Anomalous pH-gradient in Ampholyte Solution

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A mathematical model describing a steady pH-gradient in the solution of ampholytes in water has been studied with the use of analytical, asymptotic, and numerical methods. We show that at the large values of an electric current a concentration distribution takes the form of a piecewise constant function that is drastically different from a classical Gaussian form. The correspondent pH-gradient takes a stepwise form, instead of being a linear function. A discovered anomalous pH-gradient can crucially affect the understanding of an isoelectric focusing process.

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I. INTRODUCTION

The isoelectric focusing (IEF) is a classical method of a mixture separation that is widely used in medicine and biology. There is an enormous number of publications devoted to its theory and applications. We mention here only fundamental papers [1, 2, 3, 4, 5], the monographs considering various theoretical and practical aspects of the IEF [6, 7, 8], and papers [9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20] that contain mathematical models and computer simulations that are close to the topic of our paper. There are also a number of papers that study the related problems in space biotechnology [21, 22, 23, 24, 25, 26, 27].

An important part of the IEF is the creation of stable pH-gradients that are used for the setting up of a focusing process. Carrier ampholytes have been in a wide practical use for the creation of pH-gradients starting from pioneering papers [1, 2, 3, 4, 5] (see also [6, 16]). The use of immobilized pH-gradients for the same purpose has been proposed and introduced by [7, 13], while the use of borate-polyol systems for the creation of pH-gradients is considered in [28]. The mathematical models describing the formation of stable pH-gradients with the use of carrier ampholytes are given in [13, 14, 15, 19, 20]. In particular, [14, 20] introduce the notion of mixtures with the infinite number of components (the infinite-component mixtures) where the authors formally replace the discrete number $k$ in the concentration $c_k$ onto the continuous parameter $s$; in other words the discrete set $c_k, k = 1, \ldots, n$ is replaced by the distribution function $c(s)$. The first and simplest mathematical models describing the creation of linear (or almost linear) pH-gradients with the use of carrier ampholytes were given in [2, 4]. More advanced approaches that employ the governing differential equations of continuous media, mathematical modeling, and computations are given in [15, 16]. These papers show that an ampholyte concentration distribution should be close to Gaussian’s one and a pH-gradient should be linear. Similar results with the use of models for infinite-component mixtures were obtained in [19].

The statement that a concentration distribution possesses a Gaussian shape is currently accepted as a key classical result. Moreover, it is widely assumed that the increase of a potential difference (or an electric current) stabilizes a linear pH-gradient and intensifies a focusing process. On the contrary, the main results of our paper show that the increase of an electric current (starting from some threshold value) causes an anomalous steady stepwise pH-gradient and a piecewise-constant concentration distribution. In addition we present explicit analytical expressions for a pH distribution and for an electric conductivity as the functions of ampholyte concentrations. The obtained asymptotic expressions for the concentrations of ampholytes allow us to carry out the calculations of stepwise pH-gradients.

II. MATHEMATICAL MODEL

Well-known governing equations describing IEF in the aqueous solution of ampholytes in dimensionless variables are [13, 19, 20]:

$$ \frac{\partial c_k}{\partial t} + \frac{\partial i_k}{\partial x} = 0, \quad k = 1, \ldots, n, \quad (1) $$
\[ i_k = -\varepsilon \gamma_k \frac{\partial c_k}{\partial x} + \gamma_k e_k(\psi) c_k E, \]  
\[ j = \sum_{k=1}^{n} \gamma_k \left( -\varepsilon \frac{\partial (e_k(\psi) c_k)}{\partial x} + \sigma_k(\psi) c_k E \right) + 2K_w \gamma_0 \left( E - \varepsilon \frac{\partial \psi}{\partial x} \right) \cosh(\psi - \psi_0), \]  
\[ \sum_{k=1}^{n} e_k(\psi) c_k + 2K_w \sinh \psi = 0, \]  
where
\[ \gamma_0 = \sqrt{\gamma_H \gamma_{OH}}, \quad \psi_0 = \frac{1}{2} \ln \frac{\gamma_{OH}}{\gamma_H}, \]  
\[ h = K_w e^\psi, \quad pH = -\ln h = -\ln K_w - \psi \log e. \]  

