creation of a tool method - microchip for the solution of the first from the delivered problems.

2. Building adsorption isotherms

The reaction of latex agglutination and coagulation process interpreted as a second order reaction. The process of light scattering by colloidal aggregates are formed from primary particles and intermicellar liquid have covered the following modified of the Rayleigh equations [4,5]:

$$I = I_o \frac{9\pi^2 [vfN_0 M] n^4 \left(n_1^2 - n^2\right)^2 \sin^2 \alpha}{\lambda^4 r^2 V_s [N_A \rho (1-\varphi)]^2 \left(n_1^2 + 2n^2\right)^2} \tag{1}$$

where I denotes the intensity of incident beam light of wavelength λ, I_o is the intensity of light scattered in direction of angle α at distance r, n and n_1 are refraction indices of the medium and the particle respectively, N_A is the Avogadro constant, v denotes volume of aggregates, V_s is a volume of scattering system, N_o is a total number of macromolecules or primary particles in a volume V_s, M is a relative molecular mass macromolecules or primary particles, φ the ratio of intermicellar volume to total volume of the scattering particles, f the correction factor for polydispersity, ρ the density of primary particles. When entering parameters for determining numbers of particles involved in the coagulation process, namely: N_a as a number of aggregates in the volume of scattering system, N_p as a number of primary particles in the aggregates, $N_{p\infty}$ as a maximum number of primary particles taking part in coagulation, then the tot al number of primary particles N_0 in the volume of scattering system as

$$N_0 = N_a N_p , \tag{2}$$

(as suggested in [4]) the Rayleigh equation (1) can be written in the form

$$\frac{I}{I_\infty - I} = \frac{N_p}{N_{p\infty} - N_p} , \tag{3}$$

where I_∞ is an intensity of light scattering after completion of coagulation. The Eq. (3) shows that the speed of change of scattered light intensity is proportional to the speed of change in the number of primary particles that take part in the reaction; hence, it can be used to quantify the reaction of latex agglutination.

Considering the process of adsorption of protacrin enzyme on hydroperoxide latex as a latex agglutination reaction, isotherms of the adsorption can be built as follows. Let us introduce a parameter $tg\beta$:

$$tg\beta = \frac{N_p}{N_p - N_{pm}} , \tag{4}$$

where N_p is a quantity of primary particles which are centers of the adsorption, and N_{pm} stands for quantity of primary particles in points, where the temporal dependence of change of scattered light intensity during the adsorption reaction reaches its extreme (maximum). The first derivative of the mentioned functional dependence by time in such point is an average speed of change of primary particles. Knowing the maximum and time when it is reached, it is possible to calculate the quantity of primary particles in given extremal point. In the experiments on the adsorption of enzyme on latexes, the quantity of primary particles, e.g. concentration of latex in the solution, is known. When adding the solution of protacrine enzyme with chosen concentration C_i^{fm} to the solution with selected latex concentration C^{lt} and registering changes in time of the intensity of light dispersion, it is possible to find the extremal points. Further, with use of Eg. (4), it is possible to calculate $tg\beta$ and to construct functional dependence:

$$tg\beta = f\left(C^{lt} = const, C_i^{fm}\right), \tag{5}$$

where i is times of processes for enzyme solving.

Changing the latex concentration in the solution and investigating changes in time of the light intensity for each concentration with chosen titres of enzyme solving, it is possible to build the functional dependence:

$$tg\beta = f\left(C_j^{lt}, C_i^{fm}\right), \tag{6}$$

where j is times of latex solving processes. The experimentally obtained set of curves, described by (6), can be considered as set of isotherms of the adsorption of protacrin enzyme on latex.

3. The reaction of latex agglutination of protacrine determination

The research on the protacrine enzyme adsorption was conducted in water solutions of hydroperoxide latex with mass concentrations 8.83%, 4.47%, and 2.21% with the use of device described in [6]. In clean dish with 2.8 cm^3 volume, 1.8 ml of latex with given concentration was dropped with pipette, and the background spectrum of light scattering was registered during 5 minutes. Obtained data were recorded in device memory and 1 ml of protacrin enzyme solution of given concentration with chosen titre of solving was added to the dish. For that, preliminary dried protacrin enzyme was solved in distilled water until the concentration reaches 2×10^{-4} g/mole, and titres of given solutions 1, 2, 3, 4 were prepared. The time changes of light intensity spectra in the course of enzyme adsorption on latex were registered during 30 min. It was assumed that during this time the enzyme preserved its activity. The data about the time changes of light intensity, taking into account the background scattering of latex solution, were registered into the database and were visualized on the screen as graphic dependencies. Using these graphic dependencies, the quantity of latex particles was determined in points, where the speed of change of scattered light intensity gains its maximum. From the obtained data, according to (5), the isotherm of adsorp-

