

## Testing Phenol Compounds in Spices

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**Abstract:** Cardamom fruits and carnation buds were tested by high-yield liquid chromatography (HPLC) method. 12 phenol compounds were found in cardamom fruits: gallic acid, isoferulic acid, chlorogenic acid, epicatechin, chicory acid, caffeic acid, dehydroquercetin, ferulic acid, luteolin, quercetin, rutin, o-methoxycoumarin, cinnamic acid, in carnation buds - 12 phenolic compounds: gallic acid, catechin, isoferulic acid, chicory acid, coffee acid, dihydroquercetin, ferulic acid, luteolin, quercetin, rutin, o-methoxycoumarin, cinnamic acid. The methods of internal normalization show that gallic acid is prevailing compound in cardamom fruits and gallic acid and quercetin- in carnation buds. Quantity of amount of flavonoids in terms of rutin in carnation buds was measured by differential spectrophotometry. It varies from 3.55 % to 5.10%. Tanning agents in carnation buds and cardamom fruits were measured by permanganate titration, they are respectively 4.11- 5.02 % and 0.02- 0.06 %.

**Key words:** Cardamom % Clove tree % Phenol compounds

### INTRODUCTION

Treatment with phytoenous preparations becomes more and more popular now both in Russian and world practice. Research of phytoenous bioactive substances including food plants as perspective sources of vegetable crude drug is the question of pressing importance.

*Elettaria cardamomum* (L.) Maton is perennial plant of gingery family with large rhizome that forms several herb stems up to 2-3 m high. Now the plant is being cultivated in numerous tropical countries-India, Thailand, Guatemala, on Ceylon and Malay Archipelago. Seeds of ripe fruits extracted right before preparation are used. Cardamom fruits are officinal preparation in many countries [1, 2, 3].

*Syzygium aromaticum* (L.) err. et Perry is evergreen tree of myrtle family with coriaceous opposite smoothed-edge and small purple-pinkish flowers assembled in clusters. Now this plant is being cultivated in many tropical countries. Buds are used – dried flower buds that are officinal preparations in many countries [1, 4, 5].

Analysis of bibliographic data proves that chemical compound of essential oil of raw cardamom [6-11] and clove tree [12-18] is researched to relatively high extend.

The purpose of the work is testing phenol compounds and measuring this group of natural compound in cardamom fruits (*Elettaria cardamomum* (L.) Maton) and carnation buds (*Syzygium aromaticum* (L.) err. et P) as perspective sources of vegetable crude drug.

Methodic. Industrial series of cardamom fruits conforming requirements of GOST 29052-91 “Spices. Cardamom. Technical requirements” and carnation buds conforming requirements GOST 29047-91 “Spices. Carnation. Technical requirements”.

High-yield liquid chromatography (HPLC) by GILSTON, model 305 (France), small injector RHEODYNE 7125 (USA) was used to research qualitative evaluation of phenol compounds of cardamom and carnation raw materials. Research results were processed by software MultiChom for Windows. Metal column with 4,6×250 mm form-factor Kromasil C 18, sorbent particles size 5 mkm was used as fixed phase, the system methanol- ware-concentrated phosphoric acid (400:600:5)- as moving phase. Analysis was carried out in room temperature with eluent supply speed 1 ml/min. Analysis duration was 70 min. Ultraviolet detector GILSTON UV/VIS 151 model was used for detection with 254 nanometers wavelength.

Analyzed sample was reduced to fragments of such size that allows passing through the sieve with openings diameter 2 mm. About 2.5 g of raw material was introduced into retort with the volume 100 ml added 20 ml 70-percent ethyl alcohol. It was attached to reflux condenser and heated in boiling water bath for 2 hours from the moment of simmer of alcohol solution in retort. After cooling the solution was filtered through paper filter in volumetric flask with volume 25 ml. The volume of the substance was extended by 70-percent ethyl alcohol to the mark (analyzed solution). Simultaneously a series of 0.05-percent reference solutions of phenol carbonic acids, flavonoids and coumarin in 70-percent ethyl alcohol were prepared.

Than 50 mcl of analyzed solutions and reference solutions were introduced in chromatograph and were chromatographed according abovementioned method.

Separated substances were identified by comparing time of solution compound retention with time of reference compound retention on the base of not less than 5 chromatograms. Measuring of phenol substances in analyzed samples was made by peak areas by internal normalization method.

Quantitative evaluation of flavonoids in carnation buds was made by differential spectrophotometry after complexing reaction with aluminum chloride. Terms of flavonoids extraction from raw material: granulation, leach and extraction time were analyzed during development of this method. Analysis of UV absorption spectrum of spirit extraction of carnation buds shows that maximum extraction was observed with wavelength 408 nanometers, the same maximum had 0.05-percent rutin solution after complexing reaction with aluminum chloride. So 402 nanometers was taken as analytical wavelength and quantitative measurement of amount of flavonoids in terms of rutin in 3 replications was made with this wavelength.

Bud sample for analysis was reduced to fragments of such size that allows passing through the sieve with openings diameter 1 mm. About 1.0 g (exactly) of granular buds was introduced into 250 ml slice, added 50 ml 40-percent ethyl alcohol. The retort was weighed with error  $\pm 0,01$ , that it was attached to reflux condenser and heated in boiling water bath for 2 hours. Then retort was cooled to room temperature and weighed. If necessary, 40-percent ethyl alcohol was added to restore initial mass. Retort content was filtered through paper fold filter, first 25 ml of filtrate was separated (solution A). In 25 ml

volumetric flask 1 ml of solution A was added, 3 ml of 2-percent alcoholic solution of aluminum chloride was added and the volume was restored to the mark with 96-percent ethyl alcohol. Solution consisting of 1 ml of solution A and 0.1 ml concentrated acetic acid and restored to the mark with 96-percent ethyl alcohol was used as reference solution. After 40 min optical density of tested solution was measured with spectrophotometer with 408 nanometers wavelength in a pan with 10 mm thick stratum.

Content of amount of flavonoids in perfectly dry raw material in terms of rutin in percent (X) was calculated according the formula:

$$X = \frac{A \cdot 50 \cdot 25 \cdot 100}{248 \cdot a \cdot 1 \cdot (100 - W)}$$

where:

A- optical density of solution;

248- specific value of rutin complex with aluminum chloride absorption for the wavelength 408 nanometers;

a- raw material mass in gr;

W- raw material humidity in percent.

Tanning agent content was measured by permanganate titration method according to recommendations of State pharmacopeia, XI edition in 3 replications [19].

**Main Body:** The research allows finding 12 phenol compounds in cardamom fruits (Table 1). Gallic acid is the main compound of phenol compounds that was determined by the method of internal normalization. 12 phenol compounds were found in carnation buds (Table 1). Gallic acid and quercetin are the main compounds of phenol compounds as determined by the method of internal normalization.

Analysis of industrial volumes of carnation buds by method of differential spectrophotometry shown that content of amount of flavonoids in terms of rutin varies from 3.55 % to 5.10 %.

Developed methods were validated on linearity, repeatability, laboratory and correctness. Linearity was tested on 5 levels of concentrations from theoretical content of amount of flavonoids in carnation raw materials. Correlation coefficient shouldn't be lower that 0.99. In the experiment it was 0.997. Repeatability was tested on one sample of raw material in 6 replications.

Table 1: Phenol compound in cardamom fruits and carnation buds

Compound	Cardamom fruits		Carnation buds	
	Retention time, min	Quantity of compound in mixture, %	Retention time, min	Quantity of compound in mixture, %
gallic acid	2.94	30.47	3.24	15.80
catechine	-	-	3.93	8.53
isoferulic acid	4.54	3.25	4.22	21.85
chlorogenic acid	4.96	1.22	-	-
epicatechine	5.78	2.11	-	-
chicory acid	6.27	2.47	6.32	1.42
caffeic acid	7.07	2.27	6.71	3.59
dehydroquercetin	11.26	0.66	11.69	1.00
ferulic acid	13.67	2.41	13.45	4.08
luteolin	15.34	8.19	15.28	1.78
quercetin	18.38	2.41	18.52	13.63
rutin	22.15	3.04	23.39	3.00
o-methoxycoumarin	-	-	32.21	0.45
cinnamic acid	52.16	0.06	52.26	1.16

Acceptability criterion was expressed by the measure of relative standard deviation that shouldn't be higher than 10%. In the experiment it was 1.49 %. Reproducibility was tested by two chemists who used 3 samples in three replications. Acceptability criterion was expressed by the measure of relative standard deviation that shouldn't be higher than 15 %. In the experiment it was 3.77 %. Correctness of the methods was proved by measuring qualitative content of amount of flavonoids in solutions by adding necessary amount of standard rutin sample to analyzed solution. Average percentage of recovery corrected for 100% was accepted as acceptability criteria and the average measure should be (100±5)%. It was shown that it varied in the limits of 98.31 % and 104.62 % with the average 100.99 %.

Qualitative content of tanning agent per tanning for carnation buds was 4.11 – 5.02 %, for cardamom fruits 0.02 – 0.06 %.

### CONCLUSION

Research shows that cardamom fruits and carnation buds have the complex of phenol compounds in their content. Phenol complex includes flavonoids, phenol carbon acid, coumarin and tanning agents. By method of high-yield liquid chromatography, (HPLC) content was analyzed. 12 phenol compounds were found in cardamom fruits and 12 phenol compounds in carnation buds. Gallic acid is the main compound of phenol compounds in cardamom fruits and gallic acid and quercetin- in carnation

buds that was determined by the method of internal normalization.

Quantity of amounts of flavonoids in terms of in carnation buds is 3.55 – 5.10 % and tanning agents- 4.11 – 5.02 %. In cardamom fruits there are 0.02- 0.04 tanning agents.

Resume. Phenol compound of cardamom fruits and carnation buds are interesting for further research and using in domestic medical practice as the sources of valuable bioactive substances.

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## The Solution of Stiff Integro-Differential Problem of Isoelectric Focusing by the Tangent Method

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**Abstract:** The paper reports on the development of the methods for approximate analytical solution of stiff integro-differential problem on the example of modelling Isoelectric Focusing (IEF) in so-called 'anomalous' modes. While working on the model the integro-differential problem was analytically transformed to a compact form suitable for investigating by asymptotic methods. The asymptotic solution by applying the tangent method was obtained, allowing high accuracy approximation of concentration profiles of polygonal curves with the given parameters.

**Key words:** Stiff integro-differential problem . tangent method

### INTRODUCTION

Isoelectric Focusing (IEF) is one of most effective and universal modern methods for protein characterization and fractionation. IEF has been widely used in many fields of modern chemistry and biology. The method is based on the ability of biopolymers or their fragments to form charged molecular complexes [1]. During one-dimensional IEF a mixture of ampholytes (amphoteric aminoacids having high buffer capacity) is placed in the electrophoretic chamber (EC) (the cylinder with length  $l$  and radius  $r$ ). When exposed to one-dimensional IEF the pH gradient is formed ( $\text{pH} = -\lg H$ , where  $H$  is concentration of hydrogen ions  $H^+$ ), with the fractionated components for certain fixed values of pH determined by their electrochemical properties having zero migration rate and being concentrated in the corresponding regions of EC. It is known as a stationary distribution of ampholytes. The distribution of ampholytes is invariable in any axial section of EC during one-dimensional IEF. The classical theory of electrophoresis the analytical concentrations of ampholytes are used to describe the system. Their graphs are called the ampholyte concentration profiles.

The founders of the mathematical theory of IEF built a basic mathematical model according to which the distribution of ampholyte concentration is determined by the Gaussian distribution density [2-8]. Many foreign researchers obtained the Gaussian distribution of ampholyte concentrations by the computer simulation of IEF [8-12]. On the other hand, they observed a distortion of the Gaussian distribution [10-14] which was called the 'anomalous' modes of IEF [15-17] (Fig. 1).

Using complex mathematical modeling of the IEF-systems by means of methods of mathematical physics [15-17] it was found that nonstationary boundary value problem becomes stiff at high current densities due to the presence of a small parameter in front of the derivatives. As a result the system enters the 'anomalous' mode. In the normal mode with the increase of the current density, a Gaussian curve of the concentration profile is stretched vertically and when it enters 'anomalous mode' its maximum seems to be limited by some kind of a graphical "ceiling" which limits its further growth and deforms it as the current density increases. At first the so called "plateaus" appear on tops of the curves, then the concentration profile takes the form of a rectangle or trapezoid.

Therefore, the questions arise: what is the mathematical interpretation of this phenomenon? How can one calculate the geometric parameters of the trapezoid (rectangles) into which initial Gaussian curves are transformed? The aim of the present investigation is to answer these questions.