Here \( c_k(x, t) \) are molar ampholyte concentrations, \( \psi(x, t) \) is a function, linearly connected to \( \psi \) of a solution, \( E(x, t) \) is the strength of an electric field, \( i_k \) are the densities of molar ampholyte fluxes, \( j = \text{const} \) is the density of an electric current (it can also depend on time \( j = j(t) \)), \( h \) is a hydrogen ion concentration, \( \varepsilon \) is a diffusion parameter, \( \gamma_k > 0 \) are characteristic mobilities of components in an electric field, \( \varepsilon \gamma_k > 0 \) are diffusion coefficients, \( e_k(\psi) \), \( \sigma_k(\psi) \) are the molar charges and the molar conductivities of ampholytes, \( \gamma_k e_k(\psi) \) are the electrophoretic mobilities of ampholytes, \( \gamma_H, \gamma_{OH} \) are the mobilities of hydrogen \( H^+ \) and hydroxyl \( OH^- \) ions, \( \gamma_0 \) is the effective mobility of water ions, \( K_w \) is the dissociation constant for water, \( \psi_0 \) is the value of \( \psi \) when water conductivity is minimal.

The system of equations (1)–(6) describes the mass transport under the action of an electric field in a chemically active medium. Chemical reactions in the medium are assumed to be very fast (being completed almost instantly). The functions \( e_k(\psi) \), \( \sigma_k(\psi) \) are given; in each particular case they can be defined from the chemical kinetic equations (see below). The algebraic equation (1) represents the condition of mixture electroneutrality; it allows us to find \( \psi \). In fact, this equation describes the instant control of medium properties (electrophoretic mobilities and molar conductivities) by the function \( \psi \) (that is linked to the concentration of hydrogen ions). Such situation is typical only for a weak aqueous solution; in more general cases the values \( e_k, \sigma_k \) can depend on \( \psi \).

The term \( 2K_w \gamma_0 \left( E - \varepsilon \frac{\partial \psi}{\partial x} \right) \cosh(\psi - \psi_0) \) describes the contribution of water ions into mixture conductivity, while the term \( 2K_w \sinh \psi \) in (1) corresponds to the contribution of these ions into a mixture molar charge. These terms should be included into the governing equations if the autodissociation reaction is taken into account:

\[ H_2O \xrightleftharpoons[K_w]{k^-} H^+ + OH^-. \]

In this case the concentrations of ions \( H^+ \) and \( OH^- \) are related as

\[ h = [H^+], \quad [OH^-] = \frac{K_w^2}{[H^+]} = \frac{K_w^2}{h}, \]

where \( K_w^2 \) is an autodissociation constant for water, square brackets \([ \cdot ]\) denote the values of concentrations, as it is accepted in chemistry.

Let us note that the maximal values of concentrations \( c_k \) and the values \( \gamma_0, \psi_0, \gamma_k, \psi_k, \gamma_k = 1, \ldots, n \) are all of the order \( O(1) \), while the parameters \( \varepsilon \) and \( K_w \) are small.

Notice, that we denote an autodissociation constant for water as \( K_w^2 \) not \( K_w \); hence \( K_w \) represents a dissociation constant for water, its value in dimensional variables is \( K_w = 10^{-7} \text{ mol/l} \). In addition, instead of a conventionally used function \( pH \) (measure of the acidity or alkalinity of a solution) we use function \( \psi \) (linearly connected with \( pH \)) which is better adapted to our mathematical model (see (2)–(3)).

In order to make the system (1)–(6) complete, we have to prescribe the functions \( e_k(\psi) \), \( \sigma_k(\psi) \) for ampholytes. Let us consider the solution of \( n \) ampholytic substances (ampholytes) that exhibit both acid and based properties (a typical representative is an amino acid). The dissociation reactions are \( (i = 1, \ldots, n) \)

\[ H^+ R \equiv R_i^+ + H^+, \quad R_i^0 \equiv R_i^- + H^+. \]  

Here \( R_i^0 \) is a zwitterion (for instance an amino acid residue), \( A_i \) and \( B_i \) are dissociation constants for acid (\( R_i^- \)) and based (\( H^+ R_i \)) groups correspondingly. Notice, that even for polyampholytes with supplementary side groups a reaction scheme can be described by (7), assuming the \( pH \) of a solution being close to the isoelectric point of a substance [8].