tion for given concentration of latex solution was built. Fig. 1 demonstrates the time dependencies of the change of intensity of adsorption of enzyme in the solution of hydroperoxide latex with concentration 2.21% with solving titers 1, 2, 3. As we can see in the figure, the time dependencies of light dissipation intensity do not have extremes, therefore the value N_{pm} was equal to the quantity of primary particles registered after the reaction end. In this case, it corresponded to the concentration of latex particles at the 30th minute of measurements. With the use of known values of scattered light intensity at the beginning and the 30th minute of the adsorption reaction, according to the Eq. (3) and (4), an adsorption isotherm was built. Fig. 2 shows adsorption isotherms with latex concentrations 8.83%, 4.47%, and 2.21%, obtained by the above method. As can be seen from Fig. 1, increasing the enzyme solving titer led to decrease of the scattered light intensity and increase of steepness of time change curves. This result can be explained taking into account that during the reaction with equal volumes of input substances the latex concentration remains constant. Enzyme dilution is equal to an increase of primary scattering centers, which, accordingly to (3), should result in decrease of scattered light intensity at the reaction beginning, which the experiments showed.

4. Structure and implementation

One of basic shortage of traditional measuring devices and systems construction is functional rigidity of the closed-type systems, which parameters are set on a development cycle and resist any optimization while in service. That is why the concept of open systems is applied when developing a function scheme of the intellectual optic sensor analyzer. The concept characteristics are set not on a design stage. They are operatively programmed and are implemented by the user during their operation in accordance with the certain measurement

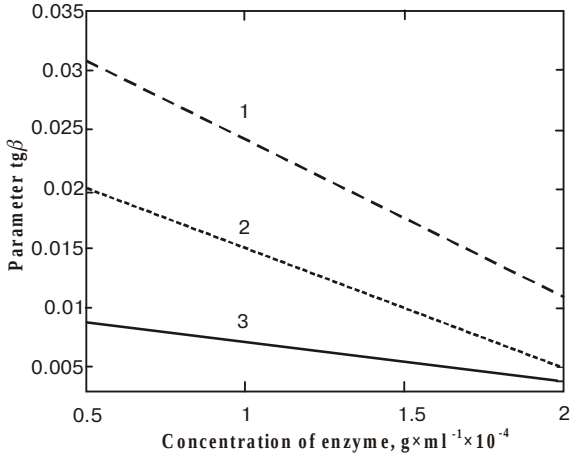

Fig. 1. Dependencies of the time change of intensity of adsorption of enzyme in the solution of hydroperoxide latex

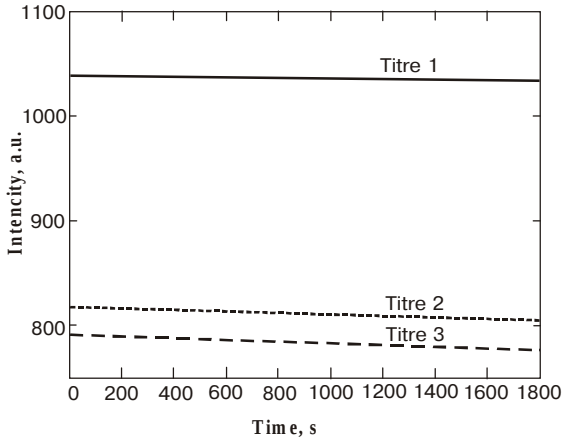

Fig. 2. Adsorption isotherms with the latex concentration 8.83%, 4.47%, and 2.21% accordingly the curves 1, 2 and 3.

mode. The open measuring system creation has the qualitatively new contents that the user receives the flexible tool that can be operatively adapted for various process parameters:

- to configure measuring system structure operatively;
- to operatively change parameters of structure: a dynamic range of measurement, discretization (sampling) period, word length of analog-digital transformation;
- to reprogram the system work algorithm as a whole.

Functional implementation scheme for intellectual optoelectronic microchip you can see in Fig. 3. The optosensor basic unit is an optical cuvette, design of which depends on the geometry of scattering and properties of liquids. The flowing cuvette is used for the mentioned purposes, where it is easy to set the certain minimal number of microparticles in considered volume of the registration zone by adjusting liquid flowing speed. The considered zone is formed in cuvette as a contiguity of a light flow of the semiconductor injection laser and the entrance window of the photodetector. An analyzed liquid is pumped through the cuvette using an automatic batcher. The particles suspended in a liquid, cross the probed zone, causing pulse changes of a light flow proportional to the particle size. The photoreceiver turns the light pulses to electrical signals that are directed to the electronic circuit for following processing.

