Facile Approach to Fabricate a Chemical Sensor Array Based on Nanocurcumin–Metal Ions Aggregates: Detection and Identification of DNA Nucleobases

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**ABSTRACT:** Here, a three-channel absorbance sensor array based on the nanocurcumin–metal ion (NCur–MI) aggregates is designed for the detection and identification of deoxyribonucleic acid nucleobases (DNA NBs) for the first time. For this purpose, the binding affinities of some of MIs (i.e., Co²⁺, Cr³⁺, Cu²⁺, Fe²⁺, Fe³⁺, Hg²⁺, Mn²⁺, Ni²⁺, V³⁺, and Zn²⁺) to the NCur to induce the aggregation were evaluated under various experimental conditions. Further studies reveal that in the presence of DNA NBs, the aggregates of NCur–Co²⁺, NCur–Ni²⁺, and NCur–Zn²⁺ show the diverse absorbance responses to the deaggregation of NCur depending on the binding affinity of each of DNA NBs to the metal ions Co²⁺, Ni²⁺, and Zn²⁺. These responses are distinguishable from one another. Thus, clear differentiation among the DNA NBs is achieved by linear discriminant analysis and hierarchical clustering analysis to generate clustering maps. The discriminatory capacity of the sensor array for the identification of the DNA NBs is tested in the ranges of 2.4–16 and 5.6–10.4 μM. Furthermore, a mixed set of the DNA NBs was prepared for multivariate multicomponent analysis. Finally, the practicability of the sensor array is confirmed by the discrimination of the DNA NBs in an animal DNA sample. It should be noted that the proposed array is the first example to fabricate an NCur-based sensor array for the simultaneous detection of DNA NBs.

1. INTRODUCTION

Nowadays, biological sensors as a novel technology offer many advantages such as simple, suitable cost and adequate time for wide range of applications. The biological sensors determine vital parameters related to diagnostics, environmental monitoring, and health. One of the most important problems in development of a biological sensor is its capability for the simultaneous detection of chemical targets. Recently, sensor array-based detection systems, which are examples of highly efficient biosensors, have been successfully used for the determination and discrimination of a wide range of chemical targets such as biological compounds,¹⁻⁵ heavy metals,⁶⁻⁹ food,¹⁰ and pesticides.¹¹

Generally, a sensor array, similar to the mammalian olfactory and gustatory system, uses cross-reactive receptor elements (REs) to create of unique patterns (i.e., fingerprints) for each target. Thus, unlike with a “lock–key system”, the cross-reactive REs involve receptors with the lower selectivity. Each RE shows the various affinity degrees toward targets. As a result, this leads to produce the non-specific interaction profiles for targets. Finally, the matrix of data obtained is analyzed by the linear discriminant analysis (LDA) and hierarchical clustering analysis (HCA).¹²⁻¹⁵ The HCA is an unsupervised approach for the grouping of target vectors according to their spatial distances in their full original vector space. The HCA is performed by the minimum variance (Ward’s) method.¹⁶ LDA is a supervised pattern recognition method for recognizing the linear combination of features that differentiate two or more classes of object. In array-based sensors, the LDA is used to differentiate quantitatively the response patterns of targets.¹⁷

The choice of a suitable number of the cross-reactive REs also is an important parameter for transfer of desired information. If the number of cross-reactive REs is very high, over-fitting phenomenon causes. This phenomenon removes the prediction ability of sensor array for unknown samples. In addition, if the number of cross-reactive REs be insufficient, the capability of sensor array to discriminate of targets decreases.¹⁸

