Sensors and Actuators B 242 (2017) 288–298

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Research Paper

A chemosensor array for the colorimetric identification of some carboxylic acids in human urine samples

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ARTICLE INFO

Article history:
Received 6 June 2016
Received in revised form 1 November 2016
Accepted 2 November 2016
Available online 4 November 2016

Keywords:
Colorimetric chemosensor array
Carboxylic acids
Principal component analysis
Linear discriminant analysis
Human urine samples

ABSTRACT

A simple colorimetric sensor array containing eight chemosensors from commercial dyes and metal salts was designed to detect a number of important carboxylic acids in human urine. Some common chemometric methods, including principal component analysis (PCA), linear discriminant analysis (LDA) and k-nearest neighbor (kNN) based hierarchical cluster analysis (HCA) were used to test the discriminatory power of the array. The eight-member sensor array perfectly identified eleven carboxylic acids in water with 100% classification accuracy. In order evaluate the potential of sensor array used in biological environments (biofluids), carboxylic acids in human urine were analyzed and 100% classification accuracy was achieved. In addition, the array's performance in the semi-quantitative identification of carboxylic acids was investigated, and the results showed that the sensor array can discriminate seven typical carboxylic acids at concentrations ranging from 100 to 1000 μmol L^{-1}. These results illustrate the potential use of the sensor array for disease diagnosis and other biomedical monitoring applications.

1. Introduction

Organic acids are intermediate metabolites of all the major groups of organic cellular components, including amino acids, lipids, carbohydrates, nucleic acids, and steroids [1]. Therefore, a comprehensive qualitative/quantitative analysis of organic acids in the body fluids has the potential for providing information on the physiological and pathophysiological status of diverse metabolic pathways as well as their interrelationships. Therefore, organic acids can play an essential role as biomarkers in the monitoring of different diseases, such as organic acidurias, metabolic diseases, diabetes, and central nervous system diseases [2].

Since a large number of organic acids are found in urine, we selected carboxylic acids, as a homogeneous group of analytes that are specific markers of organic acidurias, to develop the methodology. Fumarate, citrate, pyruvate, acetate, propionate, lactate, butyrate and etc are all short-chain organic acids [2]. Direct identification of organic acidic compounds that are as similar as carboxylic acids is a significant challenge in analytical techniques. Generally, according to the properties of these compounds including high polarity, low volatility, and high tendency towards dehydration, derivation process is required prior to chromatographic, electrophoretic or extractive analyses [3,4]. In addition, the use of these methods for general screening purposes has been impeded by the significant impediments associated with separation techniques, that are the long time required for the preparation/analysis of samples, the high cost for performing these analyses, and the need for trained personnel. Furthermore, their use is often redundant in monitoring the adhesion/progression of therapy. In determining a patient's prognosis, early and accurate diagnosis of metabolic diseases is very important. Many of these disorders are now treatable by simple dietary measures. However, these disorders can result to death or irreversible mental retardation when are undiagnosed and untreated [2].

In the recent years, chemosensors including cross-reactive arrays based sensors inspired by the mammalian olfactory system have been investigated. These sensors possess binding sites for carboxylic acids [5]. The increased popularity of array-based sensors is mainly due to their ability to recognize a large number of analytes with perfect classification accuracy [6]. However, few sensor arrays exist for carboxylic acids that function in aqueous solution [7]. To date, sensor arrays with sufficient capacity of sensing the carboxylic acids in a complex biological milieu, such as human urine, have not been developed according to the conducted researches.

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http://dx.doi.org/10.1016/j.snb.2016.11.008
0925-4005/

As previously mentioned, the use of an array with non-specific receptors, such as transition metal ions together by using pattern recognition methods, has become a popular approach in the development of sensors [8]. In the present study, the development of a colorimetric sensor array, that is capable to discriminate the eleven types of biologically-important carboxylic acids in both water and urine environments with perfect classification accuracy, was reported. This array was prepared using sensors of four non-designed or non-synthesized differential receptors and two different indicators which resulted in the fingerprinting the analyzed carboxylic acids individually [9]. The signaling relies upon indicator displacement assays (IDAs) to provide a colorimetric response based on the UV–vis spectra of the sensors with different analytes. It yields information-rich outputs that is useful for discrimination of various carboxylic acids [10].

The acquired spectral dataset are usually analyzed using common unsupervised and supervised pattern recognition methods. Principal component analysis (PCA) and k-nearest neighbor (kNN) based hierarchical cluster analysis (HCA) were implemented as some of the most common unsupervised techniques, where linear discriminant analysis (LDA) was also used as a supervised technique. PCA reduces the multi-dimensional data and the partly-correlated data to a lower number of dimensions. This is achieved by projecting the data onto fewer dimensions that represent maximum variance relationships between variables [11–13]. In addition to the PCA, the multi-dimensional responses pattern was further evaluated using LDA and kNN based HCA to explore the discriminatory power of the sensor array [14]. LDA as a capable statistical approach has been used widely for classification modeling coupled to a cross-validation routine technique (Venetian blinds) to evaluate the validity of the classification model and its overall ability to correctly classify the observations (analytes).

