The application of an array of sensors based on boronic acid derivative for the quantitative analysis of amino acids


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Abstract

The objective of this work was to perform the quantitative analysis of 4 amino acids: phenylalanine (Phe), tyrosine (Tyr), ornithine (Ort) and glutamic acid (Glu) in mixtures with the use of potentiometric sensor array (electronic tongue) and Sequential Injection Analysis (SIA) measurement system. The flow-through sensor array was composed of 6 miniaturized classical ion-selective electrodes based on polymeric membranes (plasticized PVC) containing phenylboronic acid ionophore and/or an ion exchanger. The PLS analysis of the sensor array responses, measured in amino acids mixtures prepared by the SIA system (in two buffer solutions), permitted a correct quantitative analysis of only Glu and Ort. Further chemometric treatment, involving the extraction of dynamic components of the transient response employing the Fourier transform plus training of an Artificial Neural Network (ANN) with its coefficients led to the simultaneous determination of 3 amino acids, with a separate semiquantitative estimation of Tyr.

1. Introduction

Specific inherited inborn errors in amino acid biosynthesis have been recognized as causal in many metabolism disorder. Standard metabolic diseases are detected through genetic testing which involve the analysis of mutations in the genes coding for enzymes production or by quantitative analysis of amino acids analysis; boronic acids; ion-selective electrodes; automated electronic tongue; sequential injection analysis.

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acids or their metabolites in physiological fluids. Therefore the determination of physiological amino acids using HPLC, GC/MS, HPLC/MS or tandem mass spectrometry, provides rapid diagnosis of metabolic diseases and the introduction of proper treatment [1]. The concentration of amino acids is examined mainly in plasma and in a healthy person is on the level of $10^{-4}$ M (in blood plasma) [2].

An alternative, simple method of the amino acids determination involving an array of ion-selective electrodes based on phenylboronic acid ionophore and a chemometric data processing tool was reported in this work. Boronic acids derivatives are widely used as receptors of different bioanalytes i.e. diols, anions, neurotransmitters and also amino acids [3]. Taking into account the metabolic pathways as well as the relationship between the level of amino acids in blood and several diseases, the quantitative analysis of 4 amino acids: phenylalanine (Phe), tyrosine (Tyr), ornithine (Ort) and glutamic acid (Glu) was performed.

2. Experimental

The flow-through sensor array embraced: 4 miniaturized electrodes with PVC membranes containing 4-octyloxyphenylboronic acid and a lipophilic salt TDMAC or KTFPB and 2 electrodes containing only an ion-exchanger in membrane. The method of the membranes preparation and electrodes conditioning were the same as for the standard ISEs. The membranes contained the ionophore (2 wt%), 10 mol% versus ionophore lipophilic salt, 65-66 wt% plasticizer, and 31-33 wt% high-molecular-weight PVC (the membranes based on an ion-exchanger contained 3 wt% of TDMAC or 1 wt% KTFPB). The membrane components (200 mg in total) were dissolved in 2ml of THF. A detailed architecture of the miniaturized ion-selective electrodes compatible with a flow-through module was presented in [4]. The constructed ISEs were preconditioned in a dilute solution of internal electrolyte (0.01M NaCl) for 24 hours.

The flow-through sensor array was connected to the SIA system providing the automated operation and generation of amino acid sample mixtures, thanks to the precise dosing and mixing of volumes of stock solutions. An 8-channel signal conditioning circuit connected to the National Instruments Multifunction DAQ analogue inputs (Model NI6221, USA) was used for the EMF measurements. The whole system was controlled by a PC using a virtual instrument developed in Labview.

All measurements were carried out in cells of the following type: Ag, AgCl; KCl 3M | CH₃COOLi 1M | sample solution | membrane | internal filling solution; AgCl, Ag.

Data analysis was performed in MatLab 7.1 (The MathWorks, Inc., Natick, USA) with specific routines written by the authors using its Neural Network Toolbox (v4.0.6) and Origin (Microcal Software, Inc, Northampton, USA) software. Chemical images of samples were processed using Partial Least Squares – Discriminant Analysis (PLS-DA). For the dynamic treatment, the transient response of each sensor was first compressed employing Fast Fourier Transform (FFT), and then extracted coefficients were used as inputs of the ANN model which carried out the quantification of the amino acids [5].

3. Results and discussion

The electrodes constructed by incorporating the phenylboronic acid within the PVC/DOS membrane containing anionic additives (KTFPB) were found to respond potentiometrically (Fig 1a). The highest sensitivity was recorded for Phe and Glu, whereas the values of the response slope for Ort and Tyr did not exceed 10 mV/decade. Moreover, the slope values depended on the pH of the sample solution – the acidic conditions promoted higher electrode sensitivity of the electrodes based on KTFPB additive. Surprisingly, the electrodes formulated with 4-octyloxyphenylboronic acid and cationic additive (TDMAC) displayed completely different performances (Fig 1b). Depending on the experimental pH conditions, anionic (pH=4) or cationic (pH=9) responses with slopes <10 mV/decade in quite a narrow linear range were determined towards amino acids (except phenylalanine responses in pH=4).
Fig. 1. (a) Potentiometric responses of ISEs based on PVC/DOS membranes containing 4-octyloxyphenylboronic acid (10% mol of KTFPB) towards given amino acids at pH=4; (b) potentiometric responses of ISEs based on PVC/DOS membranes with 4-octyloxyphenylboronic acid (10% mol of TDMAC or KTFPB) towards phenylalanine at various pH.

The measurements of the sensor array signals were conducted in 80 sample mixtures containing 4 amino acids in the concentration range of 0.1 – 3 mM (except tyrosine: 0.05 – 1.5 mM) prepared by the Sequential Injection Analysis system in 2 carrier solutions of different pH controlled by 1 mM MES (pH=5) and TRIS (pH=9) buffers. Data matrices involving the signals of the sensors were formed – every sample was characterized by 12 variables (6 sensors signals obtained in 2 carrier solutions). The 80x12 data matrix was divided into the train (64x12) and the test (16x12) sets, serving as inputs for Partial Least Squares (PLS) models.

The model performance was characterized after linear fitting of the real data (real concentrations of every amino acid) to the predicted data (PLS predicted concentrations of every amino acid). Values of slope (“a”), intercept (“b”) and determination coefficient (“R^2”), were calculated for the test samples and train samples. The correct quantitative analysis (for train and test sets) was achieved only for Glu and Ort, (Fig 2), whereas for Phe and Tyr, the values of a, b, and R^2 were not acceptable.

Fig. 2. Model performance characterized after linear fitting of the real data (real concentrations of amino acid) to the predicted data (PLS predicted concentrations of amino acid). Training subset (●, solid line), testing subset (○, dotted line).
Since the application of the steady-state response of each sensor was not sufficient to build a model capable of carrying out the quantification of the four amino acids, a more complete data treatment involving the use of the dynamic component of the signal was attempted. For this, the transient response of each sensor was first compressed employing the Fast Fourier Transform, which allowed the extraction of some coefficients which were used as inputs to the ANN model. As before, the same data division was performed for the train and test samples, evaluating the model performance after linear fitting of the real concentrations vs. the predicted ones by the ANN model, both for the train and test samples (Fig 3). In this way, the introduction of the dynamic component provided the correct quantitative analysis of the three amino acids (i.e. Glu, Ort and Phe), while the concentration of tyrosine could be predicted semiquantitatively in another step.

Fig. 3. Model performance characterized after linear fitting of the real concentrations of the three amino acids to the predicted data for the FFT-ANN treatment. Training subset (●, solid line), testing subset (○, dotted line) and theoretical diagonal line (dashed line).

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