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Synthesis-identification integration: One-pot hydrothermal preparation of fluorescent nitrogen-doped carbon nanodots for differentiating nucleobases with the aid of multivariate chemometrics analysis

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ABSTRACT

Most of the conventional multidimensional differential sensors currently need at least two-step fabrication, namely synthesis of probe(s) and identification of multiple analytes by mixing of analytes with probe(s), and were conducted using multiple sensing elements or several devices. In the study, we chose five different nucleobases (adenine, cytosine, guanine, thymine, and uracil) as model analytes, and found that under hydrothermal conditions, sodium citrate could react directly with various nucleobases to yield different nitrogen-doped carbon nanodots (CDs). The CDs synthesized from different nucleobases exhibited different fluorescent properties, leading to their respective characteristic fluorescence spectra. Hence, we combined the fluorescence spectra of the CDs with advanced chemometrics like principle component analysis (PCA), hierarchical cluster analysis (HCA), K-nearest neighbor (KNN) and soft independent modeling of class analogy (SIMCA), to present a conceptually novel "synthesis-identification integration" strategy to construct a multidimensional differential sensor for nucleobase discrimination. Single-wavelength excitation fluorescence spectral data, single-wavelength emission fluorescence spectral data, and fluorescence Excitation-Emission Matrices (EEMs) of the CDs were respectively used as input data of the differential sensor. The results showed that the discrimination ability of the multidimensional differential sensor with EEM data set as input data was superior to those with single-wavelength excitation/emission fluorescence data set, suggesting that increasing the number of the data input could improve the discrimination power. Two supervised pattern recognition methods, namely KNN and SIMCA, correctly identified the five nucleobases with a classification accuracy of 100%. The proposed "synthesis-identification integration" strategy together with a multidimensional array of experimental data holds great promise in the construction of differential sensors.

1. Introduction

Up to date, sensing-based analysis of multianalytes have drawn many interests from researchers due to its applications in a wide variety of areas such as environmental monitoring, food safety, clinical diagnosis, and so on [1–11]. However, the development of sensor(s) with multianalyte capability is still a current major scientific and technological challenge. Many existing sensors depend on the traditional strategy of 'lock-and-key' design to realize the identification of analyte (s) [1–6]. The multianalyte sensing mode requires the synthesis of separate, unique and highly selective artificial receptor ("lock") which can bind each analyte of interest ("key"). Obviously, the synthesis of different selective artificial receptor for individual analyte is always a time- and resource-intensive process, and usually involves complicated functionalization or chemical modification. Also, the detection mode of the one-sensor-per-analyte strategy usually suffers strict experimental conditions. More importantly, most sensors based on the 'lock-and-key' recognition mechanism more or less displayed some cross-reactivity to chemically or structurally similar substances.

To alleviate the aforementioned difficulties, differential sensing strategy has recently become a potential alternative to the 'lock-and-key' protocol [7–11]. Differential sensing strategy usually employs multiple nonspecific or weakly interacting receptors to fabricate a cross-reactive sensor array. The sensor array uses only a single-channel signal (usually signal intensity) to generate a unique fingerprint pattern in response to each analyte. Then, the large amount of data from the fingerprint pattern is processed by multivariate chemometric pattern recognition algorithms to classify and identify different analytes

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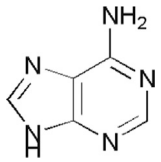
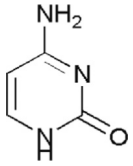
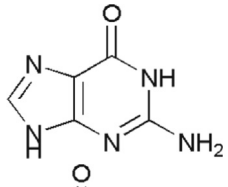
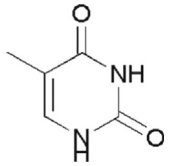
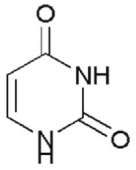
[7–11]. The conventional single-dimensional differential sensing strategy has been currently challenged by the multidimensional differential sensing strategy which extracts more than one signal from a single sensing element to form a characteristic signal pattern, because the multidimensional sensing strategy has superiority in terms of number of sensing elements, discrimination performance, sensing reproducibility, and sample consumption [12–19]. In general, multidimensional differential sensing platform can be fabricated either by mechanically incorporating several different transducers like colorimetric, fluorescent, electrochemical, and mass-sensitive, on a single-chip microsensor system [13,14], or by extracting multiple signals, such as colorimetric, fluorescent, phosphorescent and so on, from individual sensing element (known as "lab-on-a-molecule" or "lab-on-a-nanoparticle") [15–19]. The former multidimensional sensing mode needs multiple different devices to detect different signals, resulting in complicated operations, high cost and unsatisfied reproducibility. For the latter multidimensional sensing mode, materials with multidimensional information are rare or complicated for design and chemical synthesis. Hence, finding a less complicated and universal multidimensional differential sensing strategy is always attractive, especially if the strategy is performed only using a single measurement and device.