The DNA containing purine and pyrimidine bases is well known as two of the constructing blocks of DNA. They are the indispensable units of the cell programming machinery. There is a great interest in development of high-efficiency biosensors
for the monitoring of DNA nucleobases (NBs) (i.e., adenine, guanine, cytosine, and thymine). This is mainly due to vital roles of the DNA NBs in the different life process in human.\textsuperscript{19} The abnormal changes in the DNA NBs content show mutation of the immunity system that may be responsible for different diseases. Typically, the average concentration of DNA NBs and other extracellular fluids are in the range of 0.4−6 μM.\textsuperscript{20−23} The concentration of DNA NBs can serve as a key index for the detection of cancer, AIDS, and disease growth.\textsuperscript{24,25} Methods currently available for detecting DNA NBs mainly include electrochemical, surface-enhanced Raman scattering, and colorimetric methods.\textsuperscript{26−36} For example, Ng et al. reported an Au-rGO/MWCNT/graphite-based array sensor for the determination of DNA NBs by square wave voltammetry.\textsuperscript{29} Lavanya et al. developed an electrochemical method based on Cu-doped CeO\textsubscript{2} nanoparticle-modified glassy carbon electrode (Cu−CeO\textsubscript{2}/GCE) for the determination of DNA NBs.\textsuperscript{31} Our team used the electrocatalytic oxidation of DNA NBs on a glassy carbon electrode modified with SiC nanoparticles (SiCNP/GC) for the determination of DNA NBs.\textsuperscript{33} However, these methods often require sample preparation and complex instrumentation. As a result, there is an urgent need for low-cost, sensitive, and selective methods for the determination of DNA NBs. To access a rapid and reliable method, an essential idea can be using of sensor array-based sensing platforms. Liu et al. reported the first sensor array based on self-assembly of mercaptoethyamine, N-acetyl-L-cysteine, 2-dimethyl-amine-thanethiol, and thioglycolic acid-quantum dots for the identification of DNA NBs.\textsuperscript{37}

Curcumin or diferuloylmethane is the main compound extracted from the root of turmeric. The curcumin exhibits antioxidant, antineoplastic, and antimicrobial properties. In addition, the curcumin shows neuroprotective and anticancer activities.\textsuperscript{38,39} Nevertheless, the curcumin suffers from the low water solubility and poor bioavailability.\textsuperscript{40} Nanoionization of the curcumin [nanocurcumin (NCur)] is one of procedures that can help to solve this problem.\textsuperscript{41−43} Recently, the photophysical properties of the NCur as a sensing probe in optical sensors have received notable attention in chemistry of various fields.\textsuperscript{44−46}

Herein, we explore the nanocurcumin−metal ion (NCur−MIs) aggregates to fabricate an absorbance sensor array for the detection and discrimination of the DNA NBs. The MIs including Co\textsuperscript{2+}, Cr\textsuperscript{3+}, Cu\textsuperscript{2+}, Fe\textsuperscript{2+}, Fe\textsuperscript{3+}, Hg\textsuperscript{2+}, Mn\textsuperscript{2+}, Ni\textsuperscript{2+}, V\textsuperscript{3+}, and Zn\textsuperscript{2+} are selected to induce of the aggregation NCur. It was found that in the presence of DNA NBs, the deaggregation NCur with various degrees is observed for the NCur−Co\textsuperscript{2+}, NCur−Ni\textsuperscript{2+}, and NCur−Zn\textsuperscript{2+} aggregates. As a result of the deaggregation NCur, the absorbance profiles produce a unique spectral pattern for each of the DNA NBs. Moreover, we employed the proposed array for the identification of the DNA NBs in an animal DNA sample. According to our best knowledge, the proposed array is the first example to fabricate an NCur-based sensor array for the simultaneous detection of DNA NBs.

Scheme 1. Design of the Fabricated Sensor Array Using the NCur−MIs Aggregates for the Detection and Identification of DNA NBs

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2. RESULTS AND DISCUSSION

2.1. Characterization of the NCur. The photophysical properties and morphology of the NCur are investigated by the ultraviolet−visible (UV−vis) absorption, transmission electron microscopy (TEM), and Fourier-transform infrared (FT-IR), as shown in Figure S1. The NCur displays a characteristic UV−vis absorption band centered at 429 nm, and its color is yellow, as shown in Figure S1I. The TEM image in Figure S1II shows that the NCur is well dispersed in the aqueous solution. Thus, the NCur can be used as a nanoprobe for the identification of DNA NBs. Another evidence for the successful synthesis of the NCur is FT-IR analysis. Figure S1III shows the vibrational spectra of the curcumin and the NCur. The peaks of curcumin at 3200−3500 cm$^{-1}$, 1628 cm, 1515 cm$^{-1}$, 1284 cm$^{-1}$, and 1160 cm$^{-1}$ in Figure S1III(a) are attributed to the phenolic OH stretching, C=O group, aromatic C−C vibrations, aromatic C−O, and C−O−C stretching, respectively. Figure S1III(b) shows the FT-IR spectrum of the NCur. As can be seen, the NCur shows the similar vibrational frequencies to the curcumin. This result confirms in which the NCur is successfully synthesized.

