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ARTICLE

One-step analysis of glucose and acetylcholine based on intrinsic peroxidase-like activity of Ni/Co LDHs microspheres in water

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In this present study, a simple strategy is developed for Ni/Co layered double hydroxides (LDHs) as substitute for natural peroxidase. The obtained Ni/Co LDHs exhibited ease of preparation, low-cost, and water-solubility, and importantly it showed high catalytic activity at neutral pH solution (phosphate buffer, Tris-HCl buffer, and even water). Benefit by Ni/Co LDHs having similar pH and temperature with specificity oxidase, a novel one-step method for biosensor was developed in water. Glucose detection was selected as an application model system to evaluate the performance of this method, which showed the linear range from 0.5 μM to 100 μM with a detection limit (DL) of 0.1 μM. We also extended the one-step method to detect acetylcholine (ACh) by taking advantage of the specific catalytic reaction of acetylcholinesterase (AChE) and choline oxidase (ChOx) with the linear range from 10 μM to 150 μM and the DL of 1.62 μM. The proposed method had ease of operation, simple step and rapid process for the glucose and ACh detection in real samples. On the basis of these advantages and virtues, Ni/Co LDHs could become attractive nanozymes in biotechnology and bioassays, and make a great influence on the next generation of enzyme mimetics system.

1. Introduction

Natural enzymes possessed high catalytic capacity and substrate specificity under mild conditions.[1] However, the practical application of natural enzymes is hampered by their disadvantages such as high cost, low operational stability (denaturation and deactivation), and the harsh reaction condition.[2] Therefore, artificial enzymes that mimic the complexity and function of natural enzymes have been paid attention for the past two decades.[3] Nanozymes, which are nanomaterials with enzyme-like activity, have attracted researchers' enormous interest due to their unique properties, such as large surface area for further modification, size-(shape-, composition-) dependent catalytic activities, smart response to external stimuli, self-assembly capability and so on.[4-7] Up to now, a lot of nanomaterials, such as metal nanomaterial,[8-13] metal oxide nanomaterials,[14-20] carbon nanomaterials,[21-27] disulphide-based nanomaterials,[28, 29] BiOI hierarchical nanoflower[30] and so on, have been discovered to possess peroxidase-like activity. As compared to horse radish peroxidase (HRP), these nanozymes may serve as promising candidates with advantages of controlled synthesis in low cost, tunability in catalytic activities, and high stability against stringent conditions.

However, despite these favorable properties, one of the main shortcomings that these reported peroxidase mimetics faced is that its optimum reaction occurs in acidic solution, which results in the peroxidase mimetics and natural enzyme working separately to catalyze single isolated reactions rather than coupled tandem reactions. For example, Fe_3O_4 magnetic nanoparticles,[15] Au nanoparticles,[8] carbon nanodots,[24] WS_2 nanosheet[31] and so on, as peroxidase mimetic, were used to detect glucose, and their strategies of detection glucose were all carried out through separate two-step method. First step, the glucose oxidase was used to catalyze glucose with molecular oxygen to produce H_2O_2 under the mild conditions (pH=7, 37 °C). Second step, the peroxidase mimic and colorimetric substrate were added to the above solution and incubated under the acid condition. Similar to the glucose detection, the analysis of xanthine,[32] acetylcholine (ACh),[33] sarcosine,[34] and D-alanine[35] based on the peroxidase mimetics was also carried out through the separate two-step method. Though this analytical approach exhibited good sensitivity, selectivity, and accuracy, such a separation step was tedious, complex time-consuming, and even resulting in limited overall efficiency of the enzymatic reactions.

Therefore, great efforts have been devoted to overcome the above-mentioned limitation. Only a few literatures about nanozymes exhibited peroxidase-like activity at neutral pH have been reported. For example, Qu et al. reported GO-AuNCs hybrid exhibiting high peroxidase-like catalytic activity over a broad pH range,[36] and Liu and co-workers reported that Au@Ag heterogeneous nanorods had high catalytic activity at nearly neutral pH and an one-pot method for the detection of glucose was

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developed.[37] However, these noble metal-based nanozymes are typically expensive and complex. Thus, it is still necessary to develop simple and cheap peroxidase mimetics that are able to exhibit catalytic activity in neutral solution.

