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Resolution of amino acid mixtures by an array of potentiometric sensors based on boronic acid derivative in a SIA flow system

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ABSTRACT

The objective of this work was to perform the quantitative analysis of 4 amino acids mixtures: phenylalanine, tyrosine, ornithine and glutamic acid. For this purpose a potentiometric sensor array (electronic tongue) and Sequential Injection Analysis (SIA) measurement system were employed. The flow-through sensor array was composed of 6 miniaturized classical ion-selective electrodes based on polymeric membranes (plasticized PVC) containing 4-octyloxyphenylboronic acid as an ionophore and/or an ion exchanger. Partial Least Squares (PLS) analysis of the steady-state sensor array responses, measured in amino acids mixtures prepared by the SIA system (in two buffer solutions), permitted a correct quantitative analysis only of glutamic acid and ornithine. While a further chemometric treatment, involving the extraction of dynamic components of the transient response employing the Wavelet transform, the removal of less-significant coefficients by means of Causal Index pruning and training of an Artificial Neural Network (ANN) with the selected coefficients, allowed the simultaneous determination of the four amino acids.

1. Introduction

Nature uses amino acids as building blocks for the construction of proteins, and regular metabolism of amino acids is needed for the proper functioning of our organism. Specific inherited inborn errors in amino acid biosynthesis have been recognized as origin of many metabolism disorders. Metabolic diseases can be detected through genetic testing which involves the analysis of mutations in the genes coding for production of specific enzymes or by the quantitative analysis of amino acids or their metabolites in physiological fluids. Therefore, the determination of physiological amino acids provides rapid diagnosis of metabolic diseases and facilitates introduction of proper treatment [1].

The concentration of amino acids is examined mainly in plasma (in a healthy person they are on a level of 0.1 mM) or less frequently in the urine [2]. The blood and urine analysis of: phenylalanine, leucine, methionine, tyrosine, homocysteine is used to establish the diagnosis of maple syrup urine disease or hyperphenylalaninemia [3,4]. The best known disorder associated with impaired metabolism of amino acids – phenylketonuria (PKU) – is caused by a complete or nearly complete deficiency of phenylalanine hydroxylase [5]. Normal blood phenylalanine levels are 1 to 2 mg/dL (0.06–0.12 mM), whereas in the classic PKU its concentration may range from 6 to 80 mg/dL, usually exceeding 30 mg/dL (1.8 mM) [6]. When the degradation of tyrosine does not occur due to the deficiency of the enzyme fumarylacetoacetate hydrolase [7,8], the increased concentration of tyrosine in blood indicates tyrosinemia. Moreover, high concentration of ornithine is evidence of an urea cycle disorder, which can also be a consequence of tyrosinemia. High concentrations of tyrosine and glutamic acid together with low level of ornithine in the blood indicate thyroid diseases (goiterhyperactive), while high concentration of phenylalanine can also be associated with kidney disease [9].

Amino acids are commonly determined using ion-exchange chromatography, gas chromatography (GC/MS), liquid chromatography (HPLC), HPLC/MS or tandem mass spectrometry [1,2]. Efficient separation techniques like capillary electrophoresis coupled with fluorimetric detection were also proposed [10]. Such methods provide precise and accurate determination of amino acids, but frequently require time-consuming sample pre-treatment and derivatization processes. On the other hand, selective electrochemical sensors are developed for amino acids analysis, eliminating the necessity of sample preparation. Electrochemical approaches (mainly voltammetric devices) employed for the detection of amino acids were thoroughly discussed in a recent review [11]. As an example, voltammetric sensors were presented for the detection of several amino acids e.g. cysteine and tyrosine [12,13]. Various attempts were made to construct ionophore based amino acid-selective electrodes; they were firstly

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designed for the detection of amino acid esters with protonated amino and protected carboxyl groups [14–16]. Nevertheless, potentiometric sensors based on: metalloporphyrins [17,18], metallophthalocyanine [19], phosphoryl-containing receptors [20] and trifluoroacetophenone derivatives [21] were developed for the selective determination of underivatized amino acids (i.e. histidine, cysteine, phenylalanine). According to these reports, cationic or anionic amino acid species were responsible for the generation of membrane potential. Phenylalanine and glutamate biosensors based on potentiometric detection of byproducts of enzymatic breakdown of the analyte were developed [22,23]. Enantioselective sensing of some chiral amino acids by potentiometric sensors was also reported [24,25]. Recently, an electronic tongue based on potentiometric sensor array was applied for the classification of amino acids and oligopeptides [26].

As a further progress of the late work, a simple method for the amino acids quantitative determination involving an array of miniaturized potentiometric electrodes based on phenylboronic acid ionophore and chemometric data processing tools is reported in this work. Boronic acids derivatives are widely used as selective receptors of different analytes i.e. diols, anions, neurotransmitters and also amino acids [27]. Taking into account the role played by the amino acids in metabolic pathways as well as the relationship between the level of amino acids in blood and several diseases, the quantitative analysis of mixtures containing phenylalanine (Phe), tyrosine (Tyr), ornithine (Ort) and glutamic acid (Glu) was attempted.