The analytical concentrations \( c_i \), molar charges \( e_i(\psi) \), and molar conductivities \( \sigma_i(\psi) \) for (7) are:

\[ c_i = [H^+ R_i] + [R_i^0] + [R_i^-], \]
\[ e_i(\psi) = \frac{1}{\varphi_i(\psi)} \frac{d \varphi_i(\psi)}{d \psi}, \quad \sigma_i(\psi) = \frac{1}{\varphi_i(\psi)} \frac{d^2 \varphi_i(\psi)}{d \psi^2}, \]
\[ \varphi_i(\psi) = \delta_i + \cosh(\psi - \psi_i), \]
\[ \psi_i = \frac{1}{2} \ln \frac{A_i B_i}{K_w^2}, \quad \delta_i = \frac{1}{2} \left( \frac{B_i}{A_i} \right). \]

Here \( \psi_i \) is an isoelectric point (the value of \( \psi \) when the molar charge \( e_k(\psi) \) is zero, i.e., \( e_k(\psi_\varepsilon) = 0 \), \( \varphi_i \) and \( \delta_i \) are auxiliary notations. Notice, that it is convenient not to write explicit expressions for \( e_k(\psi) \) and \( \sigma_k(\psi) \) but to present them as the derivatives of functions \( \varphi_i(\psi) \).
III. FORMULATION OF STEADY PH-GRADIENT PROBLEM

Let us take \( \partial c_k/\partial t = 0 \) and introduce non-leak boundary conditions for the ampholytes at the walls of an electrophoretic chamber \((0 \leq x \leq 1)\):

\[
i_k(0) = 0, \quad i_k(1) = 0, \quad k = 1, \ldots, n. \quad (9)
\]

The total molar concentration of each ampholyte is:

\[
\int_0^1 c_k(x)dx = M_k, \quad k = 1, \ldots, n. \quad (10)
\]

The values \( \gamma_0, \psi_0, \gamma_k, \psi_k, M_k, k = 1, \ldots, n, K_w, \varepsilon \) are given. For the sake of simplicity we consider only the IEF case with a given electric current density \( j = \) const.

The problem with a given constant voltage can be solved similarly, however it is more tedious. The integration of equations (1) with boundary conditions (9) yields \( i_k = 0 \) that can be written as:

\[
-\varepsilon \frac{dc_k}{dx} + c_k(\psi)c_k E = 0, \quad k = 1, \ldots, n. \quad (11)
\]

The remaining equations keep their form:

\[
j = \sum_{k=1}^n \gamma_k \left( -\varepsilon \frac{d(c_k(\psi)c_k)}{dx} + \sigma_k(\psi)c_k E \right)
+ 2K_w \gamma_0 \left( E - \varepsilon \frac{d\psi}{dx} \right) \cosh(\psi - \psi_0), \quad (12)
\]

\[
\sum_{k=1}^n e_k(\psi)c_k + 2K_w \sinh \psi = 0. \quad (13)
\]

The system of equations (11)–(13) with additional conditions (10) allow to find the concentrations \( c_k(x), \quad k = 1, \ldots, n \), the strengths of the electric field \( E(x) \), and the function \( \psi(x) \) (i.e. pH\((x)\)). Let us emphasize here that despite of the presence of derivative \( d\psi/dx \) we do not need to introduce any supplementary condition for \( \psi \), since the function \( \psi \) has been determined by the algebraic equation (13).

IV. THE TRANSFORMATION OF THE PROBLEM

For the problem (10)–(13) we have found the change of dependent variables that allows us to obtain explicit analytical expressions for functions \( \psi(x) \) and \( E(x) \). We introduce a new ‘concentration’ \( a_k(x) \) and the ‘effective’ strength of the electric field \( E_0(x) \) as:

\[
c_k(x) = a_k(x) \varphi_k(\psi(x)),
\]

\[
E_0(x) = E(x) - \varepsilon \frac{d\psi(x)}{dx}. \quad (14)
\]