Physical and mathematical formulation of the problem. The aqueous solution of  $N$  ampholytes is placed in the EC. For each ampholyte its dissociation constants of the reactions  $K_1^{(k)}$ ,  $K_2^{(k)}$ , migration coefficient  $\mu_k$  as well as the initial quantities  $m_k$ ,  $k = 1, 2, \dots, N$  are known. When exposed to the direct current with density  $J$  in the EC a distribution of ampholyte concentration is formed, resulting in stationary distribution of hydrogen ions  $H$  concentration. The dissociation reactions of  $k$ -ampholyte are supposed to be described by the following:



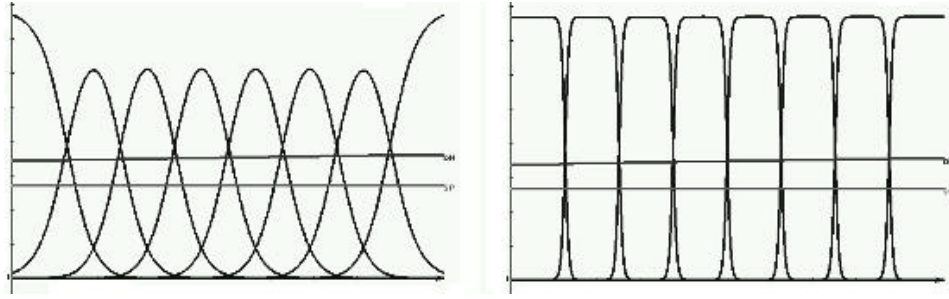


Fig. 1: Transformation of Gaussian curves to 'plateau-shaped' in 'anomalous' modes



where  $\text{NHR}\overset{\text{K}_2^{(k)}}{\text{COOH}}$ ,  $\text{NHR}\overset{\text{K}_2^{(k)}}{\text{COOH}^-}$  and  $\text{NH}_2\text{R}\overset{\text{K}_2^{(k)}}{\text{COOH}}$  are positive, negative and neutral ampholyte ions. The molar concentration of the ions are  $\xi_1^k$ ,  $\xi_{-1}^k$ ,  $\xi_0^k$ . The analytical concentration of the ampholyte is:  $\xi_k = \xi_1^k + \xi_{-1}^k + \xi_0^k$ . In the equilibrium state the concentration of the analyzed ampholyte ions are related to their analytical concentration by the equations:

$$\xi_1^k = \alpha_1^k \xi_k \quad (3)$$

$$\xi_{-1}^k = \alpha_{-1}^k \xi_k \quad (4)$$

$$\xi_0^k = (1 - \alpha_1^k - \alpha_{-1}^k) \xi_k \quad (5)$$

where  $\alpha_1^k$  and  $\alpha_{-1}^k$  are the degrees of ampholyte dissociation. From reactions (1)-(2) based on the theory of the kinetics of electrochemical reactions we obtain:  $\xi_0^k \text{H} = \text{K}_1^{(k)} \xi_1^k$ ,  $\xi_{-1}^k \text{H} = \text{K}_2^{(k)} \xi_{-1}^k$ , where H is the concentration of hydrogen ions. Their transformation on account of (3)-(4) allows one to derive the formulas expressing the degree of ampholyte dissociation through its dissociation constant and the concentration of hydrogen ions:

$$\alpha_1^k = \text{H}^2 (\text{K}_1^{(k)} \text{K}_2^{(k)} + \text{K}_1^{(k)} \text{H} + \text{H}^2)^{-1} \quad (6)$$

$$\alpha_{-1}^k = \text{K}_1^{(k)} \text{K}_2^{(k)} (\text{K}_1^{(k)} \text{K}_2^{(k)} + \text{K}_1^{(k)} \text{H} + \text{H}^2)^{-1} \quad (7)$$

In addition to these reactions of ampholyte dissociation in aqueous solution the reaction of water autoprotolysis should be taken into account:  $\text{H}_2\text{O} \Leftrightarrow \text{OH}^- + \text{H}^+$ . For the mathematical description of the system, the following functions are used [18-20]: 1)  $\xi_k$ ,  $k = 1, 2, \dots, N$ , analytical concentration of ampholytes (i.e. total concentrations of their positive, negative and

neutral ampholyte ions); 2) H, the concentration of hydrogen ions; 3) OH, the concentration of hydroxyl ions  $\text{OH}^-$ , connected with H by means of standard equation  $\text{OH}^- = k_w^2 / \text{H}$ , where  $k_w^2 = 10^{-14}$  is a constant of water auto-dissociation; 4) E, electric field intensity. The above functions are the solutions of the one-dimensional problem, consisting of N+1 differential equations, one algebraic equation and N integral equations, replacing the boundary conditions:

$$-\varepsilon \frac{d\xi_k}{dx} + \xi_k (\alpha_1^k - \alpha_{-1}^k) E = 0, k=1, 2, \dots, N \quad (8)$$

$$J = \sum_{k=1}^N \left( -D_k \frac{d}{dx} ((\alpha_1^k - \alpha_{-1}^k) \xi_k) + \mu_k (\alpha_1^k + \alpha_{-1}^k) \xi_k E \right) - D_H \frac{dH}{dx} + \mu_H H E + D_{OH} \frac{d(OH)}{dx} + \mu_{OH} OH E \quad (9)$$

$$\sum_{k=1}^N (\alpha_1^k - \alpha_{-1}^k) \xi_k + H - OH = 0 \quad (10)$$

$$\pi a^2 \int_0^l \xi_k(x) dx = m_k \quad (11)$$

where  $\varepsilon = RT/F$  is the standard electrochemical parameter (values R, T, F-absolute gas constant, temperature and Faraday constant, respectively;  $\mu_H$  and  $\mu_{OH}$  are the constants characterizing the mobility of ions  $\text{H}^+$  and  $\text{OH}^-$ ;  $D_k$ ,  $D_H$  and  $D_{OH}$  are the constants for diffusion coefficients of the ions,  $D_k = \varepsilon \mu_k$ ).

The differential equations (8) are the equation of mass transport. The differential equation (9) is the Ohm's generalized law with the diffusion (the current density is the sum of the current densities of all the ions, including both hydrogen and hydroxyl ions). The algebraic equation (10) is the charge balance equation. Finally, integral equations (11) correspond to the mass conservation law (the total quantity of all forms of the ampholyte is constant and equal to  $m_k$ ,  $k = 1, 2, \dots, N$ ). The main mathematical difficulty of numerical integration of the system (8)-(11), which was called

integro-differential problem of IEF [15-17], is the necessity to determine the value H from the the implicit function (10) to solve differential equations (8). The author have transformed the system and managed to overcome this problem.

The analytical transformation of the system. Theorem 1. The system of equations (8)-(11) with reference to N+2 unknown functions H, E,  $\xi_k(x)$ ,  $k = 1, 2, \dots, N$ , may be reduced to the boundary value problem with reference to N unknown functions  $c_k(x)$ ,  $k = 1, 2, \dots, N$ :

$$\varepsilon \frac{dc_k}{dx} \frac{1}{c_k} = \frac{\phi'_k(\psi) J}{\phi_k(\psi) \sigma} \quad (12)$$

$$\sigma = \sum_{k=1}^n \mu_k c_k \left( \phi''_k(\psi) - \frac{(\phi'_k(\psi))^2}{\phi_k(\psi)} \right) + 2k_w \mu \operatorname{ch}(\psi - \psi_0) \quad (13)$$

$$\sum_{k=1}^n c_k \phi'_k(\psi) + 2k_w \operatorname{sh} \psi = 0 \quad (14)$$

$$\phi_k(\psi) = \delta_k + \operatorname{ch}(\psi - \psi_k) \quad (15)$$

$$\int_0^1 c_k(x) \phi_k(\psi) dx = M_k, M_k = \frac{m_k}{2\pi a^2} \quad (16)$$

The old and new unknown functions are connected by the relation:

$$\xi_k(x) = c_k(x) \phi_k(\psi) \quad (17)$$

$$H = k_w \exp(\psi) \quad (18)$$

**Proof:** At the first stage of the proof the new function  $\psi$  was introduced and considered, defined by the equation (def. as (18)):  $H = k_w \exp(\psi)$ . To simplify the equations new constants are introduced:

$$\psi_k = \frac{1}{2} \ln \left( K_1^{(k)} K_2^{(k)} / k_w^2 \right) \quad (19)$$

$$\delta_k = \frac{1}{2} \sqrt{K_1^{(k)} / K_2^{(k)}} \quad (20)$$

$$\psi_0 = \frac{1}{2} \ln (\mu_{OH} / \mu_H) \quad (21)$$

$$\mu = \sqrt{\mu_H \mu_{OH}} \quad (22)$$

In the new notation, the functions involved in (8)-(10), have taken the form:

$$e_k = \alpha_1^k - \alpha_{-1}^k = \operatorname{sh}(\psi - \psi_k) (\delta_k + \operatorname{ch}(\psi - \psi_k))^{-1} \quad (23)$$

In addition

$$\sigma_k = \alpha_1^k + \alpha_{-1}^k = \operatorname{ch}(\psi - \psi_k) (\delta_k + \operatorname{ch}(\psi - \psi_k))^{-1}$$

Besides, new functions and new current density are introduced:  $\xi_k = 2k \xi_k^{\text{new}}$ ,  $J = 2k J^{\text{new}}$ . Now the system (8)-(10) can be rewritten as follows:

$$-\varepsilon \frac{d\xi_k^{\text{new}}}{dx} + \xi_k^{\text{new}} e_k E = 0 \quad (24)$$

$$J^{\text{new}} = \sum_{k=1}^N \mu_k \left( -\varepsilon \frac{d}{dx} (e_k \xi_k^{\text{new}}) + \sigma_k \xi_k^{\text{new}} E \right) + (-\varepsilon \nabla \psi + E) \mu \operatorname{ch}(\psi - \psi_0) \quad (25)$$

$$\sum_{k=1}^N e_k \xi_k^{\text{new}} + \operatorname{sh} \psi = 0 \quad (26)$$

At the second stage of the proof to the simplify the system, the new functions  $\phi_k(\psi)$  are considered (def as (15)); then,  $e_k = \phi'_k(\psi) (\phi_k(\psi))^{-1}$ ,  $\sigma_k = \phi''_k(\psi) (\phi_k(\psi))^{-1}$ . Let us represent the functions  $\xi_k(x)$  as the product of the functions  $\phi_k(\psi)$  by new unknown functions  $c_k^{\text{new}}$ :  $\xi_k^{\text{new}}(x) = c_k^{\text{new}} \phi_k(\psi)$ . With new variables the system of equations (24)-(25) takes the compact form, from which factor  $(-\varepsilon \psi'_x + E)$  is excluded:

$$\varepsilon \frac{dc_k^{\text{new}}}{dx} \frac{1}{c_k^{\text{new}}} = \frac{\phi'_k(\psi) J^{\text{new}}}{\phi_k(\psi) \sigma^{\text{new}}} \quad (27)$$

$$\sigma^{\text{new}} = \sum_{k=1}^N \mu_k c_k^{\text{new}} \left( \phi''_k(\psi) - \frac{(\phi'_k(\psi))^2}{\phi_k(\psi)} \right) + \mu \operatorname{ch}(\psi - \psi_0) \quad (28)$$

The equation (26), in its turn, with new variables takes the form:

$$\sum_{k=1}^N c_k^{\text{new}} \phi'_k + \operatorname{sh} \psi = 0 \quad (29)$$

At the third stage of the proof let us return to the old analytical concentration  $\xi_k = 2k_w \xi_k^{\text{new}}$  and the old current density  $J = 2k_w J^{\text{new}}$ . In accordance with (17) we introduce the new function  $c_k(x)$ , so that  $\xi_k(x) = c_k(x) \phi_k(\psi)$ . On account of  $c_k^{\text{new}} = c_k / 2k_w$  as well as  $\sigma^{\text{new}} = \sigma / 2k_w$ , the system of equations (27), (28), (29) takes the form of (12), (13), (15) and integral condition (11)-the form of (17)  $\Delta$ .