The intensity of a light flow is smoothly adjusted by the program of source power supply control using a digital-to-analog converter integrated into a microprocessor. The intensity regulation is necessary to compensate light absorption in analyzed liquids with various absorption factors and change sensitivity of the receiver to temperature. The illuminator is placed in the way allowing to reach a maximum of the directional diagram for radiation entering to the registration zone. The shaper of the registration zone creates the rectangular regularly lightened zone in a flow of a researched liquid. The zone comprises the flow completely and has the sizes 100x100

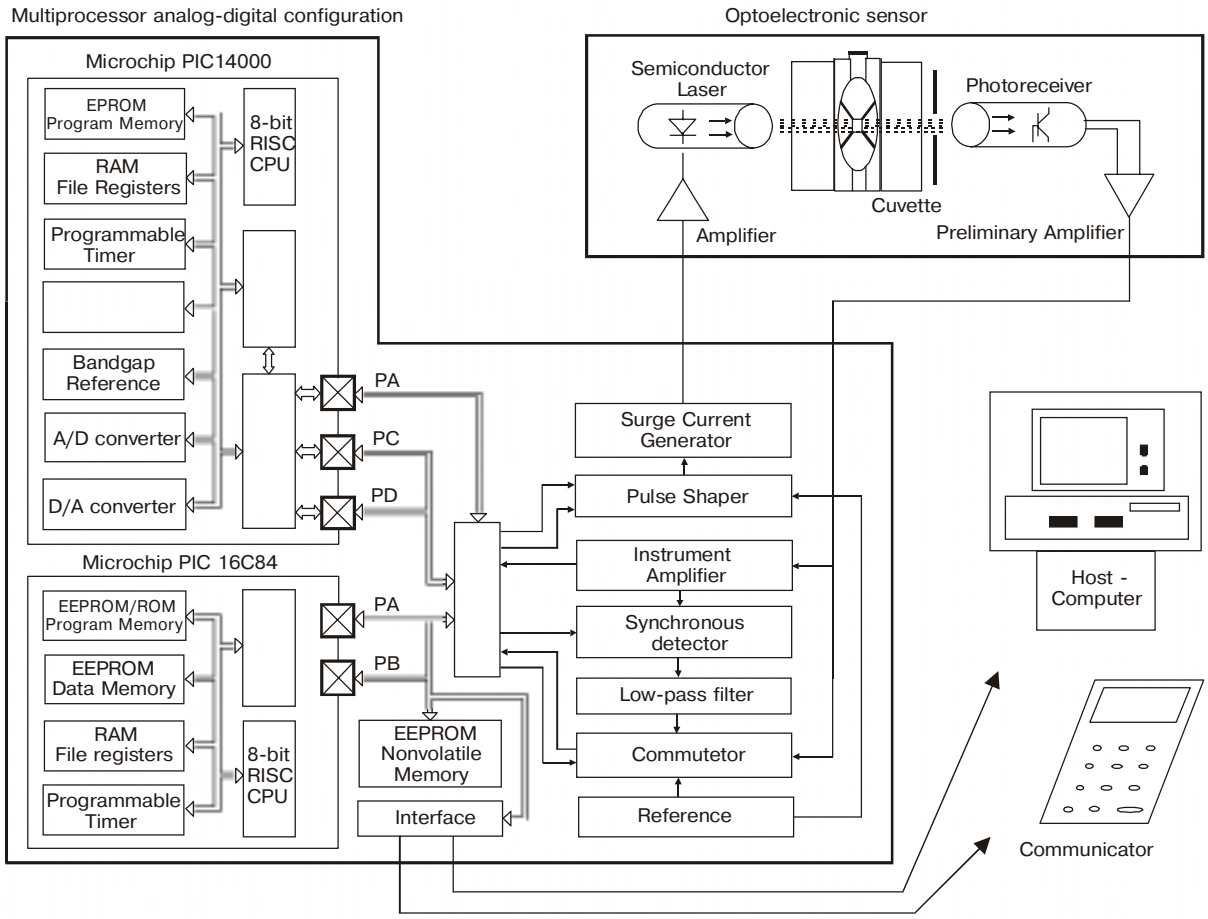

Fig. 3. A function scheme of the microchip with an optoelectronic sensor.

micron. Such zone is created in pair and placed perpendicularly to slot-hole masks, and one of the mask is a capillary wall to a liquid flow. So the registration of microparticles in all volume of a researched liquid is achieved, that raises accuracy of measurement. Small sizes of the registration zone allow to reliably register the particles sized more than 2 microns, because the bottom border of the registered particles size depends on the partition cross section size to the area of a mask window.