In this case one can easily show that \( \psi(x) \) and \( E(x) \) are defined by the explicit expressions, provided that functions \( a_k(x) \) are known:

\[
\psi(x) = \frac{1}{2} \ln \frac{2K_w + \sum_{k=1}^n a_k(x)e^{\psi_k}}{2K_w + \sum_{k=1}^n a_k(x)e^{-\psi_k}},
\]

\[
E(x) = E_0(x) + \varepsilon \frac{d\psi(x)}{dx}, \quad E_0(x) = \frac{j}{\sigma(x)}. \quad (15)
\]

where

\[
\sigma(x) = \sum_{k=1}^n \gamma_k a_k(x) \left( \varphi_k(\psi) \frac{d^2}{d\psi^2} \ln \varphi_k(\psi) \right)_{\psi=\psi(x)} + 2K_w \gamma_0 \cosh(\psi(x) - \psi_0). \quad (16)
\]

In order to find \( a_k(x) \) (taking into account the conditions (10) and the changes of variables (14)) we have the following system of 2n differential equations with \( \psi \) and \( \sigma \) defined by (15) and (16):

\[
\varepsilon_0 \frac{d}{dx} \ln a_k(x) = \frac{1}{\sigma(x)} \frac{d}{d\psi} \ln \varphi_k(\psi) \bigg|_{\psi=\psi(x)}, \quad (17)
\]

\[
\frac{d}{dx} m_k(x) = a_k \varphi_k(\psi(x)), \quad k = 1, \ldots, n. \quad (18)
\]

\[
\varepsilon_0 = \frac{\varepsilon}{j}, \quad \left( m_k(x) = \int_0^x c_k(s) ds \right),
\]

with the boundary conditions:

\[
m_k(0) = 0, \quad m_k(1) = M_k, \quad k = 1, \ldots, n. \quad (19)
\]

The additional variables \( m_k \) have the meaning of the total number of moles of an ampholyte in the interval \([0, x]\).

V. THE MAIN ASYMPTOTIC TERM AT \( K_w \to 0 \)

The nonlinear boundary problem (17)–(19) can be solved numerically with the use of a shooting method, by the reduction of (17)–(19) to Cauchy’s problem with unknown initial data \( a_k(0) \). To find \( a_k(0) \) one should solve \( n \) nonlinear transcendental equations. It can be performed for example by the Newton-Raphson method that requires good initial approximations. An initial approximation for small \( \varepsilon_0 \) and \( K_w \) can be obtained with the use of asymptotic formulas. The analysis of equations (17) show that their right side parts at \( \varepsilon_0 \to 0 \) are proportional to \( d\varphi_k(\psi)/d\psi \sim \sinh(\psi - \psi_k) \) (see (8)). It means that \( \psi(x) \to \psi_k \) for \( k = 1, \ldots, n \). It is possible only if functions \( a_k(x), k = 1, \ldots, n, \psi(x), \) and \( E(x) \) are close to stepwise ones.
Let us introduce the set of intervals \((x_{k-1}, x_k) \in [0, 1]\) that are not overlapping. We assume that
\[
a_k(x) \equiv a^0_k, \quad x \in (x_{k-1}, x_k) \quad k = 1, \ldots, n,
\]
\[
a_k(x) \equiv 0, \quad x \notin (x_{k-1}, x_k), \quad k = 1, \ldots, n \quad (20)
\]
where \(a^0_k\) are some constants. Then a particular conclusion from (18) at \(K_w \to 0\) is:
\[
\psi(x) \equiv \psi_k, \quad x \in (x_{k-1}, x_k), \quad k = 1, \ldots, n. \quad (21)
\]
Next we introduce the functions:
\[
\Phi_k(x) \equiv \frac{1}{\sigma(x)} \frac{d\varphi_k(\psi(x))}{d\psi},
\]
\[
F_k(x) \equiv \frac{1}{\varepsilon_0} \int_0^x \Phi_k(s) ds, \quad k = 1, \ldots, n. \quad (22)
\]
For an interval \((x_{i-1}, x_i)\) we get:
\[
\Phi_k(x) \equiv \frac{\epsilon_k(\psi_i)}{\gamma_k \alpha^0_i} = \Phi^{(i)}_k, \quad (23)
\]
\[
\varepsilon_0 F_k(x) = \sum_{s=1}^{i-1} \Phi^{(s)}_k'(x_s - x_{s-1}) + \Phi^{(j)}_k(x - x_{i-1}).
\]
The integrating of (17) and taking into account notations (22) yield:
\[
a_k(x) = a_k(0) e^{F_k(x)}. \quad (24)
\]
Using (22), (24), and (21), the equations \(a_k(x) = a^0_k\) for \(x \in (x_{k-1}, x_k)\) give:
\[
a_k(0) = a^0_k \exp \left( -\frac{1}{\varepsilon_0} \sum_{s=1}^{k-1} \Phi^{(s)}_k'(x_s - x_{s-1}) \right). \quad (25)
\]
The values \(a^0_k\) can be easily found from the boundary condition \(m_k(1) = M_k\) with the help of (20), (24):
\[
a^0_k = \frac{M_k}{(\delta_k + 1)(x_k - x_{k-1})}, \quad k = 1, \ldots, n. \quad (26)
\]
Expressions (20), (25), (23) represent the required initial approximation for \(a_k(0)\), provided that the lengths of intervals \((x_k, x_{k-1})\), \(k = 1, \ldots, n\) are given. The main asymptotic term at \(K_w \to 0\) is given by (24).