2.2. Scheme of the Sensor Array. The MI-induced aggregation of the NCur for formation of chemosensing aggregates by chemical interaction mechanisms has been described in several reports. On the other hand, over the past few decades, the interaction of DNA NBs with MIs (especially transition MIs) has attracted considerable interest because of their biological importance and clinical implications. Therefore, based on the competing interaction of MIs with the NCur and the DNA NBs, a multichannel sensor array can be constructed by an appropriate number of NCur−MI aggregates by displaying the absorbance changes of the NCur. As a result of the different structures of DNA NBs, the binding affinities of MIs toward the DNA NBs (typically Co$^{2+}$, Ni$^{2+}$, and Zn$^{2+}$) can be differentiated from the competition with the NCur. Subsequently, the absorbance changes of the sensor array can be recorded to produce fingerprints for each of the DNA NBs. Then, the fingerprint maps are analyzed by the LDA and HCA. This sensing strategy of the sensor array can be displayed in Scheme 1. It should be noted that the sensing strategy used in this work is very simple in the process fabrication, and there is no need for complex synthesis steps.

2.3. Steps of Fabrication of the Sensor Array. 2.3.1. Stability of the NCur. Previous studies have shown that the curcumin at the basic solutions is decomposed to other components such as vanillin, ferulic acid, and feruloyl methane by hydrolytic degradation. Therefore, the effect of the pH solution and buffer composition was examined on the stability of the NCur. Figure S2 shows the absorbance spectra and photographs of the NCur in the different pHs/buffers. The results revealed that in the phosphate buffers (PHBs, pH 11 and 12) and the ammonium buffer (AMB, pH 10), the NCur

![Figure 1. UV−vis spectra of DNA NB-induced deaggregation of NCur by NCur−Co$^{2+}$ aggregate (I): (A) adenine, (B) guanine, (C) cytosine, and (D) thymine; NCur−Ni$^{2+}$ aggregate (II): (A) adenine, (B) guanine, (C) cytosine, and (D) thymine; NCur−Zn$^{2+}$ aggregate (III): (A) adenine, (B) guanine, (C) cytosine, and (D) thymine. The UV−vis spectra of NCur−Co$^{2+}$, Ni$^{2+}$, and Zn$^{2+}$ aggregates is defined as the spectrum of blank.](https://dx.doi.org/10.1021/acsomega.0c00593)
is fully faded and the color solution changes to colorless. In general, the maximum stability of the NCur is in the range of pH 2−8. The tris buffers (TRBs, pH 9) also is an appropriate buffer for further experimental studies on the NCur at the basic solutions. Therefore, the PHBs (pH 2−10), acetate buffers (ACBs, pH 4−6), tris buffers (TRBs, pH 7−9), and ammonium buffers (AMBs, pH 8 and 9) were selected for further experiments.

2.3.2. Formation of the NCur−MI Aggregates. In the next step of designing the sensor array, the absorbance responses of the NCur to some of MIs (i.e., Co²⁺, Cr³⁺, Cu²⁺, Fe²⁺, Fe³⁺, Hg²⁺, Mn²⁺, Ni²⁺, V³⁺, and Zn²⁺) in the selected pHs/buffers were recorded, as shown in Figure S3. The results revealed that in the presence of MIs, the absorbance intensity of the NCur was decreased in different degrees with introduction of most of these MIs because of the aggregation of the NCur. As can be seen, in the range of pH 2−7, the MIs show the lowest binding affinity toward the NCur. The lowest and highest binding affinity toward the NCur is related to the ions Cr³⁺ (or V³⁺) and Hg²⁺, respectively. Moreover, the addition of the Co²⁺, Cr³⁺, Cu²⁺, Mn²⁺, Ni²⁺, V³⁺, and Zn²⁺ to the NCur solution in the PHB (pH 10) causes that the spectrum of the NCur inclines toward its spectrum in the lower basic solutions. This is probably because of the precipitation of MIs and resulting in the decrease of the pH solution. Therefore, considering the absorbance of responding extents, eight MIs under specific conditions (i.e., the Co²⁺ in TRB (pH 8), the Cu²⁺ in TRB (pH 9), the Fe²⁺ in PHB (pH 8), the Fe³⁺ in TRB (pH 7), the Hg²⁺ in TRB (pH 8), the Mn²⁺ in AMB (pH 9), the Ni²⁺ in TRB (pH 9), and the Zn²⁺ in AMB (pH 8)) were selected as primary candidates for the fabrication of the NCur−MI aggregates. The results are presented in Figure S3. From the results shown in Figure S3, about 44 μM Co²⁺, 40 μM Cu²⁺, 44 μM Fe²⁺, 44 μM Fe³⁺, 24 μM Hg²⁺, 48 μM Mn²⁺, 44 μM Ni²⁺, and 44 μM Zn²⁺ μM during times of 11, 10, 11, 11, 6, 12, 11, and 11 min were observed to achieve the maximum aggregation of the NCur, respectively.