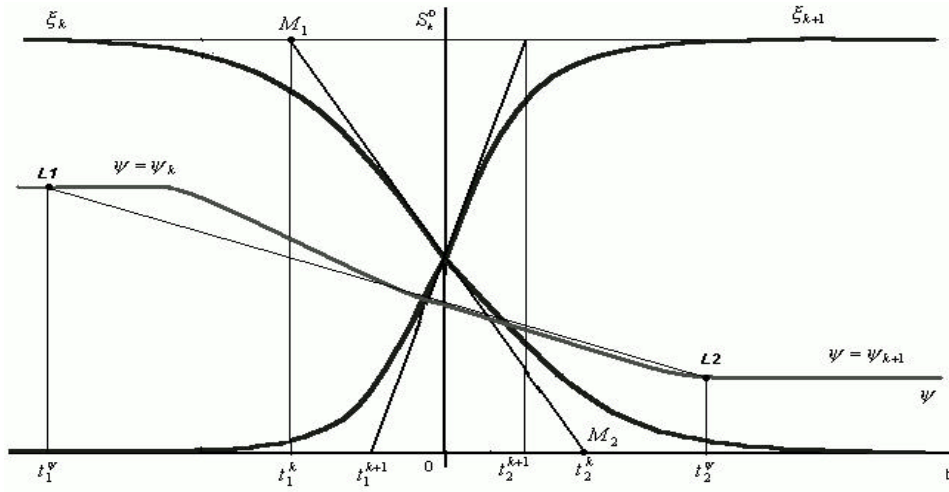


Fig. 2: The tangent to the profiles of two neighbouring ampholytes at the point of their intersection

Investigation of the system by tangent method. To solve the problem we used the tangent method which is based on the tension of a graph along the coordinate abscissa axis by replacing the variable:  $t = x/\varepsilon$  ( $\varepsilon$  is a small value). The abscissa corresponding to the point of intersection of  $k$  and  $k+1$  ampholyte profiles was taken as a new origin of coordinates (Fig. 2).

$$\xi_k(0) = \xi_{k+1}(0) \quad (30)$$

In the new coordinates the following assumptions are taken for the unknown functions:

$$\xi_k(-\infty) = S_k^0 \quad (31)$$

$$\xi_k(+\infty) = 0 \quad (32)$$

$$\xi_{k+1}(-\infty) = 0 \quad (33)$$

$$\xi_{k+1}(+\infty) = S_{k+1}^0 \quad (34)$$

where  $S_k^0$  and  $S_{k+1}^0$  are unknown parameters to be determined (parameters of IEF problem). Besides, for the function  $\psi$  the boundary conditions should be added:

$$\psi(-\infty) = \psi_k \quad (35)$$

$$\psi(+\infty) = \psi_{k+1} \quad (36)$$

where  $\psi_{k+1}, \psi_k$  are the constants to be determined.

**Theorem 2:** Functions  $\xi_k(0), \xi_{k+1}(0)$  at  $t = 0$  are defined by the equations:

$$\xi_k(t) = c_k(t)\phi_k(\psi), \xi_{k+1}(t) = c_{k+1}(t)\phi_{k+1}(\psi) \quad (37)$$

where functions  $c_k(t), c_{k+1}(t)$  are determined from the boundary value problem, consisting of two differential and one algebraic equations:

$$\frac{1}{c_k} \frac{dc_k}{dt} = \frac{\phi'_k(\psi) J}{\phi_k(\psi) \sigma}, i = k, k+1 \quad (38)$$

$$\sigma = \sum_{i=k}^{k+1} \mu_i c_i (\phi_i''(\psi) - \frac{(\phi_i'(\psi))^2}{\phi_i(\psi)}) \quad (39)$$

$$c_k \phi'_k(\psi) + c_{k+1} \phi'_{k+1}(\psi) = 0 \quad (40)$$

with two initial conditions

$$c_k(0) = 0.5 S_k^0 / \phi_k(\psi(0)), i = k, k+1 \quad (41)$$

The value  $\psi(0)$  in the formulas (41) is defined from algebraic equation:

$$\phi'_k(\psi(0))\phi_{k+1}(\psi(0)) + \phi'_{k+1}(\psi(0))\phi_k(\psi(0)) = 0 \quad (42)$$

**Proof:** At the first stage. Equations (12), (13), (14), on the basis of Fig. 2, are written for the two functions  $c_k(t), c_{k+1}(t)$ . At the second stage. Equation (14) under  $t \rightarrow \pm\infty$ , on account of (31)-(37) show that the term  $2k_w \cdot \text{sh}(\psi_{k+1})$  can be neglected for being small. Consequently, equations (12), (13), (14) may be written in the form (38)-(40), in particular, the equation (40) in the general form:

$$\sum_{k=1}^n c_k \phi'_k(\psi) = 0 \quad (43)$$



The equation (43), in its turn, by the multiplication by  $\psi'_i$ , on the basis of (37), (38) and (43), we obtaine:

$$\xi_k + \xi_{k+1} = \text{const} \tag{44}$$

The conditions (30)-(34), (44) show that  $S_k^0 + 0 = \text{const}, 0 + S_{k+1}^0 = \text{const}$ . Consequently, in this assumption,

$$S_k^0 = S_{k+1}^0 = \text{const} \tag{45}$$

From the initial condition (30) we obtain the following equality:  $2\xi_k = S_k^0$  and, consequently,

$$\xi_k(0) = \xi_{k+1}(0) = 0.5S_k^0 \tag{46}$$

At the third stage. Equations (30), (37) and (43), written for  $t = 0$ , provide the system of two linear homogeneous algebraic equations with reference to two unknowns  $c_k(0)$  and  $q_{k+1}(0)$ . The system is joint if its determinant equals zero, which yields the equation (42) for determining the value  $\psi(0)$ . Hence, on the basis of (37) and (46) the initial conditions (41) are obtained  $\Delta$ .

Now, let us draw the tangent to the profiles at the point  $t = 0$  (Fig. 2). Obviously, they are set by the equations:

$$\xi_i(t) = \xi_i(0) + \xi'_i(0)t, i = k, k + 1 \tag{47}$$

$$\psi(t) = \psi(0) + \psi'(0)t \tag{48}$$

**Theorem 3:** In equations (47) and (48) the coefficients  $\xi_k(0)$ ,  $\xi_{k+1}(0)$  and  $\psi(0)$  are determined by means of the obtained formulas (46) and (42) and slopes of the tangent are given by:

$$\xi'_k(0) = S_k^0 / \Delta t_k \tag{49}$$

$$\xi'_{k+1}(0) = -S_k^0 / \Delta t_k \tag{50}$$

$$\psi'(0) = 2\Phi_{k, k+1}(\psi(0)) / \Delta t_k \tag{51}$$

$$\Delta t_k = \frac{2\sigma}{J} \left( \frac{\Phi_k(\psi(0))}{\Phi'_k(\psi(0))} - \Phi_{k, k+1}(\psi(0)) \right) \tag{52}$$

$$\Phi_{k, k+1}(\psi(0)) = \left( \frac{\Phi'_{k+1} - \Phi'_k}{\Phi_{k+1} - \Phi_k} \right) \left( \frac{1 + \delta_k \Phi''_k}{\Phi_k^2} + \frac{1 + \delta_{k+1} \Phi''_{k+1}}{\Phi_{k+1}^2} \right) \Big|_{\psi=0} \tag{53}$$

**Proof**

1. Figure 2 shows that the tangent for  $\xi_k(t)$  intersects the straight line  $\xi = S_k^0$  at the point  $M_1(t_1^k, S_k^0)$  and

abscissa axes  $\xi = 0$  at the point  $M_2(t_2^k, 0)$ . On the basis of (47) the equality is obtained:

$$t_1^k - t_2^k = (\xi_k(-\infty) - \xi_k(+\infty))(\xi'_k(0))^{-1} \tag{54}$$

Similar relations for  $\xi_{k+1}(t)$  and  $\psi(t)$ , on the basis of equations (47), (48) take the form:

$$t_1^{k+1} - t_2^{k+1} = (\xi_{k+1}(-\infty) - \xi_{k+1}(+\infty))(\xi'_{k+1}(0))^{-1} \tag{55}$$

$$t_1^\psi - t_2^\psi = (\psi(-\infty) - \psi(+\infty))(\psi'(0))^{-1} \tag{56}$$

We introduce the denotations

$$t_1^k - t_2^k = \Delta t_k, t_1^{k+1} - t_2^{k+1} = \Delta t_{k+1} \tag{57}$$

$$t_1^\psi - t_2^\psi = \Delta t_\psi, \psi_k - \psi_{k+1} = \Delta \psi_k \tag{58}$$

As a result, the equations (57)-(61), with the conditions (30)-(36), (45), take the form:

$$\xi'_k(0) = S_k^0 / \Delta t_k \tag{59}$$

$$\xi'_{k+1}(0) = -S_k^0 / \Delta t_{k+1} \tag{60}$$

$$\psi'(0) = \Delta \psi_k / \Delta t_\psi \tag{61}$$

Thus, the formulas (59)-(61) allow us to calculate the values  $\xi'_k(0)$ ,  $\xi'_{k+1}(0)$ ,  $\psi'(0)$ , if values  $\Delta t_k$ ,  $\Delta t_{k+1}$ ,  $\Delta t_\psi$  are known. The equation (37) shows that

$$\xi'_k(0) = (c'_k \Phi_k(\psi) + c_k \Phi'_k(\psi) \psi') \Big|_{t=0}$$

As a result, the equations (59), (60), using (38) can be given in the form:

$$S_k^0 = \Delta t_k c_k(0) \Phi'_k(\psi(0)) (J/\sigma + \Delta \psi_k / \Delta t_\psi) \tag{62}$$

$$S_k^0 = -\Delta t_{k+1} c_{k+1}(0) \Phi'_{k+1}(\psi(0)) (J/\sigma + \Delta \psi_k / \Delta t_\psi) \tag{63}$$

It is obvious, that  $J/\sigma + \Delta \psi_k / \Delta t_\psi \neq 0$ , otherwise magnitude  $S_k^0$  would be zero. This means, that the system of equations (62), (63), with (76), leads to the important equality:

$$\Delta t_{k+1} = \Delta t_k \tag{64}$$

2. The equation of tangent for  $\xi_k(t)$ , written for the points  $M_1$  and  $M_2$ , gives a system of equations. Its

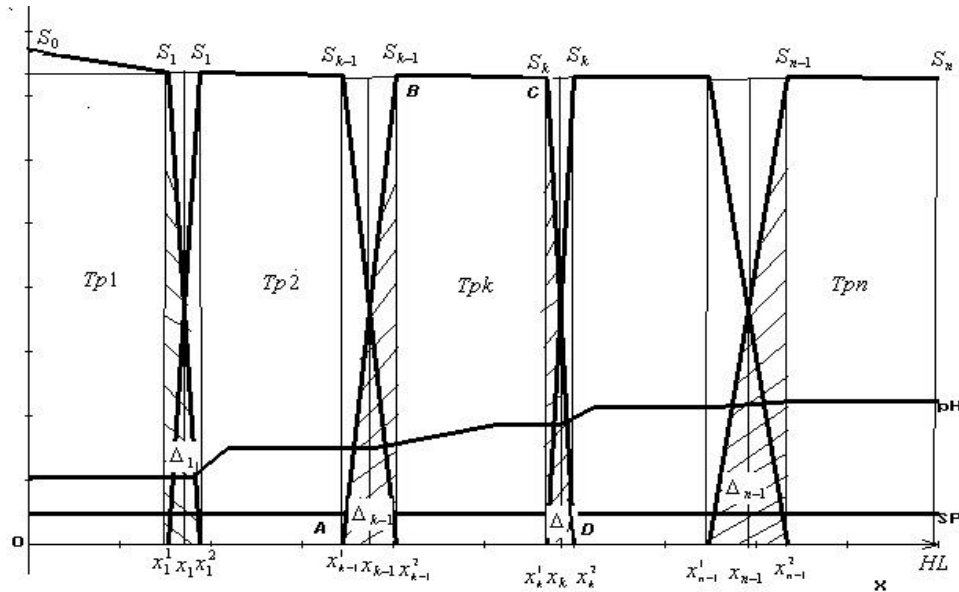


Fig. 3: Tangent approximation of the concentration profile of IEF

transformation with (44) and (58) leads to the equations:

$$t_1^k = 0.5\Delta t_k, \quad t_2^k = -0.5\Delta t_k \quad (65)$$

Similarly, the equation of tangent for  $\xi_{k+1}(t)$  with (45) and (60) helps to obtain the equation:

$$t_1^{k+1} = 0.5\Delta t_k, \quad t_2^{k+1} = -0.5\Delta t_k \quad (66)$$

Consequently,  $t_1^k = t_1^{k+1}, t_2^k = t_2^{k+1}$ . Differentiation of (43) with (45), (59), (60) gives equation (51), (53). Substituting (51) in (62) with (41) gives equation (52).