At present, with the advancement of science and technology, many modern instruments can generate a multidimensional array of experimental data per sample. For example, optical device like spectrophotometry or spectrofluorimetry usually records a UV–Vis or fluorescence spectrum in a single measurement, which is represented as an array of absorbance or fluorescence intensity derived from multiple wavelengths; electrochemical device can record a voltammogram in a single measurement, which is expressed as an array of current at multiple potentials. Obviously, in the sensing process, different analyte usually gives rise to different spectral signature of response signals. Hence, theoretically, a characteristic spectrum collected using a single measurement and device is sufficient as data input to construct a simple, cost-effective, and universal multidimensional differential sensing platform for detection and discrimination of different analytes.

On the other hand, compared with other analytical techniques, fluorescence-based technique has recently showed great potentials for designing a differential sensor because of its simple instrument, ease of operation, and rich optical information [20–27]. However, the application of most differential fluorescence sensors currently needs two steps, namely, (i) synthesis of functional fluorescent probe(s), (ii) identification of different analytes: mixing of analytes and functional fluorescent probe(s) to bring about their interactions which lead to the characteristic fluorescence change of the probe(s) [21–27]. The two above-mentioned steps are rather time-consuming, and the preparation of high-quality functional fluorescent probe(s) usually requires specific separation and purification, which increase the complexity of the differential sensing assay. Therefore, a conceptual demonstration of a differential fluorescence sensor, which simultaneously realizes the synthesis of fluorescent probes(s) and identification of different analytes, will be of great significance for both fundamental research and applications.

In the present study, we described a conceptually novel "synthesis-identification integration" strategy to construct a differential fluorescence sensing platform. The differential sensors can simultaneously realize the synthesis of fluorescent probes(s) and identification of different analytes. We used the strategy for identification of five different nitrogen-containing nucleobases, namely, adenine (A), cytosine (C), guanine (G), thymine (T), or uracil (U) (chemical structures: Table 1). These nucleobases are the main ingredients of nucleic acid, and play an important role in the carriers of genetic information and the material basis of genetic expression [28,29]. Therefore, it is appealing to develop a method for recognizing the chemically or structurally similar nucleobases and reporting their presence. Most importantly, the new sensing platform took advantage of a one-step hydrothermal reaction to construct a differential sensor. We found that under hydrothermal conditions, sodium citrate (CANa) could react directly with various nucleobases to produce different nitrogen-doped carbon nanodots (CDs). The CDs synthesized from different nucleobases showed different fluorescent properties, resulting in their respective characteristic fluorescence spectra. Herein, we directly utilized the unique spectra to develop a new multidimensional differential sensing strategy for nucleobase discrimination. The strategy only employed a single measurement and device. We measured the three-way fluorescence excitation–emission matrix (EEM) spectra of the sensory system, and represented the EEM spectral data as a multidimensional array of fluorescence intensity at multiple wavelengths. The multidimensional data array was then analyzed using multivariate chemometrics tools including unsupervised pattern recognition (principal component analysis – PCA, hierarchical cluster analysis – HCA) and supervised pattern recognition (K-nearest neighbors – KNN, soft independent modeling of class analogy – SIMCA). The results showed that the developed differential sensor had the strong discrimination ability for the chemically or structurally similar nucleobases, confirming the validity of the new strategy. In addition, we demonstrated that the discrimination ability of the differential sensor could be improved by increasing the number of the data input of fluorescence spectra. The brief schematic for the multidimensional differential sensing of nucleobases was depicted in Scheme 1.

Table 1
Chemical structures of the nucleobases.

Compound	Molecular formula	Molecular weight	Structure
Adenine	$C_5H_5N_5$	135.13	
Cytosine	$C_4H_5N_3O$	111.10	
Guanine	$C_5H_5N_5O$	151.13	
Thymine	$C_5H_6N_2O_2$	126.11	
Uracil	$C_4H_4N_2O_2$	112.09	

2. Materials and methods

2.1. Reagents and chemicals

Adenine (A), cytosine (C), guanine (G), thymine (T), uracil (U), and trisodium citrate dihydrate (CANa) were purchased from Sinopharm