2.4. Sensor Array Responses to DNA NBs. The results revealed that in the presence of DNA NBs from among the primary candidates, the three of NCur−Co²⁺, NCur−Ni²⁺, and

Figure 2. (A) Color change patterns of the solutions using three of the NCur−Co²⁺, NCur−Ni²⁺, and NCur−Zn²⁺ aggregates as REs for DNA NBs at 5.6 μM. (B) Color map corresponding to (A).

Scheme 2. Probable Mechanism in the Proposed Array for the Detection and Identification of DNA NBs
NCur−Zn$^{2+}$ aggregates can produce different deaggregation responses. Thus, a three-channel sensor array was fabricated in this work. To better determine the sensing conditions, the effect of the incubation time was also explored. Figure S4 indicates that the maximum peak intensity of the NCur stabilizes after 100 min of the incubation of the DNA NBs with Co$^{2+}$, Ni$^{2+}$, and Zn$^{2+}$, respectively. The DNA NBs were exposed to the three of NCur−Co$^{2+}$, NCur−Ni$^{2+}$, and NCur−Zn$^{2+}$ aggregates in the range of 0.8−10.4 μM. Then, the absorbance responses were measured, as shown in Figure 1I−III. As can be observed, the adenine and guanine can induce the deaggregation of the NCur for the NCur−Co$^{2+}$ aggregate. However, in the presence of cytosine and thymine, the insignificant spectral changes occur, as shown in Figure 1I. This confirms that the adenine and guanine show the more binding affinity for the Co$^{2+}$ compared to the NCur. However, the situation is opposite for the cytosine and thymine. For the NCur−Ni$^{2+}$ aggregate, no significant deaggregation was seen with the addition of the adenine or cytosine, as shown in Figure 1II. Nonetheless, in the presence of guanine and thymine, the deaggregation of the NCur occurs. The behavior of the NCur−Zn$^{2+}$ aggregate is somewhat different compared to the NCur−Co$^{2+}$ or NCur−Ni$^{2+}$ aggregate, as shown in Figure 1III. The adenine, cytosine, and thymine can induce the deaggregation of the NCur. The weaker complexation of Zn$^{2+}$ with the NCur compared to the Co$^{2+}$ or Ni$^{2+}$ leads to the more selectivity of the DNA NBs for Zn$^{2+}$. All these results suggest that the sensor array can produce the unique absorbance profiles (i.e., fingerprints) to each of the DNA NBs. These fingerprints can be used for the identification of DNA NBs. Furthermore, the colorimetric responses of the sensor array to DNA NBs are shown in Figure 2A. Also, a color difference map is provided to qualitatively visualize the colorimetric patterns of the sensor array, as shown in Figure 2B. As can be seen from Figure 2A,B, the DNA NBs exhibit different colorimetric patterns. These colorimetric patterns demonstrate the ability of the sensor array to discriminate the DNA NBs.

The determination of binding sites the ions of Co$^{2+}$, Ni$^{2+}$, and Zn$^{2+}$ to the DNA NBs theoretically and empirically have been investigated in several studies. Based on our observations and accordingly previous studies, the probable mechanism in our array can be illustrated in Scheme 2. The ions of Co$^{2+}$, Ni$^{2+}$, and Zn$^{2+}$ bind to the NCur via the acetylacetone functional group. This results in the aggregation of the NCur−Zn$^{2+}$ aggregate.
of the NCur. The stronger binding of ions of the Co$^{2+}$, Ni$^{2+}$, and Zn$^{2+}$ to the DNA NBs (i.e., the Co$^{2+}$ to adenine and guanine, the Ni$^{2+}$ to guanine and thymine to and the adenine, and Zn$^{2+}$ to cytosine and thymine) leads to release of them from the NCur. Thus, the deaggregation of the NCur occurs depending on the binding affinity of each the DNA NBs for the aforesaid ions. To end, it is necessary to point out that there is not significant interaction among the DNA NBs and the NCur in the range of worked concentrations.