To tackle these challenges, here we report a facile chemical coprecipitation method to prepare Ni/Co layered double hydroxides (LDHs), which exhibits peroxidase-like activity at neutral pH solution (phosphate buffer, Tris-HCl buffer, and even water). Although other LDHs, including Co-Al LDHs,[38] DNA–LDH,[39] and Fe-Ni LDHs-hemin[40] possessed peroxidase-like activity were reported, the preparation process of these LDHs was complex. As peroxidase mimetics, Ni/Co LDHs showed several advantages such as ease of preparation, low-cost, and water-solubility and so on. To demonstrate the promising application of the synthesized Ni/Co LDHs, a novel one-step method for glucose detection was developed in water. Furthermore, we also extended this one-step method to the detection of ACh by taking advantage of the specific catalytic reaction of acetylcholinesterase (AChE) and choline oxidase (ChOx). The promising one-step method had ease of operation, simple step and rapid process.

2. Experimental

2.1. Materials

30% H_2O_2, $Co(NO_3)_2{\cdot}6H_2O$, $Ni(NO_3)_2{\cdot}6H_2O$, $NH_3{\cdot}H_2O$ were purchased from Tianjin Guangfu Chemical Reagent Factory (Tianjin, China). 3,3′,5,5′-tetramethylbenzidine (TMB), 2,2′-azinobis (3-ethylbenzothiazo-line-6-sulfonic acid) diammonium salt (ABTS), o-phenylenediamine (OPD), glucose oxidase (GOx, 50 KU), acetylcholinesterase (AChE, from Electrophorus electricus) and choline oxidase (ChOx, from Alcaligenes sp.) were purchased from Sigma-Aldrich. Glucose, fructose, lactose and maltose were purchased from Sangon Biotech (Shanghai). Acetylcholine chloride (ACh) was purchased from Acros. Glycine, cystine, tryptophan, threonine, proline were purchased from Adamas.

All other chemicals were of analytical reagent grade and used without further purification. Deionized water was used throughout the experiment.

2.2. Apparatus and Characterization

A TU-1900 double beams UV-vis spectrophotometer (Beijing Purkine General Instrument Co. LTD., China) was used to implement kinetic experiments and measure absorbance. Transmission electron microscopy (TEM) images of Ni/Co LDHs were obtained using a Tecnai G^2 F30 instrument. The surface morphology of Ni/Co LDHs was taken on a field emission scanning electron microscopy (FE-SEM Hitachi S-4800, Japan). X-ray diffraction (XRD) pattern of Ni/Co LDHs was carried out with X'Pertpro Philips X-ray diffractometer with Cu Kα radiation (λ=0.154056). ESR (Electron Spin Resonance) measurements were acquired using a JES-FA200 electron spin resonance spectrometer.

2.3. Preparation of Ni/Co LDHs

The Ni/Co LDHs microspheres were fabricated by a facile one-step chemical coprecipitation way. 10 mL 0.1 mol L^{-1} $Co(NO_3)_2{\cdot}6H_2O$ and 20 mL 0.1 mol L^{-1} $Ni(NO_3)_2{\cdot}6H_2O$ were uniformly mixed, and 1 mL 35% $NH_3{\cdot}H_2O$ was added into the above mixed solution drop by drop. The precursor was sealed in a 50 mL glass bottle for 3 h at room temperature. The sample was collected and washed by centrifugation for several cycles and the as-prepared Ni/Co LDHs sample was obtained by deionized water and absolute ethanol washed for several times and dried in a vacuum oven at 60 °C overnight.