- Figure 2 shows that tangent (48) for  $\psi(x)$  intersect the straight line  $\psi = \psi_k$  at the point  $L_1(t_1^\psi, \psi_k)$  and straight line  $\psi = \psi_{k+1}$  at the point  $L_2(t_2^\psi, \psi_{k+1})$ . Using the equation (48) at these points we obtain the formula:

$$t_1^\psi = 0.5\Delta t_k \frac{\psi_k - \psi(0)}{\Phi(\psi(0))}, \quad t_2^\psi = 0.5\Delta t_k \frac{\psi_{k+1} - \psi(0)}{\Phi(\psi(0))} \Delta \quad (67)$$

Let us consider the problem on the whole interval  $[0,1]$ . The following notations will be used:  $x_1$  is the point of intersection of the 1st and 2nd ampholyte profiles;  $x_2$  is the point of intersection of the 2nd and 3rd ampholyte profiles;  $x_{N-1}$  is the point of intersection of (N-1) and Nampholyte profiles. Let us consider the graphical approximation of ampholyte concentration profiles by the trapezoid system (Fig. 3).

The Fig. 3 shows that k-profiles of concentration: 1) on the segment  $[x_{k-1}^2, x_k^1]$  are approximated by means of a straight line passing through the points  $(x_{k-1}^2, S_{k-1})$  and  $(x_k^1, S_k)$ ; 2) on the intervals  $[x_{k-1}^1, x_{k-1}^2]$  and  $[x_k^1, x_k^2]$  by means of tangents passing through the points  $(x_{k-1}^1, 0)$ ,  $(x_{k-1}^2, S_{k-1})$  and  $(x_k^2, S_k)$ ,  $(x_k^1, 0)$ ; 3) at all other points of the interval  $[0,1]$  the concentration is assumed to be zero. The return to the initial variable  $x$  is realized on the basis of the formulas:

$$x_k^1 = x_k + 0.5\Delta t_k \cdot \varepsilon, \quad x_k^2 = x_k - 0.5\Delta t_k \cdot \varepsilon \quad (68)$$

The integral conditions (9) were applied to the functions  $\xi_k$ ,  $k = 1, 2, \dots, N$ . Integration is realized by means of simple summation of the areas of trapezoids and leads to the following conclusion.

**Theorem 4:** Parameters of IEF problem  $S_k^0$ ,  $k = 1, 2, \dots, N$  and  $x_1, x_2, \dots, x_N$  are determined by a system of  $N+1$  linear algebraic equations:

$$\begin{cases} m_1 = \Delta x_1 \cdot S + 0.5(S_0 + S)(x_1 - \Delta x_1) \\ m_k = \Delta x_{k-1} \cdot S_{k-1} + \Delta x_k \cdot S_k + 0.5(S_{k-1} + S_k)(x_k - x_{k-1} - \Delta x_{k-1} - \Delta x_k) \\ k = 2, 3, \dots, N-1 \\ m_N = \Delta x_{N-1} \cdot S_{N-1} + 0.5(S_{N-1} + S_N)(L - x_{N-1} - \Delta x_{N-1}) \end{cases} \quad (69)$$

and  $N-1$  simplest (elementary) integral equation:

$$S_k = S_0 - 2k_w \int_0^{x_k} \text{sh}(\psi) \left( \frac{J}{\varepsilon \sigma} + \psi'_x \right) dx, \quad k = 1, N-1 \quad (70)$$

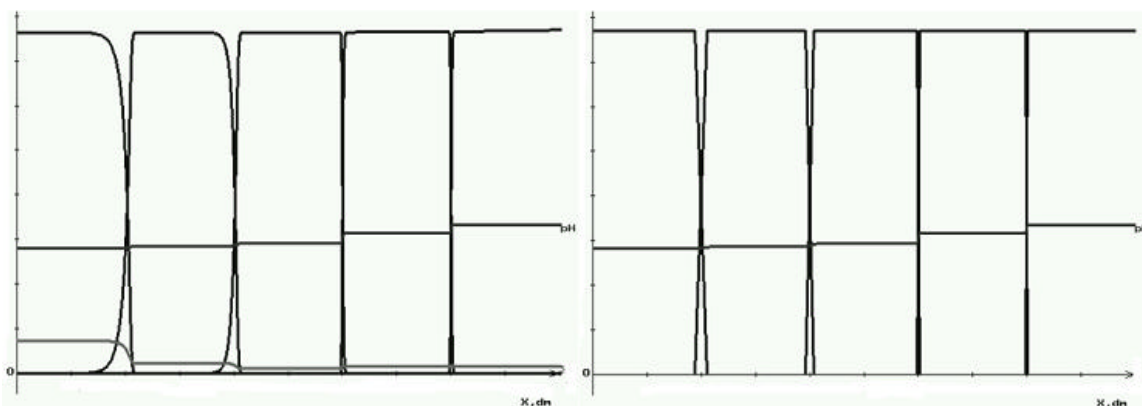


Fig. 4: Calculated and asymptotic concentration profiles the system with pH>7: His -His, His-Gly, His, β-Ala-His, Tyr-Arg, J = 0.539

Summing up the above, using the formulas (39), (41), (76), (51)-(53), (67), (68), the equations take the general form:

$$\xi_k = \begin{cases} 0, & x \in [0, x_{k-1}^1] \\ S_{k-1} \cdot \frac{(x - x_{k-1}^1)}{(x_{k-1}^2 - x_{k-1}^1)}, & x \in [x_{k-1}^1, x_{k-1}^2] \\ S_{k-1} + (S_k - S_{k-1}) \cdot \frac{(x - x_{k-1}^2)}{(x_k^1 - x_{k-1}^2)}, & x \in [x_{k-1}^2, x_k^1] \\ S_k - S_k \cdot \frac{(x - x_k^1)}{(x_k^2 - x_k^1)}, & x \in [x_k^1, x_k^2] \\ 0, & x \in [x_k^2, H] \end{cases} \quad (71)$$

$$x_k^1 = x_k + 0.5\Delta t_k \cdot \varepsilon, \quad x_k^2 = x_k - 0.5\Delta t_k \cdot \varepsilon, \quad i = k - 1, k$$

where values  $S_i^0, x_k^1$  are determined by formulas (69), (70);

$$\Delta t_i = \frac{\Theta_i}{J} \left( \frac{\phi_i}{\phi_i'} - \phi_{i+1} \right) \Big|_{\psi=\psi(x_i)} \quad (72)$$

$$\Theta_i = \mu_i \frac{S_i}{\phi_i'} \left( \phi_i'' - \frac{(\phi_i')^2}{\phi_i} \right) + \mu_{i+1} \frac{S_{i+1}}{\phi_{i+1}'} \left( \phi_{i+1}'' - \frac{(\phi_{i+1}')^2}{\phi_{i+1}} \right) \Big|_{\psi=\psi(x_i)} \quad (73)$$

$$\Phi_{i,i+1}(\psi(x_i)) = \left( \frac{\phi_{i+1}'}{\phi_{i+1}} - \frac{\phi_i'}{\phi_i} \right) \left( \frac{1 + \delta_i \phi_i''}{\phi_i'^2} + \frac{1 + \delta_{i+1} \phi_{i+1}''}{\phi_{i+1}'^2} \right) \Big|_{\psi=\psi(x_i)} \quad (74)$$

the value  $\psi(x_i)$  in the formulas (73)-(75) is derived from algebraic equation:

$$\phi_i'(\psi(x_i))\phi_{i+1}(\psi(x_i)) + \phi_{i+1}'(\psi(x_i))\phi_i(\psi(x_i)) = 0 \quad (75)$$

Accordingly, the function  $\psi$  is approximated by a broken line:

$$\psi = \begin{cases} \Psi_k, & x \in [x_{k-1}^\psi, x_k^\psi] \\ \Psi_{k+1} + (\Psi_k - \Psi_{k+1}) \cdot \frac{(x - x_k^\psi)}{(x_{k+1}^\psi - x_k^\psi)}, & x \in [x_k^\psi, x_{k+1}^\psi] \\ \Psi_{k+1}, & x \in [x_{k+1}^\psi, x_{k+2}^\psi] \end{cases} \quad (76)$$

$$x_k^\psi = x_k + \Delta t_k \frac{\Psi_k - \psi(x_k)}{\Phi(\psi(x_k))} \cdot \varepsilon$$

$$x_{k+1}^\psi = x_k + 0.5\Delta t_k \frac{\Psi_{k+1} + 0.5\psi(x_k)}{\Phi(\psi(x_k))} \cdot \varepsilon$$

Model testing. The calculations were carried out on the assumption that: the length of EC is  $l = 2(\text{dm})$  and its radius is  $r = 0.2(\text{dm})$ . The current density is measured in  $\text{A}/\text{sq.dm}$ . The values of dissociation constants  $K_1^{(k)}, K_2^{(k)}$  and migration coefficients are taken from [1]. The system of five standard ampholytes has been considered: His -His, His-Gly, His, β-Ala-His, Tyr-Arg. Asymptotic profiles have been constructed on the basis of formulas (72) (77). Fig. 4 shows that in ‘anomalous’ mode the calculated and asymptotic concentration profiles provide a high degree of consistency.

## CONCLUSIONS

The asymptotic solution of the integro-differential problem of IEF has been obtained by using the tangent method. The concentration profiles are approximated by straight lines, whose coefficients are determined by the electrochemical parameters of the system. It has been found that the geometrical ‘ceiling’ for the system of concentration profiles is the broken line, with the parameters determined by the equations (58)-(59), plays the role of such a ‘ceiling’. The complex IEF model

developed in this paper has theoretical significance: using the suggested transformation methods and the asymptotic solution of stiff integro-differential problem, similar problems in mathematical physics, having large parameters, could be solved.

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## The Investigation of Stiff Integro-Differential Problem of Isoelectric Focusing by Means of Singular Asymptotic Method

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**Abstract:** In the current work the stiff integero-differential problem of Isoelectric Focusing (IEF) in "anomalous" regimen was investigated by means of asymptotic methods. The solution of problem was obtained by means of singular asymptotics which showed a high degree of convergence with the calculated solutions of the problem. The physical sense of IEF "anomalous" regimen was obtained. The software was developed, which allows to simulate real IEF-systems in terms of the received asymptotic solution.

**Key words:** Stiff integero-differential problem . singular asymptotics . "anomalous" regimen

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### INTRODUCTION

The Isoelectric Focusing (also known as electrofocusing, IEF) is one of most important methods of modern electrochemistry [1]. It allows to separate with high accuracy the solution of ampholytes (amphoteric aminoacids) into fractions when exposed to the electric field. Under the IEF in solution the stable gradient pH is extended from anode to cathode. Ampholyte molecule migrates in solution by means of surface charge so far as to achieve the zone, where its electric conductivity equals zero, i.e.  $pH = pI$ , where  $pI$  is so called isoelectric point of ampholyte. As a result, ampholytes segregate into the fractions according to the increase of  $pI$  from anode to cathode (the steady-state distribution of ampholytes is formed). The method has immensely wide possibilities for the substance fractionation (primarily albumins); it has wide application in biological and medical investigations.

One of the most significant problems of mathematical modeling of IEF is the creation of mathematically laconic and descriptive models, which allow to make clear the physical sense of complicated biochemical processes in EC. The investigation of the so-called "anomalous" regimen of IEF by means of mathematical physics is of great scientific interest.

The originators of IEF, [2-8], have created a simplified mathematical model of IEF, according to which the concentrations of ampholytes are described by the density function of Gaussian distribution:  $C = C_0 \exp(-pEx^2/2D)$ , where  $E$  is electric field strength,  $D$  coefficient of diffusion,  $p = -\frac{du}{dx}$  - gradient of electrophoretic mobility of ampholyte. The solution

of integro-differential IEF problem, as a special case of electrophoresis, was obtained on the basis of general models of the homogenetic multicomponent electrochemically active mediums in the applied external fields [10, 11]. The IEF modeling in terms of closed systems of basic balance equations has shown, that classical Gaussian distribution of concentration is the solution of the problem under the low and average current density. However, it was established, that under higher current density, the corresponding integro-differential problem becomes stiff because of small parameter derivatives and the problem gets a number of features that impede its solution by conventional numerical and asymptotic methods.

The Gaussian distribution of ampholytes concentrations was discovered by many foreign scientists in the course of the computer simulation of IEF [11-14]. However, the distortions of Gaussian distribution have been recorded in [13-16], which are called "anomalous" regimen of IEF [17-20]. At the high values of electric current the concentration distribution takes "plateaus"-shaped form, which drastically differs from the Gaussian distribution as well as other classical distributions. The physical and mathematical sense of this phenomena has not been fully revealed in [13-16], which are the applied electrochemical research.

