

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

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

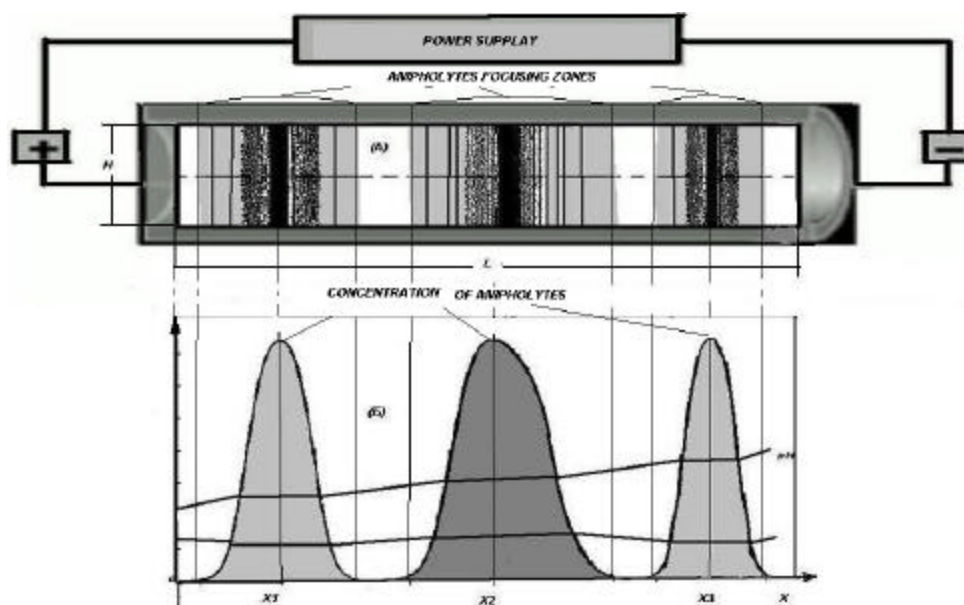


Fig. 1: (a): The stationary distribution of three ampholytes in EC; (b): The profiles of ampholytes

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 μ_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 ξ_1^k , ξ_{-1}^k , ξ_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 α_1^k and α_{-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 ξ_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):

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

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

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

$$\pi \int_0^1 \xi_k(x) dx = m_k \quad (12)$$

where $\varepsilon = RT/F$ is standard electrochemical parameter, where magnitudes R , T , F are absolute gas constant, temperature and Faraday constant, respectively; μ_H and μ_{OH} are constants, characteristic of ions mobility H^+ and OH^- ; D_k , D_H and D_{OH} constants are the ions diffusion coefficients, $D_k = \varepsilon \mu_k$; α_1^k and α_{-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/ε 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 ψ 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^{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) Δ .

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 ψ , 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 ψ 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 ψ 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. Δ 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

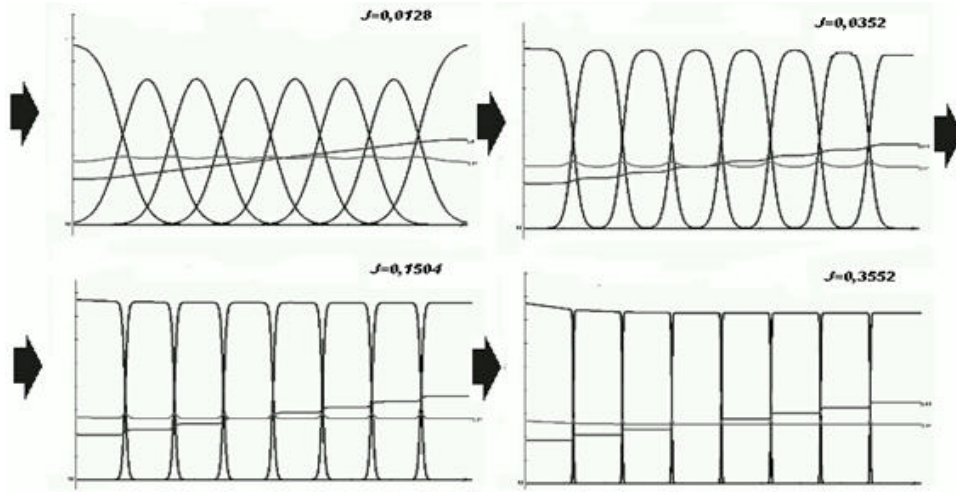


Fig. 2: Calculated and asymptotic concentration profiles of IEF system

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 pH and σ are step-shapped.