Analog-digital processing of the measured signals is provided on the basis of the multisignal RISC-controller of the Garvard type, such as PIC14000 of the Microchip firm. The microcontrollers of the indicated type intended for construction of micropowerful highly effective measuring systems. They also contain all analog and digital components for that purpose. A high degree of integration, functional saturation by analog components and flexibility of their switching program have allowed to produce an effective system of measurements and processing of signals at relative simplicity of the basic circuit. The integration of analog units of integrating ADC directly in a controller crystal minimizes hardware expenses for the process implementation of AD-transformation and reduces influence of external factors of instability (electromotive force -induced handicap, noise of discrete elements, etc.). The methods of program access to these units allow optimal control of analog-digital converting of information signals process. The optimality of management of the transformation process consists in the previous estimation of peak meaning of the measured signal. That signal is the basis for programming such transformation steepness of ADC, that provides a mode of measurement at the end of a scale of the transformation characteristic, i.e. with the maximal separate ability. In order to reduce the time of transformation, the antecedent information on these signals is used at measurement of a number of diagnostic parameters (displacement voltage, factor of transfer). The correction of an absolute error of transformation in a final point of a scale and displacement voltage of zero on an input of the converter is carried out by a digital method by taking away the multiplicate and additive component of an error from the measurement result. The additive error is defined in the first phase of each measuring cycle by submission of a zero signal on an ADC input. The multiplicate error is defined analytically in the second phase of a ADC running cycle by internal switching of an ADC input on the integrated source of a reference voltage.

To control additive and multiplicate errors of external analog units, as well as errors of temperature drift, the procedure of calibration of a path is provided in each measuring cycle. Compensation of errors is carried out by measuring-computing methods using direct processor additional measurements of the following parameters:

- signal of zero potential;
- signal of a source of an external basic voltage;
- signal integrated in the controller of a sensor control of climatic temperature.

The controller PIC16C84 provides program generation of control logic signals and pulse sequences for digital-to-analog units of a measuring path. The lines of ports programmed on a control signals output mode. The lines initiate each cycle of work of the measurement channel, provide synchronous operation of the system as a whole. The codes which set the order of change of control signals are written down in external re-programmed non-volatile device (EEPROM).

The basic software is the integrated package of the subroutines and drivers with the external parameters of initialization. The algorithm of the device work is carried out by a method of serial call of the necessary subroutines together with initializing parameters, according to the order established by the configuration program placed in the Atmel 24C256 flash-memory. Record, control, and updating of the configuration program in non-volatile flash-memory are carried out in one of service operations modes of the sensor, and can be activated by the user through the direct access method usired a programmer, host-computer or communicator. The possibility to make changes in control codes in the non-volatile remembering device allows to change widely the algorithm of work of the whole measuring path, which is significant for implementation of diagnostic subroutines, calibration, and configuration.

The program part is an open system expanded by the user on a level of the cross-linker. The developed basic program module MPLIB is a complex of the interacting programs of the real time scale that are implemented in Assembler for the architectures based on the PIC-controllers produced by Microchip. The modularity and structuredness of the program package secure transparency of the logical structure of all its elements.

By the functional destination, the library of subprograms includes:

- auxiliary programs of the universal destination that are used by other program modules (operations of movement, transfer, logical data analysis, etc.);
- programs of the basic mathematical operations with multidigit binary and binary-decimal numbers with fixed and floating point;
- programs of the polynomial approximation of characteristics of primary and secondary transducers, programs of analytical processing of the measurement results;
- drivers of the program generation of number sequences and time intervals;
- subprogram for reprogramming flash-memory of the controllers;
- communication, service and diagnostic programs.

Operative calling to one or another subprogram of the package, formation of the task algorithm from the called subprograms is carried out by the text or graphic interpreter functioning in the elaborated shell on the basic of the IBM-compatible personal computer. The service working regime provides work with asm-programs on the level of their symbolic names with the parameters of initialization.

The interaction of the gauge with the host-computer or communicator is carried out by standard communication methods (RS232, USB).

There is an implemented program of graphic visualization on the measured data monitor screen and results of their mathematical processing with automatic scaling and user choice of one or another functions as arguments for the comparative analysis. Elaborated are original mathematical methods and computer software for uniform approximation of discrete data by several functional dependences, separately by algebraical and rational polynomials, exponential, logarithmical, power and other nonlinear expressions.

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