"Anomalous" regimen were also recorded by the author of this investigation in the course of numeric solution of the correspondent integro-differential problem of IEF [17-20]. The aim of the present research is to create the mathematical model, which would allow to establish the physical (electrochemical) sense of "anomalous" regimen of IEF through visual analysis of dependencies. In the course of work on the model the

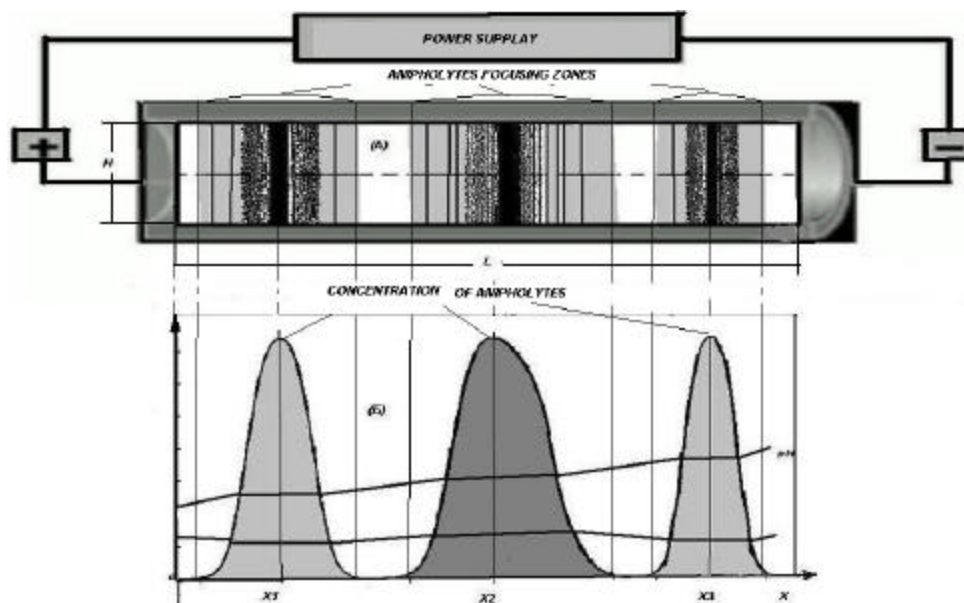


Fig. 1: (a): The stationary distribution of three ampholytes in EC; (b): The profiles of ampholytes

following problems were solved: the initial integro-differential problem was analytically transformed into the ordinary boundary-value problem, which is suitable for the numeric solution by means of Runge-Kutta method together with errors accumulation control; the initial integro-differential problem was investigated by asymptotic methods and its singular asymptotic solution was obtained; the software was created, which allows to construct numeric and asymptotic solution and showed its full compliance to the predicted values in "anomalous" regimen; by means of electrochemical interpretation of obtained formulas, the physical sense of "anomalous" regimen was established; the complex mathematical model of IEF was developed, which allowed to perform computations for real IEF-systems.

Physical and mathematical statement of the problem. The aqueous solution of  $K$  ampholytes is placed into the EC, which has a cylinder shape with the length  $l$  and radius  $r$ . Initial quantities of ampholytes equal:  $m_k$ ,  $k = 1, 2, \dots, N$ . For each of ampholytes is dissociation constants  $K_1^{(k)}$ ,  $K_2^{(k)}$  and characteristic mobility  $\mu_k$  are known. The temperature  $T$  in EC is constant. In this model the longitudinal axial cross-section of EC is considered, which is a rectangular with the length  $l$  and the width  $2r$  (Fig. 1). When exposed to the constant current density  $J$  the intrinsic pH-gradient is formed in EC, i.e. stationary distribution of hydrogen ions  $H^+$  concentrations is obtained. Under the influence of the constant current density  $J$  in EC the stationary (constant in time) distribution of amino acids is formed.

It is supposed that the dissociation reactions of  $k$ -s ampholyte are described by means of equations:



where  $NHR_2COOH$ ,  $NHR_2COOH^-$  and  $NH_2RCOOH$  are positive, negative and neutral ions of ampholyte. Molar concentration of corresponding ions are  $\xi_1^k$ ,  $\xi_{-1}^k$ ,  $\xi_0^k$ . The total or so-called analytical concentration is:  $\xi_k = \xi_1^k + \xi_{-1}^k + \xi_0^k$ .

In equilibrium state the concentrations of the analyzed ampholyte ions are connected by means of equations:

$$\xi_1^k = \alpha_1^k \xi_k \quad (3)$$

$$\xi_{-1}^k = \alpha_{-1}^k \xi_k \quad (4)$$

$$\xi_0^k = (1 - \alpha_1^k - \alpha_{-1}^k) \xi_k \quad (5)$$

where  $\alpha_1^k$  and  $\alpha_{-1}^k$  are the degrees of ampholyte dissociation. From reaction (1)-(2) on the basis of dissociation reactions its magnitude is connected by means of equations in equilibrium state of electrolyte:  $\xi_0^k H = K_1^{(k)} \xi_1^k$ ,  $\xi_{-1}^k H = K_2^{(k)} \xi_0^k$ , where  $H$  is hydrogen concentration. Its transformation, on account of (3)-(5), leads to the formulas, which express degrees of ampholyte dissociation by means of its dissociation constants and concentration of hydrogen ions. The formulas result from this system:

$$\alpha_1^k = H^2(K_1^{(k)}K_2^{(k)} + K_1^{(k)}H + H^2)^{-1} \quad (6)$$

$$\alpha_{-1}^k = K_1^{(k)}K_2^{(k)}(K_1^{(k)}K_2^{(k)} + K_1^{(k)}H + H^2)^{-1} \quad (7)$$

The isoelectric point is the state of the system, in which the total charge of the system is zero. Consequently, on account of formulas (3)-(5) the equations which characterize the isoelectric point may be obtained:

$$(\alpha_1^k - \alpha_{-1}^k)\xi_k = 0 \quad (8)$$

In addition to these ampholytes dissociations in aqueous solution, the reaction of autodissociation should be taken into account:  $H_2O \rightleftharpoons OH^- + H^+$ .

For the mathematical description of the system, according to the mathematical theory of electrochemical processes [10, 11], the following unknown functions must be used: 1) functions  $\xi_k$ ,  $k = 1, 2, \dots, N$ , analytical concentration of ampholytes; 2) function  $H$ , the concentration of hydrogen; 3) function  $OH$ , the concentration of ions  $OH^-$ , connected with  $H$  by means of standard equation  $OH = k_w^2/H$ , where  $k_w^2 = 10^{-14}$  is the autodissociation constant of water; 4) function  $E$ , the electric field strength. The unknown functions are connected by closed balance system, which includes the equation of mass transport, generalized Ohm's law, the law of conservation of mass and the law of conservation of mass for each of ampholytes. In the suppositions made the basic equation of mass transport theory for each ampholyte takes the form:  $j_k = 0$ . It means, that the functions are the solutions of one-dimensional integral-differential problem, which consists of  $N+1$  differential equations (equation of mass transport), one algebraic equation (generalized Ohm's law) and  $N$  integral equations, which replace boundary conditions (law of conservation of mass for each of ampholytes):

$$-\varepsilon \frac{d\xi_k}{dx} + \xi_k(\alpha_1^k - \alpha_{-1}^k)E = 0, k = 1, 2, \dots, N \quad (9)$$

$$J = \sum_{k=1}^N \left( -D_k \frac{d}{dx} ((\alpha_1^k - \alpha_{-1}^k)\xi_k) + \mu_k(\alpha_1^k + \alpha_{-1}^k)\xi_k E \right) - D_H \frac{dH}{dx} + \mu_H H E + D_{OH} \frac{d(OH)}{dx} + \mu_{OH} OH E \quad (10)$$

$$\sum_{k=1}^N (\alpha_1^k - \alpha_{-1}^k)\xi_k + H - OH = 0 \quad (11)$$

$$\pi \int_0^l \xi_k(x) dx = m_k \quad (12)$$

where  $\varepsilon = RT/F$  is standard electrochemical parameter, where magnitudes  $R$ ,  $T$ ,  $F$  are absolute gas constant, temperature and Faraday constant, respectively;  $\mu_H$  and  $\mu_{OH}$  are constants, characteristic of ions mobility  $H^+$  and  $OH^-$ ;  $D_k$ ,  $D_H$  and  $D_{OH}$  constants are the ions diffusion coefficients,  $D_k = \varepsilon \mu_k$ ;  $\alpha_1^k$  and  $\alpha_{-1}^k$  -functions of  $H$ , so called degrees of ampholyte dissociation, determined by the equations:

$$\alpha_1^k = \frac{H^2}{K_1^{(k)}K_2^{(k)} + K_1^{(k)}H + H^2}, \alpha_{-1}^k = \frac{K_1^{(k)}K_2^{(k)}}{K_1^{(k)}K_2^{(k)} + K_1^{(k)}H + H^2}$$

The differential equations (9) are the equations of mass transport, obtained on basis of ampholyte current equation. The differential equation (10) is generalized Ohm's law with account of diffusion and electromigration transport of all kind of ions. The algebraic equation (11) is the equation of electrochemical neutrality. Finally, the integral equation (12) is the law of conservation of mass (the summary quantity of all forms of ampholyte is constant and equals  $m_k$ ,  $k = 1, 2, \dots, N$ ).

The main mathematical difficulties of system numerical integration (9)-(12), known as IEF integral-differential problems [17-20], are: a) the necessity to convey the magnitude  $H$  from nonlinear algebraic equation (11) for solution of differential equations (9); b) the necessity to exploit the integral condition (12) instead of usual boundary conditions. These difficulties hamper the numerical solution of problem by means of Runge-Kutta methods.

For large  $J$  values, as it follows from equations (1), the large parameter  $J/\varepsilon$  appears before functions  $\xi_k(x)$  ( $\varepsilon \approx 0.0257$ ) (9). It leads to the further problems, which are typical for the stiff integero-differential problem: c) little changes of  $\xi_k(x)$  lead to the large changes of their derivatives which can bring the uncontrollable accumulation of computational errors; d) as a consequence, in the region of "plateaus", where values of derivatives are about zero, the cycling of Runge-Kutta method followed by the incorrect solution are possible; e) in other points the derivatives of unknown functions strive to the infinity; it may lead to the jump of solution with its outcome to negative, (without physical meaning) solutions.

As a result, the preliminary analysis of integero-differential problem and its solutions in "anomalous" regimen has shown that: for the problem in standard formulation (9)-(12) the risk of uncontrollable accumulation of computational errors is large. Therefore, the authors of the current research have performed the transformation of problem to overcome the difficulties a)-e).

The reduction of system to the boundary-value problem. Theorem 1. The system of equations (9)-(12) with respect to N+2 unknown functions H, E,  $\xi_k(x)$ ,  $k=1,2,\dots,N$ , may be reduced to the boundary-value problem with respect to N unknown function  $c_k(x)$ ,  $k=1,2,\dots,N$ :

$$\varepsilon \frac{dc_k}{dx} \frac{1}{c_k} = \frac{\varphi'_k(\psi) J}{\varphi_k(\psi) \sigma} \quad (13)$$

$$\sigma = \sum_{k=1}^n \mu_k c_k \left( \varphi''_k(\psi) - \frac{(\varphi'_k(\psi))^2}{\varphi_k(\psi)} \right) + 2k_w \mu \operatorname{ch}(\psi - \psi_0) \quad (14)$$

$$\sum_{k=1}^n c_k \varphi'_k(\psi) + 2k_w \operatorname{sh} \psi = 0 \quad (15)$$

$$\int_0^1 c_k(x) \varphi_k(\psi) dx = M_k, M_k = \frac{m_k}{2\pi r^2} \quad (16)$$

$$\varphi_k(\psi) = \delta_k + \operatorname{ch}(\psi - \psi_k) \quad (17)$$

The old and new unknown functions are connected by the equalities:

$$\xi_k(x) = c_k(x) \varphi_k(\psi) \quad (18)$$

$$H = k_w \exp(\psi) \quad (19)$$

Proof. At the first stage the new function  $\psi$  was considered on basis of equality (def. as (17)):  $H = k_w \exp(\psi)$ . For convenience new constants were introduced:

$$\psi_k = \frac{1}{2} \ln \left( \frac{K_1^{(k)} K_2^{(k)}}{K_w^2} \right) \quad (20)$$

$$\delta_k = \frac{1}{2} \sqrt{K_1^{(k)} / K_2^{(k)}} \quad (21)$$

$$\psi_0 = \frac{1}{2} \ln(\mu_{OH} / \mu_H) \quad (22)$$

$$\mu = \sqrt{\mu_H \mu_{OH}} \quad (23)$$

In the new notation, the functions involved in (9)-(12), have taken the form of:

$$e_k = \alpha_1^k - \alpha_{-1}^k = \operatorname{sh}(\psi - \psi_k) (\delta_k + \operatorname{ch}(\psi - \psi_k))^{-1} \quad (24)$$

$$\sigma_k = \alpha_1^k + \alpha_{-1}^k = \operatorname{ch}(\psi - \psi_k) (\delta_k + \operatorname{ch}(\psi - \psi_k))^{-1}$$