2.5. Analysis of TEM Images. It is obviously revealed that the NCur solution is well dispersed, as shown in Figure S1II. Next, the morphology changes of the NCur−Co$^{2+}$, NCur−Ni$^{2+}$, and NCur−Zn$^{2+}$ aggregates were surveyed in the absence and presence of the DNA NBs, as shown in Figure 3. In the presence of Co$^{2+}$, Ni$^{2+}$, and Zn$^{2+}$, without the DNA NBs, the NCur is irregularly shaped to different degrees. This is mainly due to the differential interactions of Co$^{2+}$, Ni$^{2+}$, and Zn$^{2+}$ with the NCur, the value of pH solution, and the buffer composition. The Zn$^{2+}$ has the minimum capability to the absorbance quenching of the NCur, followed by the Ni$^{2+}$ and Co$^{2+}$, as shown in Figure 3I,VI,XI. In the presence of DNA NBs, the diverse extents of the deaggregation NCur can be seen depending on the binding affinity of each of the DNA NBs toward ions of the Co$^{2+}$, Ni$^{2+}$, and Zn$^{2+}$. Figure 3II−V shows the TEM images of the NCur−Zn$^{2+}$ aggregate in the presence of DNA NBs. These observations suggest the substitution of Zn$^{2+}$ from the surface NCur. Resulting, the deaggregation of NCur occurs. About the NCur−Ni$^{2+}$ and NCur−Co$^{2+}$aggregates, the selectivity of DNA NBs to induce the deaggregation of NCur is further clear. This indicates that the binding affinity of the NCur toward the Ni$^{2+}$ and Co$^{2+}$ is comparable with the DNA NBs. For example, in the presence of guanine and thymine, the NCur−Ni$^{2+}$ aggregate indicates the maximum selectivity to induce the deaggregation of NCur, as shown in Figure 3VIII,X. However, for the NCur−Co$^{2+}$ aggregate, the cytosine and thymine indicate the minimum selectivity to induce of the deaggregation NCur, Figure 3XIV,XV. Similarity, for the Ni$^{2+}$−NCur aggregate, in the
presence of the adenine and cytosine, the aggregation NCur almost is constant, as shown in Figure 3VII,IX. For the NCur–Co\(^{2+}\) aggregate, in the presence of adenine and guanine, the deaggregation of NCur occurs, as shown in Figure 3XII,XIII.

### 2.6. DNA NB Discrimination by LDA and HCA

The LDA and HCA analyses were employed for quantitative evaluation of the absorbance responses of the sensor array. Considering the spectral regions having the most spectral changes, 14 variables in the range of 352–612 with 20 nm intervals (i.e., 352, 372, 392, 412, 432, 452, 472, 492, 512, 532, 552, 572, 592, and 612 nm) were selected. Then, the differences between the absorbance values of the NCur in the absence and presence of the DNA NBs, \(\Delta A\), were defined as the analytical signals. Consequently, vectors with size 14 variables \(\times\) 3 REs were organized according to \(\Delta A\) values for further analyses. Because of the large number of absorbance responses and probably overlapping of them, all the concentration range were divided into two ranges of 2.4–4.8 \(\mu M\) (I) and 5.6–10.4 \(\mu M\) (II) for (A) adenine, (B) guanine, (C) cytosine, and (D) thymine \((n = 3)\).

**Figure 6.** Values of EDs vs different concentrations of DNA NBs in the ranges of 2.4–4.8 \(\mu M\) (I) and 5.6–10.4 \(\mu M\) (II) for (A) adenine, (B) guanine, (C) cytosine, and (D) thymine \((n = 3)\).

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Subsequently, the limits of detections (LODs) were calculated using the well-known...
also for the quantification of DNA NBs, Table 1. Accordingly, the LODs of 0.51, 0.41, 0.81, and 0.59 μM were obtained for the adenine, guanine, cytosine, and thymine, respectively. These results confirm that the sensor array can be used not only for the identification but also for the quantification of the DNA NBs.