2. Materials and methods

2.1. Experimental

All the used chemicals were purchased from Merck Company as analytical grade and used without further purification. All stock solutions (1 mmol L^{-1}) of receptors, indicators, and carboxylic acids were prepared using double distilled water. Besides, the diluted solutions were prepared from stock solutions. The appropriate amount of boric acid (Merck) was dissolved in distilled water to prepare the borate buffer, and the pH was adjusted to 7.0 with a 5.0 N solution of NaOH.

All UV–vis measurements were performed on a JASCO V-570 spectrometer. To measure the pH, a Metrohm 632 pH-meter was used. After an equilibration period, all the measurements were conducted at room temperature (298 K). By mixing the receptors and indicators in a test cell in appropriate ratios (obtained from titration data), each sensor solution was prepared after that the solutions were diluted with buffered aqueous solution at pH = 7.0 so that the final volume in the test cell was 2 mL.

After preparing the sensor solutions, 100 μmol L^{-1} of each carboxylic acid were added to test cells that contained the sensor solution, and the UV–vis spectra (200–900 nm) were recorded after allowing the system at least 5 min to reach to equilibrium. Urine samples were obtained from sick individuals in the Pars Hospital's laboratory (Tehran, Iran). The ages of the individuals (from both genders) in the studied groups ranged from 2 months to 13 years. The urine samples were frozen after collecting, and thawed immediately before the analysis.

2.2. Software

In this research, the PLS Toolbox ver. 4.1.1 (*Eigenvector Research*, Inc., Manson, Washington, USA) which is an advanced chemometric software in MATLAB® environment, was used to implement genetic algorithm (GA) for variable/feature selection. Other program routine codes (e.g., PCA, HCA and LDA) were written in the house using MATLAB® R2009a ver. 7.8.0 software (*MathWorks*© Inc., Natick, MA, USA).

3. Results and discussion

3.1. Array design

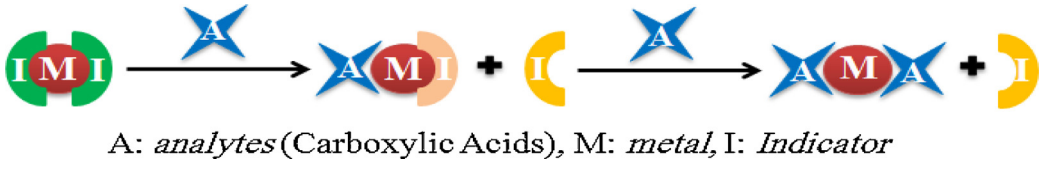
3.1.1. Selection of non-designed receptors

In this study, since highly selective binding was unnecessary for the pattern recognition, the use of an array of non-designed receptors for discrimination of structurally similar analytes was explored and examined. Simple metal salts were considered as non-designed receptors, because they are commercially available and there are many coordination sites at a metal center [10]. The cross-reactivity states originated from the different coordination modes (i.e., MI and MI_2) are considered to be main procedure in the sensors as shown in Scheme 1.

Moreover, the complex guest-receptor binding stoichiometry in the IDAs for pattern recognition can create useful information for the discrimination. Recently, some studies have been performed on the discrimination of amino acids or dipeptides *via* IDAs by Buryak and Severin [15] using non-designed receptors such as a single Cp^*Rh complex ((Cp^*) η5-pentamethylcyclopentadienyl) or a mixture of $CuCl_2$ and $NiCl_2$. In contrast to the use of a single-receptor system, it is known that arrays consisting of many types of receptors are more effective for discrimination process [16]. Therefore, four types of metal salts were used for the array as mentioned in the following items: $Fe(NO_3)_2.9H_2O$, $Fe(NO_3)_3.9H_2O$, $Cu(NO_3)_2.3H_2O$, and $VOSO_4.5H_2O$.

3.1.2. Indicators and analytes

The carboxylic acids (CA1-CA11) as shown in Fig. 1 are the most important types of the short chain organic acids in human urine [18]. They were chosen to represent analytes with very similar structures that remain as substantial scientific challenges for molecular recognition and the practical methods can be developed to detect and discriminate them. The suited indicators for an IDA should fulfill the following criteria: (a) they should bind to the metals with different affinities; (b) the spectroscopic changes upon coordination should occur in different regions of the UV–vis spectrum. In the present study, through a screening process of commercially available dyes, pyrocatechol violet (PV or PCV) and Eriochrome Cyanine R (ECR) emerged as potential indicator candidates (Fig. 1) [17].



Scheme 1. Coordination modes as 1:2 and 1:1 complexes for the discrimination of analytes.