Besides, new functions and new current density were introduced:  $\xi_k = 2k \xi_k^{\text{new}}$ ,  $J = 2k_w J^{\text{new}}$ . Now the system (13)-(15) may be transformed to the following form, which has two advantages-compact form and absence of small parameter  $k_w$ :

$$-\varepsilon \frac{d\xi_k^{\text{new}}}{dx} + \xi_k^{\text{new}} e_k E = 0 \quad (25)$$

$$J^{\text{new}} = \sum_{k=1}^N \mu_k \left( -\varepsilon \frac{d}{dx} (e_k \xi_k^{\text{new}}) + \sigma_k \xi_k^{\text{new}} E \right) + (-\varepsilon \nabla \psi + E) \mu \operatorname{ch}(\psi - \psi_0) \quad (26)$$

$$\sum_{k=1}^N e_k \xi_k^{\text{new}} + \operatorname{sh} \psi = 0 \quad (27)$$

At the second stage the new functions were introduced for the simplification of the proof (def. as (15)):

$$\varphi_k(\psi) = \delta_k + \operatorname{ch}(\psi - \psi_k)$$

then

$$\frac{d\varphi_k(\psi)}{d\psi} = \varphi'_k(\psi) = \operatorname{sh}(\psi - \psi_k)$$

$$\frac{d^2\varphi_k(\psi)}{d\psi^2} = \varphi''_k(\psi) = \operatorname{ch}(\psi - \psi_k)$$

consequently,

$$e_k = \varphi'_k(\psi) (\varphi_k(\psi))^{-1}, \sigma_k = \varphi''_k(\psi) (\varphi_k(\psi))^{-1}$$

Let us represent functions  $\xi_k(x)$  in the form of the product of functions  $\varphi_k(\psi)$  and new unknown functions  $c_k^{\text{new}}$ :  $\xi_k^{\text{new}}(x) = c_k^{\text{new}} \varphi_k(\psi)$ . In new variables the system of equations (25)-(26) takes the form of:

$$\varepsilon \frac{dc_k^{\text{new}}}{dx} = e_k c_k^{\text{new}} (-\varepsilon \psi'_x + E)$$

$$J^{\text{new}} = (-\varepsilon \psi'_x + E) \left[ \sum_{k=1}^N \mu_k c_k^{\text{new}} (\varphi''_k - e_k \varphi'_k) + \mu \operatorname{ch}(\psi - \psi_0) \right]$$

and amounts to the compact form, from which factor  $(-\varepsilon \psi'_x + E)$  is excluded:

$$\varepsilon \frac{dc_k^{\text{new}}}{dx} \frac{1}{c_k^{\text{new}}} = \frac{\varphi'_k(\psi) J^{\text{new}}}{\varphi_k(\psi) \sigma^{\text{new}}} \quad (28)$$

$$\sigma^{\text{new}} = \sum_{k=1}^N \mu_k c_k^{\text{new}} \left( \varphi''_k(\psi) - \frac{(\varphi'_k(\psi))^2}{\varphi_k(\psi)} \right) + \mu \operatorname{ch}(\psi - \psi_0) \quad (29)$$



The equation (16), in turn, in new variables has the form:

$$\sum_{k=1}^N c_k^{new} \phi'_k + sh\psi = 0 \tag{30}$$

At the third stage of proof let us return to the previous analytical concentration  $\xi_k = 2k_w \xi_k^{new}$  and previous current density  $J = 2k_w J^{new}$ . According to (18) let us introduce new function  $c_k^{new} = c_k / 2k_w$  and the equality  $\sigma^{new} = \sigma / 2k_w$ , to the consideration, then the system of equations (28), (29), (30) transforms to the form (13), (14), (17) and integral condition (12) to the form (16)  $\Delta$ .

As a result, the system was reduced to a more compact form with reduced the number of unknown functions, in addition, as can be seen from (13)-(19), the type of algebraic and integral-differential dependency of unknown functions  $c_k$  is clearly indicated. The algebraic functions now depend on an auxiliary variable  $\psi$ , that is, on the concentration of hydrogen ions  $H^+$ (which follows from equation (19)). The coordinates dependence of the function  $c_k$  is expressed by the differential and integral relations, respectively, in equations (13) and (16).

**Theorem 2:** The system of equations (13)-(16) with integral conditions, with respect to  $N$  unknown functions  $a_k(x)$ ,  $k = 1, 2, \dots, N$ , may be reduced to the usual boundary problem, with respect to  $2N$  unknown function: 1)  $N$  function  $c_k(x)$ , determined from  $N$  differential equations (13), (14); 2)  $N$  auxiliary function  $n_k(x)$  determined from  $N$  differential equations and  $2N$  boundary conditions:

$$\frac{dn_k(x)}{dx} = a_k \phi_k(\psi) \tag{31}$$

$$\psi = 0.5 \ln \left( 1 + \sum_{k=1}^n a_k \exp(\psi_k) \right) - 0.5 \ln \left( 1 + \sum_{k=1}^n a_k \exp(-\psi_k) \right) \tag{32}$$

$$n_k(0) = 0 \tag{33}$$

$$n_k(1) = \frac{m_k}{\pi r^2}, \quad k = 1, 2, \dots, N \tag{34}$$

**Proof:** At the first stage the function  $\psi$  was expressed in terms of functions  $c_k(x)$ . Suppose that

$$F(\psi) = \sum_{k=1}^n a_k sh(\psi - \psi_k) + sh\psi \tag{35}$$

Then,  $F(\psi) = F'(\psi)$ . Evidently, the solution of this equation is the function:  $F(\psi) = A sh\psi + B ch\psi$ . From (4) the two equations are the following:

$$F(0) = A = -\sum_{k=1}^n a_k sh\psi_k, \quad F'(0) = B = -\sum_{k=1}^n a_k ch\psi_k + 1$$

The (15) is equivalent to the equation  $F(\psi) = 0$ . Consequently,  $th\psi = -A/B$  and expression of function  $\psi$  in terms of functions  $c_k(x)$  is (def. as (32)).

At the second stage the integral conditions (16) were transformed to the boundary conditions. New auxiliary functions were introduced:

$$n_k(x) = \int_0^x a_k \phi_k(\psi) dx \tag{36}$$

which satisfy the following boundary-value problem (def. as (31), (33), (34)) and allow to avoid essential numeric problem-integral conditions.  $\Delta$  As result, the problems of solution a)-b) are overcome.

**The numerical implementation of boundary-value problem:** To overcome problems of solution c)-e), the numerical solution of problem required the preliminary conversion and the creation of special algorithms. In order to avoid the negative (without physical meaning) solutions, the unknown functions  $a_k$  were represented as exponentials:

$$a_k = b_k \exp(1/\epsilon F_k(x)), \quad k = 1, 2, \dots, N \tag{37}$$

where  $b_k$  is constant (was defined as 1). The parameter  $1/\epsilon$  (is small magnitude) ensures high accuracy of calculation, because small increment of function  $F_k(x)$  corresponds to small increment of function  $a_k(x)$ .

For the numerical problem solving the special algorithms were developed. The first algorithm was constructed on basis of modified Runge-Kutta method and Newton's method. The second algorithm was based on parameter marching method. It allows the calculations in the wide interval of current density  $J$  without essential accumulation of errors. These algorithms were implemented in Turbo Pascal using the standard module Graph.

For example, the system of eight abstract ampholytes was considered (Fig. 2). The values of isoelectric points  $pI = 0.5(pK_1^{(k)} + pK_2^{(k)})$  fill out the interval from 4.0 to 7.5 in constant step  $\Delta pI = 0.5$ ;  $\Delta pK = 2$ ,  $pK_{1,2}^{(k)} = pI_k \pm \Delta pK$ . The initial quantities of ampholytes are  $m_k = 0.1$  (mol). Calculations were carried out under the assumptions that: the length of EC

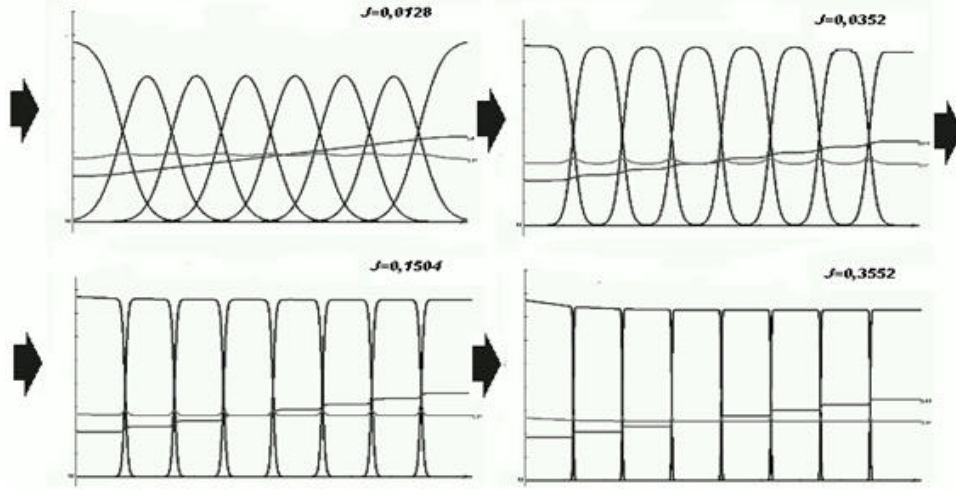


Fig. 2: Calculated and asymptotic concentration profiles of IEF system

is  $l = 2(\text{dm})$  and its radius is  $r = 0.2(\text{dm})$ ; the temperature is  $T = 298(\text{K})$ . The unit of current density measurement is  $\text{A}/\text{sq.dm}$ .

The graphs show, that at low and medium current density, the concentration profiles are similar to those of Gaussian distribution. At high current densities the system works in "normal" mode, which can not be described by Gaussian distribution. The curves have a "plateaus"-shaped form, which is very different from a Gaussian distribution. The more current density  $J$  is, the wider the "plateaus" is. The curves  $\text{pH}$  and  $\sigma$  are step-shaped.

Obtaining of singular asymptotics. Lemma 1: The system of differential equations (13)-(15) may be reduced to the form, which is independent of the variable  $x$ :

$$-\frac{1}{a_k} \frac{da_k}{d\psi} = \theta_k \frac{\sum_{i=1}^N a_i \theta_i' + 2k_w \text{ch}(\psi)}{\sum_{i=1}^N a_i \theta_i^2} \quad (38)$$

$$\sigma = \sum_{i=1}^N \mu_i a_i \theta_i' + 2k_w \mu \text{ch}(\psi - \psi_0) \quad (39)$$

$$\sum_{i=1}^N a_i \theta_i + 2k_w \text{sh}(\psi) = 0 \quad (40)$$

by means of insertion of two new functions:

$$a_k(x) = c_k \varphi_k(\psi) \quad (41)$$

$$\theta_k(\psi) = \frac{\varphi_k'(\psi)}{\varphi_k(\psi)} \quad (42)$$

**Proof:** Lemma 1 is proved in four steps:

- 1) The transition in (13) to the derivative with respect to  $\psi$ :

$$\varepsilon \frac{1}{c_k} \frac{dc_k}{d\psi} \psi_x' = \frac{\varphi_k' J}{\varphi_k \sigma}$$

- 2) Derivation of equation (15) with respect to  $x$  and with account of (13), we obtain:

$$\psi_x' = -\frac{J}{\sigma \varepsilon} \left( \sum_{i=1}^N c_i \frac{(\varphi_i')^2}{\varphi_i} \right) \left( \sum_{i=1}^N c_i \varphi_i'' + 2k_w \text{ch}(\psi) \right)^{-1} \quad (43)$$

- 3) Transformation of the latter two equations with account of (13) is reduced to the form:

$$-\frac{1}{c_k} \frac{dc_k}{d\psi} = \frac{\varphi_k'}{\varphi_k} \left( \sum_{i=1}^N c_i \varphi_i'' + 2k_w \text{ch}(\psi) \right) \left( \sum_{i=1}^N c_i \frac{(\varphi_i')^2}{\varphi_i} \right)^{-1} \quad (44)$$

- 4) Function (41), (42) are substituted into the equations (14), (17) and (44).