The analytical performance of the sensor array was compared with some of previously electrochemical methods employed for the determination of DNA NBs. The details are presented in Table 2. This comparison shows the ability of the sensor array to accomplish the determination of DNA NBs within a single test. As can be seen, the sensitivity of the proposed array is comparable with reported electrochemical methods. It is worthwhile to note that the most obvious feature of our array is its cost-effectiveness.

2.7. Discrimination of DNA NB Mixtures. The discrimination of DNA NBs mixture has specific importance because of their quantitative amounts reflect diagnostic criteria for different diseases. The absorbance responses of the sensor array were measured for two binary mixtures (i.e., adenine/thymine and guanine/thymine), two ternary mixtures (i.e., adenine/guanine/cytosine and adenine/guanine/thymine), and a quadruple mixture of DNA NBs. The concentration of all of the DNA NBs was 5 μM. It was observed that the absorbance responses of DNA NB mixture are different than pure forms of them, as shown in Figure S5. The results of LDA and HCA show that all mixtures as well as two pure forms of the guanine and cytosine are well resolved, as shown in Figure S6.

2.8. Study of Selectivity. To evaluate the performance of the sensor array, its absorbance responses to a variety of interfering substances were measured. It was found that the NO₃⁻, Cl⁻, K⁺, Na⁺, Ca²⁺, and Mg²⁺ at a concentration of 10 μM did not interfere in the identification of the DNA NBs, Figure S7I. As can be observed, the DNA NBs and these substances are clustered to two separated groups. However, it is clear that some of biological compounds and MIs may cause the sensor array’s spectral changes. The effects of arginine, ascorbic acid, cysteine, dopamine, and glucose (all a concentration 10 μM) and Cd²⁺, Cu²⁺, Fe³⁺, Mn²⁺, and Pb²⁺ (all a concentration 10 μM) also were investigated. The results revealed that although these biological compounds and MIs can cause the absorbance responses, they are obviously resolved from the DNA NBs, Figure S7II,III. The HCA dendrograms are presented in Figure S8. They suggest that the sensor array can effectively discriminate the DNA NBs in the presence of other interference substances.

2.9. Analysis of an Animal DNA Sample. Eventually, the applicability of the sensor array for the identification of DNA NBs was assessed in a rat DNA sample. Figure 7 indicates that the rat DNA sample produces a specific array’s pattern, whereas four NBs-spiked DNA samples were grouped into four different clusters. The first two factors consisted the 96.8 and 2.2% of the total variance 99%, respectively. These results show that the sensor array had potential to distinguish the DNA NBs in real samples.

3. CONCLUSIONS

The three of NCur–Co²⁺, NCur–Ni²⁺, and NCur–Zn²⁺ aggregates were used as REs in the development a sensor array for the identification and discrimination of DNA NBs for the first time. Depending on experimental conditions, the MIs...
(i.e., Co^{2+}, Ce^{3+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, Mn^{2+}, Ni^{2+}, V^{3+}, and Zn^{2+}) can induce the aggregation of NCur. Further studies revealed that in the presence the DNA NBs, the three of NCur—Co^{2+}, NCur—Ni^{2+}, and NCur—Zn^{2+} aggregates show the different deaggregation responses of the NCur. These responses can produce a fingerprint pattern for each of the DNA NBs, and it allows the discrimination of the DNA NBs and their mixtures. Finally, it was found that the sensor array can detect the DNA NBs in an animal real sample. We believed that the sensor array presents a novel approach for the development of highly selective arrays for biological applications.

4. EXPERIMENTAL SECTION

4.1. Materials. Curcumin (≥92.5%), dichloromethane (CH_{2}Cl_{2} ≥ 98%), Triton X-100 (5%), adenine (≥99%, HPLC), guanine (≥99%, HPLC), cytosine (≥99%, HPLC), and thymine (≥97%, UV) were purchased from Sigma-Aldrich Co (www.sigmaaldrich.com). Na_{2}HPO_{4}, H_{2}O, CH_{3}COONa·3H_{2}O, H_{2}NC(CH_{2}OH)_{3} and NH_{4}Cl, respectively.

4.2. Equipment and Software. An ultrasound bath (230/240V-50 Hz-30 W, Italy) was utilized for the synthesis of the NCur. A rotary evaporator (Heidolph, Germany) was used for solvent elimination. The pH values were recorded of each of the DNA NBs; UV−vis spectra of the NCur, (a) and NCur (b); UV−vis spectrum of the NCur, (II) the TEM image of the NCur, and (III) the FT-IR spectrum of the curcumin.