VI. NUMERICAL RESULTS

The main difficulty in the use of formulas (25), (26) is the fact that the lengths of the intervals \((x_{k-1}, x_k), k = 1, \ldots, n\) are unknown, they can be determined by the next asymptotic terms. However our numerical experiments have shown that (at least for \(n > 5\)) it is sufficient to take their lengths equal: \((x_k - x_{k-1}) = 1/n\). We have shown that asymptotic formulas and numerical results are in good agreement beginning from \(\varepsilon_0 \approx 2 \cdot 10^{-4}\) and \(K_w \approx 10^{-4}\). In particular, the numerical results obtained by a shooting method for the values of parameters \(n = 8, \delta_k = 99, \psi_k = 3.95 - 0.6k, M_k = 160, \varepsilon_0 = 5.25 \cdot 10^{-6}, \gamma_k = 0.15, \psi_0 = -0.274, \gamma_0 = 7.077, K_w = 10^{-5}\) are given in Table I where for the sake of convenience we present the values \(z_k = -10 \varepsilon_0 \ln a_k(0)\). The values \(z^*_k\)

| \(k\) | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|------|---|---|---|---|---|---|---|
| \(z_k\) | 0.0042 | 0.0138 | 0.0325 | 0.0662 | 0.1256 | 0.2264 | 0.3894 |
| \(z^*_k\) | 0.0639 | 0.0137 | 0.0324 | 0.0664 | 0.1262 | 0.2276 | 0.3914 |

TABLE I: The comparison between the numerical and asymptotic values

in the second raw correspond to their asymptotic values. One should notice that the formulas (25), (26) do not work well for \(a_1(0)\), so for the obtaining of its asymptotic value one has to use an additional procedure that are similar to Sect. (11) for \(a_k(1)\). Fig. 1 shows the concentration distributions \(a_k(x)\) and function \(\psi(x)\) for two values \(\varepsilon_0 = 5.25 \cdot 10^{-5}\) (left) and \(\varepsilon_0 = 5.25 \cdot 10^{-6}\) (right). It is clearly seen that \(a_k(x)\) are located successively on the \(x\)-axis in the interval \((x_{k-1}, x_k)\). The concentration maxima correspond to the isoelectric points \(\psi_k\). In a classical case when \(\varepsilon_0\) is not too small, function \(\psi(x)\) is monotonically decreasing and concentration distributions are close to Gaussian ones (the left frame). The decreasing of \(\varepsilon_0\) (or the increasing of current density) causes the ‘almost’ stepwise distribution of \(\psi(x)\) i.e. \(\psi(x) \approx \psi_k\) in the intervals \((x_{k-1}, x_k)\) and ‘almost’ constant concentrations \(a_k(x) \approx a^0_k\) (the right frame).