2.4. Kinetic Analysis

The peroxidase activity of Ni/Co LDHs was carried out at 37 °C using 0.1 mL 1 mg mL^{-1} Ni/Co LDHs in a reaction volume of 3.0 mL deionized water with ABTS as substrate, and the concentration of H_2O_2 was fixed, unless otherwise stated. Kinetic measurements were carried out in time course mode by monitoring the absorbance change at 417 nm for 5 min on TU-1900 UV-vis spectrophotometer. The Michaelis-Menten constant was calculated using a Lineweaver-Burk plot:

$$1/v = K_m/V_{max}\,(1/[S] + 1/K_m) \quad (1)$$

Where v is the initial velocity, V_{max} represents the maximal reaction velocity, and [S] is the substrate concentration. K_m is Michaelis-Menten constant, which is an indicator of enzyme affinity for its substrate. The smaller the value of K_m is, the stronger the affinity between the enzyme and the substrate.

2.5. Assay of Glucose

Glucose detection was performed by a facile one-step method. The mixed solution which included 5 μL 10 mg mL^{-1} GOx, 100 μL of different concentration of glucose, 50 μL 10 mg mL^{-1} ABTS, 100 μL 1 mg mL^{-1} Ni/Co LDHs and 2.75 mL deionized water, was incubated at 37 °C for 30 min and then the absorbance at 417 nm was measured.

The serum samples were treated by ultrafiltration on a 10 kDa ultrafiltration membrane and centrifugation at 10000 rpm for 40 min, and then the supernatant was diluted 40 times before measurement.

2.6. Assay of ACh

The detection of ACh was also performed by the one-step method. The coupled reaction was carried out by adding 40 μL 2 U mL^{-1} ChOx, 60 μL 2 U mL^{-1} AChE, 100 μL of different concentration of ACh, 50 μL 10 mg mL^{-1} ABTS, 100 μL 1 mg mL^{-1} Ni/Co LDHs and 2.65 mL deionized water at 37 °C for 1 h and then the absorbance at 417 nm was measured.

The preparation of milk sample was as following: 20 g milk sample was added into 10 mL of 3.0 M HCl and mixed uniformly. The mixture was incubated at 70 °C for 3 h and shaken every half an hour. And then the solution was adjusted to pH 3.5~4 with NaOH. After filtration with 0.45 μm ultrafiltration membrane, the solution was quantitatively diluted with water to 50 mL. And then the solution of milk was diluted 40 times before measurement.

3. Results and discussion

3.1. Characterization of Ni/Co LDH

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The crystalline structure of as-obtained Ni/Co LDHs was examined by XRD characterization. From the XRD curves (Figure S1), The four characteristic peaks at 11.1°, 22.5°, 34.3°, and 60.9° are successfully attributed to (003), (006), (012), and (110) lattice plane of layered hydroxides phase, which are same with the standard XRD peaks for Ni/Co LDHs.[41, 42] The SEM morphologies of Ni/Co LDHs were shown in Figure 1a, which indicates that the products consist of uniform microspheres with the average size of about 470 nm. The detailed feature of the products is shown in Figure 1b, indicating the 3D flower-like hierarchical architectures of the microsphere. To obtain more structural insight for Ni/Co LDHs, TEM results were recorded on the as-synthesized products. In Figure 1c, it can be seen that Ni/Co LDHs samples are spherical and consist of thin crinkly nanosheets, which grow out from the core of the microspheres and form the flower-like feature. Details of the flower-like feature are given in Figure 1d, which indicates that the hybrid ultrathin nanosheets show a flat, compact, and transparent feature. The SAED pattern (Figure S2) shows well define rings, which is an indication of the polycrystalline nature of Ni/Co LDHs. The EDX spectrum in Figure S3 reveals the single LDHs nanosheet mainly contains Ni, Co, and O elements. Furthermore, a Brunauer–Emment–Teller (BET) analysis of Ni/Co LDHs gives a specific surface area of 77.8 $m^2 g^{-1}$ (Figure S4). It confirms that this LDHs possesses a relative large specific surface area caused by the unique flower-like hierarchical configuration, which is beneficial to the catalytic property.