Consequently, the original system of equations is reduced to the system, which is formally independent of the variable  $x$ .  $\Delta$

In addition, a new function  $a_k$ ,  $k = 1, 2, \dots, N$ , was introduced into the consideration; it formally coincides with concentration function of  $\xi_k$ . Symbols  $a_k$  denote the asymptotic solution of the problem, which corresponds to the functions  $\xi_k$ . Functions  $a_k(\psi)$  should satisfy the following requirements: 1) to be continuous in all region of consideration, i.e. on the segment between isoelectric points of first and  $N$ -st ampholytes,  $a_k \in C[\psi_1, \psi_N]$ ,  $k = 1, 2, \dots, N$ ; 2) to be at least twice

continuously-differentiable on each of the segments between two adjacent isoelectric points, i.e.  $a_k \in C^2[\psi_n, \psi_{n+1}]$ ,  $k = 1, 2, \dots, N$ ,  $n = 1, 2, \dots, N-1$ .

The asymptotic solution was presented as a series in the small parameter (the square root of the ionic product of water):

$$a_k(\psi) = a_k^0(\psi) + k_w a_k^1(\psi) + k_w^2 a_k^2(\psi) \dots \quad (45)$$

Based on the supposition of the smoothness of function  $a_k$ , it is natural to assume that the functions  $a_k^0$  must satisfy the following requirements: 1)  $a_k^0 \in C[\psi_1, \psi_N]$ ,  $k = 1, 2, \dots, N$ ; 2)  $a_k^0 \in C^2[\psi_n, \psi_{n+1}]$ ,  $k = 1, 2, \dots, N$ ,  $n = 1, 2, \dots, N-1$ .

**Lemma 2:** The system of differential equations for definition functions  $a_k^0$ ,  $k = 1, 2, \dots, N$ , which are zero terms of (45), has the form:

$$-\frac{da_k^0}{d\psi} = a_k^0 \theta_k \frac{\sum_{i=1}^N a_i^0 \theta_i}{\sum_{i=1}^N a_i^0 \theta_i^2} \quad (46)$$

$$\sum_{i=1}^N a_i^0 \theta_i = 0 \quad (47)$$

$$\sum_{i=1}^N a_i^0 = a_0 \quad (48)$$

where  $a_0$  is constant, (to be determined later).

The proof is given in four steps: 1) obtaining of equations (46) and (47) by means of substitution of series (45) with subsequent reduction of homothetic terms; 2) obtaining equations (48) by means of summation of (46) with account of (47):

$$\frac{d}{d\psi} \left( \sum_{i=1}^N a_i^0 \right) = 0 \cdot \Delta$$

**Lemma 3:** The system of differential equations (46)-(48) to determine the functions  $a_k^0$ ,  $k = 1, 2, \dots, N$ , that are zero terms of (45), is homogeneous with respect to variables  $\theta_k$ ,  $k = 1, 2, \dots, N$ .

The proof is given by means of transfer of the (46) to the differentials:

$$da_k^0 = -\frac{a_k^0 \theta_k}{r} \sum_{i=1}^N a_i^0 d\theta_i, \quad r = \sum_{i=1}^N a_i^0 \theta_i^2$$

It follows that

$$\frac{\partial a_k^0}{\partial \theta_i} = -\frac{a_k^0 \theta_k}{r} a_i, \quad i, k = 1, 2, \dots, N$$

The summation of the latest equations with account of (48) leads to:

$$\sum_{i=1}^N \theta_i \frac{\partial a_k^0}{\partial \theta_i} = 0$$

It means, that the system (46)-(48) is homogeneous with respect to  $\theta_k$ ,  $k = 1, 2, \dots, N$ .  $\Delta$

Lemma 3 implies that in the system (46)-(48), a transition to a new variable, which provides a more convenient form for study entry, is possible.

**Lemma 4:** The system of differential equations (46)-(48) to determine the functions  $a_k^0$ ,  $k = 1, 2, \dots, N$ , which are zero terms of (45) and satisfy smoothness conditions 1)  $a_k^0 \in C[\psi_1, \psi_N]$ ,  $k = 1, 2, \dots, N$ ; 2)  $a_k^0 \in C^2[\psi_n, \psi_{n+1}]$ ,  $k = 1, 2, \dots, N$ ,  $n = 1, 2, \dots, N-1$ , by successive changes of variables can be reduced to a system with the total differential equality for each of the segments  $\psi \in [\psi_n, \psi_{n+1}]$ ,  $n = 1, 2, \dots, N-1$ .

The proof (for  $N = 3$ , for simplification).

1) At first stage the first substitution was used:

$$\omega_1 = \theta_1 / \theta_3, \quad \omega_2 = \theta_2 / \theta_3 \quad (49)$$

In the new variable the system (46)-(48) reduces to:

$$-\frac{da_1^0}{d\psi} = a_1^0 \omega_1 F(\omega_1, \omega_2) \quad (50)$$

$$-\frac{da_2^0}{d\psi} = a_2^0 \omega_2 F(\omega_1, \omega_2) \quad (51)$$

$$a_3^0 = -a_1^0 \omega_1 - a_2^0 \omega_2 \quad (52)$$

where

$$F(\omega_1, \omega_2) = (a_1^0 \omega_1' - a_2^0 \omega_2') (a_1^0 \omega_1 (\omega_1 - 1) - a_2^0 \omega_2 (\omega_2 - 1))^{-1}$$

Namely the substitution (49) imposes the requirement  $\psi \neq \psi_3$ , i.e.  $\psi \in [\psi_1, \psi_2]$ .

2) The second substitution of variable is made:

$$R_1 = (\omega_1 - 1)(1 - \omega_2)^{-1}, \quad R_2 = (1 - \omega_2)^{-1} \quad (53)$$

The system (50)-(52) is reduced to:

$$-\frac{da_1^0}{d\psi} = \frac{R_1 + R_2}{R} a_1^0 (a_1^0 R_1' + a_2^0 R_2') \quad (54)$$

$$a_2^0 = a_0 R_2 + a_1^0 R_1 \quad (55)$$

$$a_3^0 = a_0 (1 - R_2) - a_1^0 (1 + R_1) \quad (56)$$

where

$$R = a_1^0 R_1 (R_1 + 1) + a_0 R_2 (1 - R_2)$$

3) The equation (54) may be given as following:

$$da_1^0 = (R_1 + R_2) R^{-1} a_1^0 (a_1^0 dR_1 + a_2^0 dR_2) \quad (57)$$

Consequently, the original system (46)-(48) is reduced to the system of algebraic equations and one total differential equality.  $\Delta$

**Lemma 5:** The solution of the system of differential equations (46)-(48) on the each of segments

The solution of this quadratic equation has the form:

$$(a_1^0)_{1,2} = \frac{a_0}{2R_1(R_1 + 1)} (R_1 - 2R_1R_2 - R_2 \pm ((R_2 + 2R_1R_2 - R_1)^2 + 4R_1R_2(R_1 + 1)(1 - R_2))^{0.5})$$

The return to the formulas (49) and (53) leads to the following equalities:

$$(a_1^0)_{1,2} = \frac{a_0}{2(\theta_1 - \theta_3)(\theta_1 - \theta_2)} [\theta_3(\theta_2 - \theta_1) + \theta_2(\theta_3 - \theta_1) \pm (\theta_3(\theta_2 - \theta_1) - \theta_2(\theta_3 - \theta_1))]$$

Taking into account formulas (55) and (56) two solution of the original system (46)-(48) were obtained:

$$(a_1^0)_1 = -a_0 \frac{\theta_3}{\theta_1 - \theta_3}, (a_2^0)_1 = 0, (a_3^0)_1 = a_0 \frac{\theta_1}{\theta_1 - \theta_3} \quad (60)$$

and

$$(a_1^0)_2 = -a_0 \frac{\theta_2}{\theta_1 - \theta_2}, (a_2^0)_2 = a_0 \frac{\theta_1}{\theta_1 - \theta_2}, (a_3^0)_2 = 0 \quad (61)$$

Obviously, the solution (60) does not correspond to the form of profiles in "anomalous" regimen. Consequently, we conclude that there is the solution (61) at  $\psi \neq \psi_3$ . Similarly, at  $\psi \neq \psi_2$ :

$$a_1^0 = 0, a_2^0 = -a_0 \frac{\theta_3}{\theta_2 - \theta_3}, a_3^0 = a_0 \frac{\theta_2}{\theta_2 - \theta_3} \quad (62)$$

The graphics show, that functions  $a_1^0, a_2^0, a_3^0$  must be continuous. The continuity is ensured, if solution is defined by formula (61) at  $\psi \in [\psi_1, \psi_2]$  and formula (70) at  $\psi \in [\psi_2, \psi_3]$ . In fact,

$$a_1(\psi_2 - 0) = 0 = a_1(\psi_2 + 0), a_2(\psi_2 - 0) = a_0 = a_2(\psi_2 + 0), a_3(\psi_2 - 0) = 0 = a_3(\psi_2 + 0)$$

Similarly, the assertion is proved in the case of an arbitrary number of ampholytes.  $\Delta$

$\psi \in [\psi_n, \psi_{n+1}], n = 1, 2, \dots, N-1$ , can be represented as a function  $a_k^0 \in C^2[\psi_n, \psi_{n+1}], k = 1, 2, \dots, N$ , defined by following formulas:

$$a_n^0 = -a_0 \frac{\theta_{n+1}}{\theta_n - \theta_{n+1}}, a_{n+1}^0 = a_0 \frac{\theta_n}{\theta_n - \theta_{n+1}}, a_k^0 = 0, k \neq n, n+1 \quad (58)$$

The proof It follows from (57), that

$$\frac{\partial a_1^0}{\partial R_1} = -\frac{(a_1^0)^2 (R_1 + R_2)}{r}, \frac{\partial a_1^0}{\partial R_2} = -\frac{a_0 a_1^0 (R_1 + R_2)}{r}$$

Consequently, the condition must be met

$$\frac{\partial}{\partial R_2} \left( \frac{\partial a_1^0}{\partial R_1} \right) = \frac{\partial}{\partial R_1} \left( \frac{\partial a_1^0}{\partial R_2} \right)$$

It has the form:

$$R_1(R_1 + 1)(a_1^0)^2 + a_0 a_1^0 (R_2 + 2R_1R_2 - R_1) + a_0^2 R_2 (R_2 - 1) = 0 \quad (59)$$

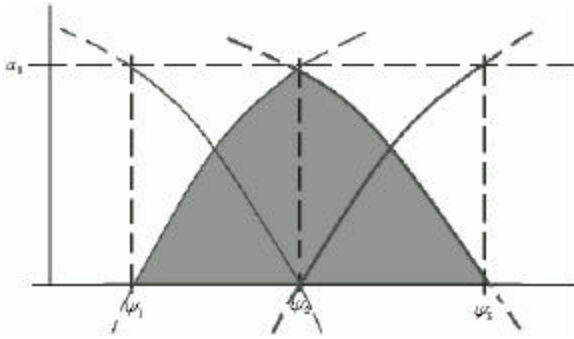


Fig. 3: Approximation of concentration profile by two fragments of unlimited function

**Lemma 6:** The dependence of zero term of the series (45)  $a_k^0$  of the variable  $x$  in each of the intervals  $\psi \in (\psi_n, \psi_{n+1})$ ,  $n = 1, 2, \dots, N-1$  is expressed by means of the following differential equation:

$$\frac{dx}{d\psi} = \frac{\varepsilon a_0}{J} \frac{\mu_n \theta'_n \theta_{n+1} - \mu_{n+1} \theta_n \theta'_{n+1}}{\theta_n - \theta_{n+1}} \left( 1 + \frac{\theta'_n \theta_{n+1} - \theta_n \theta'_{n+1}}{\theta_n - \theta_{n+1}} \right) \quad (63)$$

The proof is carried out by:

- 1) Transformation of equation (50) with account of (49) to the form:

$$\frac{\varepsilon}{J} \left( \frac{da_k}{d\psi} \frac{1}{a_k} - \theta_k \right) \psi'_x = \frac{\theta_k}{\sigma} \quad (64)$$

- 2) The summation of the equations (64)  $k = 1, 2, \dots, N$  with account of (38)-(40):

$$-\frac{\varepsilon}{J} \left( \sum_{i=1}^N \mu_i a_i \theta'_i + 2k_w \mu \operatorname{ch}(\psi - \psi_0) \right) \frac{\sum_{i=1}^N a_i (\theta'_i + \theta_i^2) + 2k_w \operatorname{ch}(\psi)}{\sum_{i=1}^N a_i \theta_i^2} = \frac{dx}{d\psi} \quad (65)$$

- 3) Obtaining the equation for the zero term of the series for  $x$  from (65):

$$-\frac{\varepsilon}{J} \left( \sum_{i=1}^N \mu_i a_i^0 \theta'_i \right) \left( \sum_{i=1}^N a_i^0 (\theta'_i + \theta_i^2) \right) = \frac{dx}{d\psi} \left( \sum_{i=1}^N a_i^0 \theta_i^2 \right) \quad (66)$$

- 4) Obtaining the equation (63) from (66) and (13).  $\Delta$

**Note:** The equation (63) shows, that in denominator of the fraction there is the product of functions  $\theta_n \theta_{n+1}$ , which are zero in the points  $\psi = \psi_k$  and  $\psi = \psi_{k+1}$  respectively. Thus, this formula is not applicable in the region of points  $\psi = \psi_k$  and  $\psi = \psi_{k+1}$ ; in these points calculation should be made with other asymptotic formulas; for example, tangent or saddle-point methods [20].