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FIG. 1: The concentration distribution \( a_k(x) \), \( k = 1, \ldots, 8 \) and \( \psi(x) \) (the dashed line)

[1] O. Vesterberg, Acta. Chem. Scand. 23, 2653 (1969).
[2] H. Haglund, Meth. Biochem. Anal. 19, 1 (1971).
[3] H. Rilbe, Sci. Tools. 23, 18 (1976).
[4] H. Rilbe, in Isoelectric focusing (Acad. Press, New York–London, 1976), p. 14.
[5] O. Vesterberg, in Isoelectric focusing (Acad. Press, New York–London, 1976), p. 53.
[6] P. G. Righetti, Isoelectric Focusing: Theory, Methodology and Application (Elsevier Biomedical Press, New York–Oxford: Elsevier, 1983).
[7] P. G. Righetti, Immobilized pH-gradient: Theory and Methodology. Laboratory Techniques in Biochemistry and Molecular Biology (Elsevier Biomedical Press, Amsterdam–New York–Oxford: Elsevier, 1990).
[8] A. Stoyanov, M. Yu, Zhukov, P. G. Righetti, The Proteome Revisited: Theory and Practice of All Relevant Electrophoretic Steps (J. Chromatography. Elsevier, 2001), Vol. 63, p.1.
[9] Zs. Busas, L. M. Hjelmeland, A. Chrambach, Electrophoresis. 4, 27 (1983).
[10] D. A. Saville, O. A. Palusinski, AIChE Journal. 32, 207 (1986).
[11] J. R. Cann, in Electrophoresis and Isoelectric Focusing of Interacting System. Electrokinetic Separation Methods, edited P. G. Righetti, C. J. van Oss, J. W. Vanderhoff (Biomedical Press, Elsevier/North-Holland, 1979), p. 369.
[12] Nga Y. Nguyen, A. Chrambach, Electrophoresis. 1, 14 (1980).
[13] R. A. Mosher, M. Bier, P. G. Righetti, Electrophoresis. 6, 5 (1985).
[14] R. A. Mosher, W. Thorman, A. Graham, M. Bier, Electrophoresis. 6, 545 (1985).
[15] R. A. Mosher, D. A. Saville, W. Thorman, The Dynamics of Electrophoresis (VCH Publishers, New York, 1992).
[16] R. A. Mosher, W. Thorman, Electrophoresis. 6, 595
[17] A. Tsai, R. A. Mosher, M. Bier, Electrophoresis. 7, 487 (1986).
[18] W. Thormann W, T. Huang, J. Pawliszyn, R. A. Mosher, Electrophoresis. 25, 324 (2004).
[19] V. G. Babskii, M. Yu. Zhukov, V. I. Yudovich, Mathematical theory of electrophoresis (Plenum Publishing Corporation, New York, 1989).
[20] M.Yu. Zhukov, Mass Transfer by an Electric Field (RGU Press, Rostov-on-Don, Russia, 2005), (in Russian).
[21] M. Yu. Zhukov, O. A. Tsyvenkova, Fluid Dynamics. 29, 717 (1994).
[22] M. Yu. Zhukov, O. A. Tsyvenkova, Fluid Dynamics. 30, 652 (1995).
[23] M. Yu. Zhukov, N. V. Petrovskaya, Fluid Dynamics. 32, 631 (1997).
[24] M. Yu. Zhukov, L. I. Sazonov, Diff. Uravn. 3, 470 (1997) (Translated in English, Diff. Equations, 1997).
[25] V. G. Babskii, M. Yu. Zhukov, A. D. Myshkis, N. D. Kopachevskii, L. A. Slobuzhanin, A. D. Tyuptsov, Methods of Solving Problems of Hydromechanics in Zero Gravity (Naukova Dumka: Kiev, 1992) (in Russian).
[26] M. S. Bello; V. I. Polezhaev, Fluid Dynamics, 25, 174 (1990).
[27] V. I. Polezhaev, M. S. Bello, N. A. Verezub, Convection in zero gravity (Nauka: Moscow, 1991) (in Russian).
[28] G. V. Troitskii, G. Yu. Azitskii, Isoelectric Focusing of Proteins in Self-organizing and Artificial pH-gradients (Naukova Dumka: Kiev, 1984) (in Russian).
[29] J. T. Edsall in Proteins, Amino Acids and Peptides, edited E. J. Conh and J. T. Edsall (Reinhld, New York, 1943), p. 444.
[30] One can consider this step as being too radical. However the introduction of new notations aimed to simplify mathematical expressions belong to the highest achievements in sciences. For instance, the timely replacement of Latin terms for ‘plus’, ‘minus’, and ‘equals’ by the symbols +, −, and = as well as the replacement of Roman figures for numbers by Arab digits initiated an extraordinary progress in sciences.