4.3. Synthesis of the NCur. The curcumin solution was prepared by dissolving the 125 mg curcumin in the 25 mL CH_{2}Cl_{2}. Then, aqueous solution was prepared by the addition of the 2 mL of curcumin solution to the aqueous solution of the 10 mL of Triton X-100 (5%) to 90 mL of boiling water. The pH values were recorded of each of the DNA NBs; UV−vis spectra of the NCur, (a) and NCur (b); UV−vis spectrum of the NCur, (II) the TEM image of the NCur, and (III) the FT-IR spectrum of the curcumin.

4.4. MI-Induced Aggregation of the NCur. The MI-induced aggregation of the NCur by Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, Mn^{2+}, Ni^{2+}, V^{3+}, and Zn^{2+} was screened in the PHBs (10 mM, pH 9), TRBs (10 mM, pH 8), and AMBs (10 mM, pH 8) as the following: the 30 μL of the NCur solution was added to 0.5 mL of the as-prepared buffers. Then, the 1 μL volumes of MI (Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, Mn^{2+}, Ni^{2+}, V^{3+}, and Zn^{2+}) with different concentrations were added to each of the NCur solution. In each pH, the absorption spectra were measured after standing for 1 min in the range of 325–650 nm. In each pH, the addition of the MI is continued until that the constant aggregation was achieved.

4.5. Assay Procedure. The fabrication of the sensor array was carried out as the following: the 0.5 mL of TRB (10 mM, pH 8) containing the 44 μM of Co^{2+}, the 0.5 mL of TRB (10 mM, pH 9) containing the 44 μM Ni^{2+}, and the 0.5 mL of AMB (10 mM, pH 8) containing the 44 μM of Zn^{2+} were separately incubated with the different concentrations of DNA NBs (0.8–10.4 μM) for t = 100 min. In each solution, the absorption spectra were measured in the presence of 30 μL of NCur solution for t = 12 min before the detection procedure. The selectivity of the sensor array was studied by addition of various interfering substances. All the measurements were performed in triplicates. Finally, the LDA and HCA were employed for the purposes of the detection and identification of the DNA NBs.

4.6. Acid Hydrolysis and DNA Identification in an Animal DNA Sample. For real sample analysis, an animal DNA sample from brain tissues of male Wistar rat was used for the identification of DNA NBs. For this purpose, the DNA sample is provided by Dr. S. Ahmadi Animal Physiology Laboratory in Department of Biological Science, University of Kurdistan, Sanandaj, Iran. Briefly, rat was decapitated and the whole brain rapidly was removed from the skull and prefrontal cortex. The isolated brain tissue was instantly incubated with RNAlater RNA stabilization agent at 4 °C. After a period of 24 h, the RNAlater solution was aspirated and the tissue was kept at −80 °C. The RNasey Mini Kit was employed for the total RNA extraction. Quality of the extracted RNA is determined by electrophoresis. Finally, reverse transcription of the total RNA is performed by Thermo Scientific RevertAid first strand cDNA synthesis kit. For acid hydrolysis, the DNA sample was incubated with 5 mL of H_{2}SO_{4} (8 M) in a glassy tube. The solution was sonicated at 150 °C for 2 h, followed by neutralization with 10 mL of NaOH solution (8 M). After cooling to room temperature, the absorbance responses of the sensor array to acid hydrolyzed-spiked DNA samples (all DNA NBs at 3 μM), as described in the Section 4.5, were recorded. Subsequently, these responses were analyzed by the LDA and HCA.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c00593.

UV−vis spectrum of the NCur, (II) the TEM image of the NCur, and (III) the FT-IR spectrum of the curcumin (a) and NCur (b); UV−vis spectra and photographs of NCur solution; UV−vis spectra of MI-induced aggregation of NCur; effect of incubation time on determination of each of the DNA NBs; UV−vis spectra of DNA NB
mixture-induced deaggregation of NCur; illustrated results of LDA and HCA for the different mixtures of DNA NBs; illustrated results of LDA for the discrimination of DNA NBs in the presence of various interfering compounds; and illustrated results of HCA for the discrimination of DNA NBs (PDF)

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Notes
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