This fact, as well as the piecewise form of the solution lead to some questions. What are the unlimited functions, which fragments of the solution are they formed from and how do they coordinate with the calculated solution to the problem? Why does the "singularity" arise, which does not allow to use the given formulas  $\psi(x)$  in the entire range of integration?

The proofs of lemmas show, that the solution is obtained by means of rigorous mathematical transformations of the original system. The solution contains only two obvious suppositions, compared with the original problem. These are the suppositions of pair wise profiles of ampholytes in "anomalous" regimen and the possibility to neglect terms with  $k_w$ . However, there is still another factor: the absence of the requirement of nonnegative solutions.

The physical sense of problem requires the nonnegativity of the unknown concentration functions. The search of the numerical solution of integro-differential problem takes the form of exponential function (37). This method cuts off all negative solutions, which are sure to have the differential equations. In the asymptotic solution of problem this requirement was absent. As a result, in the transition to the total differential equation the partial solution of problem was obtained, which coincide with the calculated one in local region, but does not have sense in other regions of integration. In fact, the area of application of the developed asymptotics is limited by its positive values. In isoelectric points asymptotic formulas reduce to zero; hence, in these points the asymptotics may be applied only in the limiting case.

**Lemma 7:** The coefficient  $a_0$  in formulas (63) is defined by equation:

$$a_0 = \frac{1}{L} \sum_{i=1}^N M_i, \quad M_i = m_i \pi r^2 \quad (67)$$

The proof consists of three stages. At the first stage, in the integral conditions (16) there is a formal transition to the variable  $\psi$ :

$$\int_{\psi_1}^{\psi_N} a_k(\psi) x'_\psi d\psi = M_k, \quad M_k = \frac{m_k}{\pi r^2} \quad (68)$$

At the second stage the series of functions  $x$  and  $a_k$  for degrees  $k_w$  are substituted in the conditions (68) and equations for the zero order are then added together:

$$\int_{\psi_1}^{\psi_2} (a_1^0 + a_2^0) x'_\psi d\psi + \int_{\psi_2}^{\psi_3} (a_2^0 + a_3^0) x'_\psi d\psi + \int_{\psi_3}^{\psi_4} (a_3^0 + a_4^0) x'_\psi d\psi + \dots + \int_{\psi_{N-2}}^{\psi_{N-1}} (a_{N-2}^0 + a_{N-1}^0) x'_\psi d\psi + \int_{\psi_{N-1}}^{\psi_N} (a_{N-1}^0 + a_N^0) x'_\psi d\psi = \sum_{i=1}^N M_i \quad (69)$$

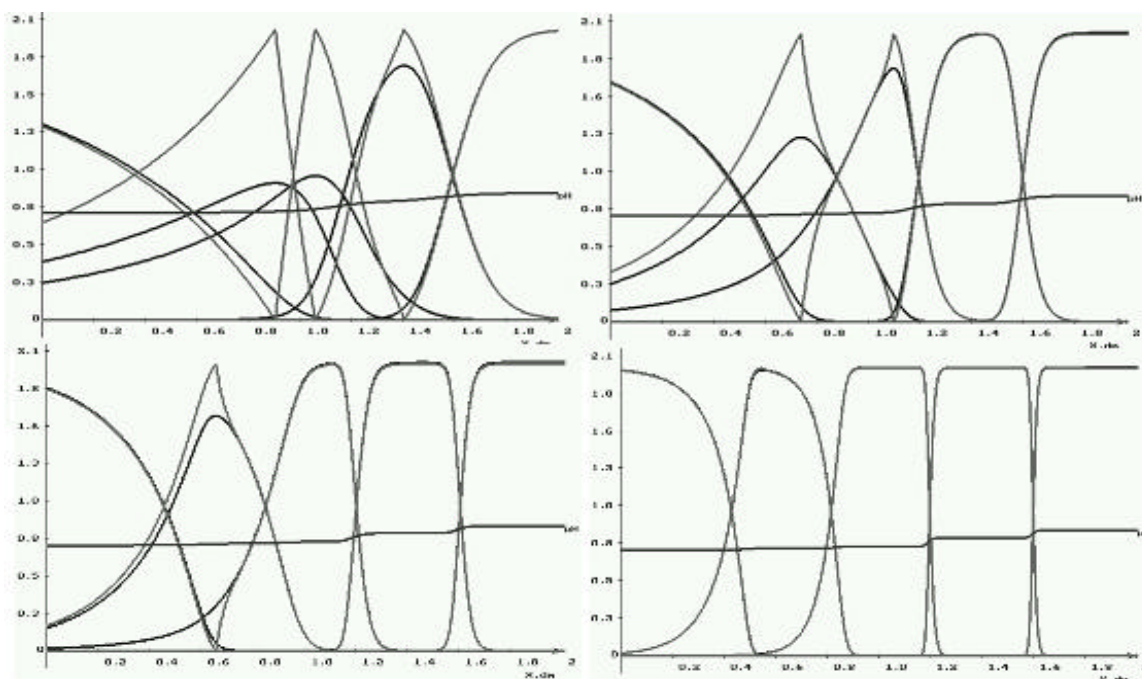


Fig. 4: Calculated and asymptotic profile of concentration of IEF system His -His, His -Gly, His,  $\beta$ -Ala-His, Tyr-Arg

At the third stage, given that  $\psi \in [\psi_n, \psi_{n+1}]$   $a_n^0 + a_{n+1}^0 = a_0$  each of the integrals in the last equation reduces to the simple integral by means of return to the variable  $x$ ; resulting in the equation (68).  $\Delta$

Note: The solution obtained is a particular solution of the equation (58), which is a special case of the differential equation of Abel.

Thus, this study has shown that the "anomalous" regimen mode solution of (13)-(15) is described by the zero-order terms of the series (44). The physical meaning of this fact is made clear by comparing the singular asymptotic formula (58) with (24), which determines the difference of the degrees of dissociation. In fact, the formula should be.

**Theorem 3 (The physical sense of "anomalous" regimen):** In "anomalous" regimen the distribution of two ampholytes between its isoelectric points is expressed by means of difference between its degrees of dissociation  $n$ -s and  $(n+1)$ -s ampholytes :

$$a_n^0 = -a_0 \frac{e_{n+1}}{e_n - e_{n+1}}, a_{n+1}^0 = a_0 \frac{e_{n+1}}{e_n - e_{n+1}}, a_0 = (\pi^2 L)^{-1} \sum_{i=1}^N m_i \quad (70)$$

where

$$e_k = \alpha_1^k - \alpha_{-1}^k = \text{sh}(\psi - \psi_k)(\delta_k + \text{ch}(\psi - \psi_k))^{-1}$$

$m_k$  are initial quantities of ampholytes,  $r$  and  $L$  are radius and length of EC. The gradient  $\text{pH}$  is also the

function of difference between its degrees of dissociation and concentrations of hydrogen  $H^+$ .

The proof is implemented by comparing of (58), Lemma-6 and Lemma-7 with formulas (24) for the difference between degrees of dissociation.

The study of the asymptotic solutions by means of graphical methods. The calculations were made under the assumptions that: the length of EC is  $l = 2(\text{dm})$  and its radius is  $r = 0.2(\text{dm})$ ; temperature is  $T = 298(\text{K})$ . Initial quantities of all ampholytes are  $m_k = 1(\text{mole})$ . The unit of current density measurement is  $\text{A}/\text{sq.dm}$ . Characteristics of ampholytes, used in experiment, are given in [1] (Table 1).

On the figures the concentration profiles obtained earlier by numerical methods are shown in black and the profiles corresponding to the asymptotic solution are in gray. The asymptotic solution is obtained by (58) on the basis of the values obtained by numerical methods.

The system of five standard ampholytes with  $\text{pH} > 7$  was considered: His-His, His-Gly, His,  $\beta$ -Ala-His, Tyr-Arg (Fig. 3). Figure 2 illustrates the following tendencies identified: 1) at low and medium current density singular asymptotics accurately reflects the ampholytes localization in EC, but has a series of divergence in the form of profiles; 2) the forming of "plateaus"-shape profile (i.e. exit to the "anomalous" regimen) leads to the full compliance of profile with asymptotics, which remains with the further increase of  $J$ ; 3) at high current densities there is full compliance for all profiles of ampholytes.

Table 1: Characteristics of ampholytes

N	Ampholyte	pK <sub>1</sub> <sup>(k)</sup>	pK <sub>2</sub> <sup>(k)</sup>	pI	ΔpK	Mobility×10 <sup>-4</sup>
1	His-His	6.80	7.80	7.30	1.00	1.49
2	His-Gly	6.27	8.57	7.42	2.30	2.40
3	His	6.00	9.17	7.59	3.17	2.85
4	β-Ala-His	6.83	9.51	8.17	2.68	2.30
5	Tyr-Arg	7.55	9.80	8.68	2.25	1.58

Consequently, created singular model have had successful verification by the calculated experiment.

Asymptotic behavior remains the same for different IEF systems regardless of the number of ampholytes, their mass and characteristics.

### CONCLUSION

1. It is found that in "anomalous" regimen modes the initial integro-differential problem is expressed by the asymptotic methods and its singular asymptotic solution was received. The solution by the system of differential equations (46)-(48) on the each of the segments  $\psi \in [\psi_n, \psi_{n+1}]$ ,  $n = 1, 2, \dots, N-1$ , has the form of function  $a_k^0 \in C^2[\psi_n, \psi_{n+1}]$ ,  $k = 1, 2, \dots, N$ , defined by the formulas;

$$a_n^0 = -a_0 \frac{\theta_{n+1}}{\theta_n - \theta_{n+1}}, a_{n+1}^0 = a_0 \frac{\theta_n}{\theta_n - \theta_{n+1}}, a_k^0 = 0, k \neq n, n+1$$

The dependence of functions  $a_k^0$  from variable  $x$  on each of the intervals  $\psi \in (\psi_n, \psi_{n+1})$ ,  $n = 1, 2, \dots, N-1$ , is expressed by means of differential equation:

$$\frac{dx}{d\psi} = \frac{\epsilon a_0}{J} \cdot \frac{\mu_n \theta'_n \theta_{n+1} - \mu_{n+1} \theta_n \theta'_{n+1}}{\theta_n - \theta_{n+1}} \left( 1 + \frac{\theta'_n \theta_{n+1} - \theta_n \theta'_{n+1}}{\theta_n \theta_{n+1} (\theta_n - \theta_{n+1})} \right)$$

where  $\epsilon = RT/F$  is a standard electrochemical parameter, where magnitudes  $R$ ,  $T$ ,  $F$  are absolute gas constant, temperature and Faraday constant, respectively.

2. The software was created, which allowed to find numeric and asymptotic solution and show its compliance in the "anomalous" regimen; by means of electrochemical interpretation of obtained formulas the physical sense of "anomalous" regimen was established; the complex mathematical model of IEF was developed, which allowed to carry out computations for real IEF-systems. Calculated experiment shows, that singular asymptotics has full compliance with explicit solution of mathematical IEF-problem (13)-(16) in the "anomalous" regimen.

Software developed on the basis of the constructed asymptotics has made it possible to establish a high degree of consistency between asymptotic solutions and singular solutions of the original stiff integral-differential problem.

3. Singular asymptotics leads to the important physical (electrochemical) conclusion: in "anomalous" regimen the distribution of two ampholytes between its isoelectric points is expressed by means of difference between its degrees of dissociation  $n$ -s and  $(n+1)$ -s of ampholytes:

$$a_n^0 = -a_0 \frac{e_{n+1}}{e_n - e_{n+1}}, a_{n+1}^0 = a_0 \frac{e_{n+1}}{e_n - e_{n+1}}, a_0 = (2\pi^2 L)^{-1} \sum_{i=1}^N m_i$$

where  $e_k = \alpha_1^k - \alpha_{-1}^k = \text{sh}(\psi - \psi_k)(\delta_k + \text{ch}(\psi - \psi_k))^{-1}$ ,  $m_k$  are initial quantities of ampholytes,  $r$  and  $L$  are radius and length of EC. The gradient  $pH$  is also the function of difference between its degrees of dissociation and concentrations of hydrogen ions  $H^+$ .

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