2. Experimental

2.1. Chemicals and membrane materials

All amino acids used (phenylalanine, tyrosine, glutamic acid, alanine and ornithine hydrochloride), 1-morpholinoethanesulfonic acid (MES), tris(hydroxymethyl)amino- methane (TRIS), sulfuric acid were of analytical grade and were purchased from Fluka. The solutions of amino acids (0.1 M or 0.01 M; except tyrosine: 1 mM), MES and TRIS buffer solution (0.01 M and 1 mM) were prepared with deionised water. The pH was adjusted by the addition of sulfuric acid or sodium hydroxide solution. High-molecular-weight poly(vinyl chloride) (PVC), plasticizers: o-nitrophenyl octyl ether (o-NPOE), bis(2-ethylhexyl) sebacate (DOS), lipophilic salts: potassium tetrakis [3,5-bis(trifluoromethyl)phenyl] borate (KTFPB) and tridodecylmethylammonium chloride (TDMAC) were obtained from Fluka (Selectophore). The synthesis of the 4-octyloxyphenylboronic acid was described previously [28]. Freshly distilled tetrahydrofuran (Fluka) was used as a solvent for the membrane components.

2.2. Sensor array

The flow-through sensor array embraced: 4 miniaturized electrodes based on PVC membranes containing the ionophore 4-octyloxyphenylboronic acid and a lipophilic salt TDMAC or KTFPB (plasticized using DOS or o-NPOE) and 2 electrodes containing only an appropriate ion-exchanger (TDMAC or KTFPB) in the membrane, exhibiting generic anion and cation response, respectively. The method of the membranes preparation and the electrodes conditioning were the same as for the standard ISEs. The membranes contained: 2 wt% ionophore, 65–66 wt% plasticizer, 31–33 wt% high-molecular-weight PVC and 10 mol% (vs ionophore) lipophilic salt (the membranes based on an ion-exchanger contained 3 wt% TDMAC or 1 wt% KTFPB, see Table 1). The membrane components (200 mg in total) were dissolved in 2 mL of THF. A detailed architecture of the miniaturized ion-selective electrodes compatible with a single flow-through module was presented in [29], whereas the design of the modular flow-cell system is a subject of a polish patent application [30]. NaCl solution (0.01 M) was used as an internal filling. The constructed sensors were preconditioned overnight in a dilute solution of internal electrolyte for at least 24 h.

2.3. Instrumentation and EMF measurements

All measurements were carried out in flow-through mode with cells of the following type: Ag, AgCl; KCl 3 M|CH_3COOLi 1 M|sample solution||membrane||internal filling solution; AgCl, Ag.

Potentiometric multiplexer (EMF 16 Interface, Lawson Labs Inc., Malvern, USA) was used for the characterization of the sensors. The calibration curves of the electrodes were examined by measuring the EMFs in 0.01 M MES or TRIS buffer solution, increasing the concentration of the amino acid in steps of 0.5 log c (concentration range: $10^{-5.5}$–10^{-1} M for Ort and Phe, $10^{-5.5}$–10^{-2} M for Glu and $10^{-5.5}$–10^{-3} M for Tyr). Potentiometric selectivity coefficients ($\log K_{Glu,X}$) of the electrodes were determined by the separate solution method (SSM) [31] using 0.01 M buffered solutions of amino acids (except tyrosine: 1 mM). Moreover, the $\log K_{Glu,X}$ values for an additional amino acid i.e. alanine (Ala) were also determined.

For the quantitative resolution of amino acids mixtures, the flow-through sensor array was connected to the Sequential Injection Analysis (SIA) system providing the automated operation and generation of amino acids samples mixtures, thanks to the precise dosing and mixing of volumes of stock solutions. The SIA system was formed by two differentiated parts: the fluidic system and the measurement system [32,33]. The first part was the fluid system which consisted of an automatic microburette (Crison 2030 microburette, Crison, Spain) equipped with a 5-mL syringe (Hamilton, Switzerland), a holding coil (5 m × 1 mm i.d. PTFE tubing, Bioblock, France), a 8-way Hamilton MVP valve (Hamilton, Switzerland) and a 7 mL Perspex mixing cell (home built) with a magnetic stirrer. The multiport valve is connected to the burette with holding coil placed in between. The burette is fed through a carrier solution reservoir. By the time the common port may access any of the other ports which led to sample, standard stock solutions, mixing chamber or sensor array by an electrical rotation of the valve. All the elements were connected together using low pressure liquid chromatography connectors. The second part was the measurement system that comprised the sensor array, a reference electrode (miniaturised silver/silver chloride electrode with a double junction) and an 8-channel signal conditioning circuit connected to the data acquisition analog inputs (National Instruments NI6221 Multifunction DAQ, TX, USA). The whole system was controlled by a PC using a virtual instrument developed in Labview [32], where the other active elements were commanded through RS-232 communication lines.

2.4. Data analysis

Data analysis was performed in MatLab (The MathWorks, Inc., Natick, USA) and Origin (Microcal Software, Inc, Northampton, USA) software. Chemical images of samples were processed using Partial Least Squares (PLS) analysis. For the dynamic treatment, the transient response of each sensor was first compressed employing Discrete Wavelet Transform (DWT), and then extracted coefficients were used as inputs of the Artificial Neural Network (ANN) model [34,35]. Prior to building the quantification model, removal of less-significant coefficients that barely contribute to the network was carried out by means of Causal Index (CI) pruning of the inputs based on a Back Propagation ANN (BPANN) [36,37]. This method usage for variable selection is the finding of an optimal set of inputs that can successfully classify or predict the desired outputs. It could be divided into two steps, first an ANN model is built in order