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 ψ :

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

- 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 . Δ

In addition, a new function a_k , $k = 1, 2, \dots, N$, was introduced into the consideration; it formally coincides with concentration function of ξ_k . Symbols a_k denote the asymptotic solution of the problem, which corresponds to the functions ξ_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 θ_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 θ_k , $k = 1, 2, \dots, N$. Δ

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. Δ

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. Δ

$\psi \in [\psi_n, \psi_{n+1}], n = 1, 2, \dots, N-1$, 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:

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

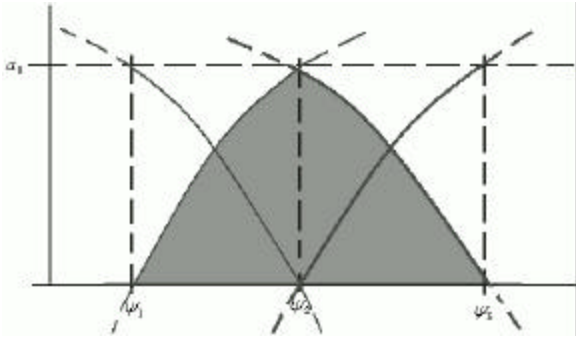


Fig. 3: Approximation of concentration profile by two fragments of unlimited function

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). Δ

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 ψ :

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

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:

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

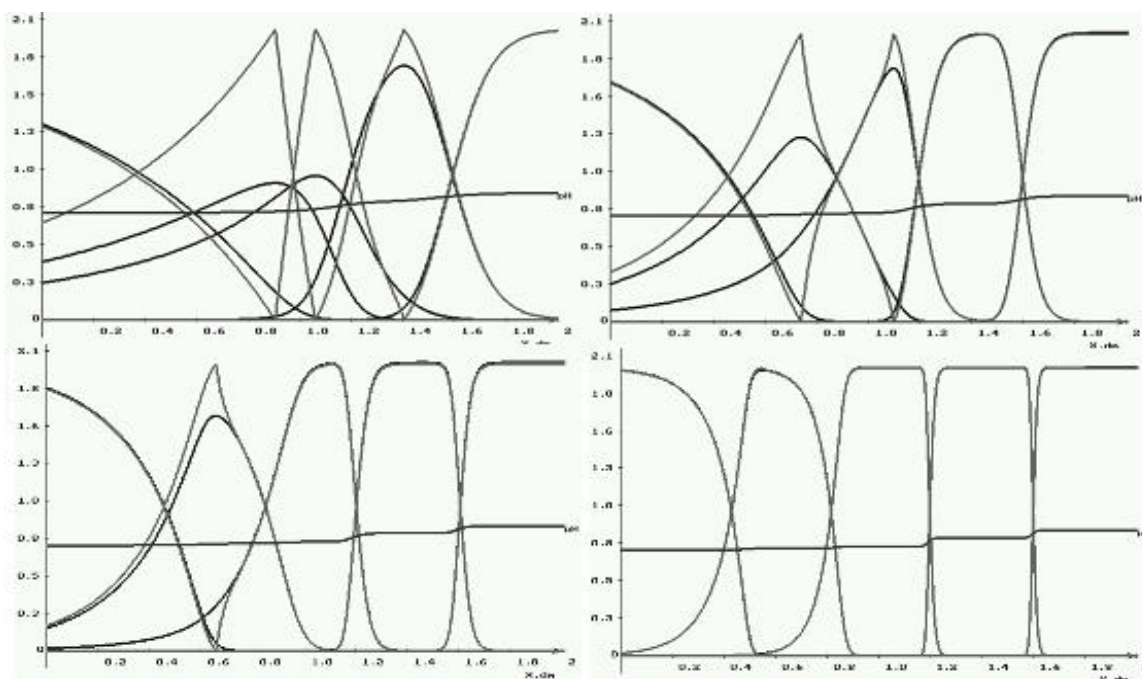


Fig. 4: Calculated and asymptotic profile of concentration of IEF system His -His, His -Gly, His, β -Ala-His, Tyr-Arg

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). Δ

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 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 $l = 2$ (dm) and its radius is $r = 0.2$ (dm); temperature is $T = 298$ (K). Initial quantities of all ampholytes are $m_k = 1$ (mole). The unit of current density measurement is A/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 $pH > 7$ was considered: His-His, His-Gly, His, β -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 ₁ ^(k)	pK ₂ ^(k)	pI	ΔpK	Mobility×10 ⁻⁴
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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