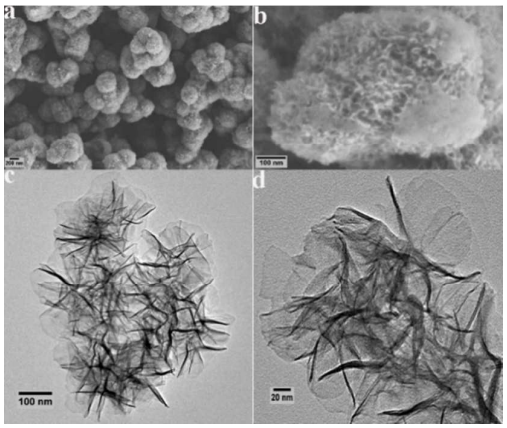

Figure 1 SEM images of Ni/Co LDHs (a and b). TEM images of Ni/Co LDHs (c and d).

3.2. The peroxidase-like catalytic activity of Ni/Co LDHs

To demonstrate the proof of principle, the peroxidase-like catalytic activity of Ni/Co LDHs was investigated under three different neutral pH solutions, compared with that of the absence of Ni/Co LDHs. As shown in Figure 2a-c, the absorbance at 417 nm increased in the presence of Ni/Co LDHs, while there was no absorbance at 417 nm in the absence of Ni/Co LDHs, indicating Ni/Co LDHs possesses peroxidase-like activity at neutral pH. In order to determine the influence of neutral pH solution on the peroxidase-like activity of Ni/Co LDHs. phosphate buffer (PBS), Tris-HCl buffer and deionized water were selected to apply in the contrast experiment. Firstly, as shown in Figure 2a, Ni/Co LDHs could catalyze the reaction of the peroxidase substrate 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonicacid) (ABTS) in the presence of H_2O_2 at pH 7.0 PBS. However, the rate of catalyze reaction was very slowly and changed at near 200 s. This was probably because the Co and Ni ions in the surface of Ni/Co LDHs conjugated with PO_4^{3-} from PBS and exhibited strong coordination and low solubility ($Co_3(PO_4)_2$ K_{sp}=10^{-35},

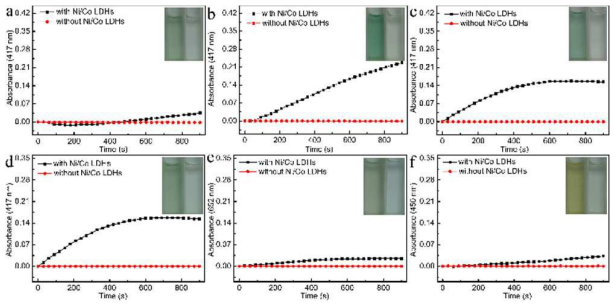

Figure 2 The time-dependent absorbance changes at 417 nm in the absence (red) or presence (black) of Ni/Co LDHs in pH 7.0 phosphate buffer (a), pH 7.0 Tris-HCl buffer (b) and deionized water (c) at 37 °C. The time-dependent absorbance changes at 417 nm in the presence ABTS and H_2O_2 (d), 652 nm in the presence TMB and H_2O_2 (e) and 450 nm in the presence OPD and H_2O_2 (f) with or without Ni/Co LDHs in deionized water at 37 °C. Inset shows the color change under different conditions.

$Ni_3(PO_4)_2$ K_{sp} =10^{-32}), which may spoil the native structure of Ni/Co LDHs and decrease the catalytic performance. Secondly, the influence of Tris-HCl buffer (pH=7.0) on the peroxidase-like activity of Ni/Co LDHs was shown in Figure 2b. The absorbance at 417 nm always increased linearly in 900 s in the presence of Ni/Co LDHs, however, the rate of catalyze reaction was fast in Tris-HCl buffer so that the terminating agent was to add in order to terminate the on-going reaction, which lead to the complex operation. At last, the influence of deionized water on the peroxidase-like activity of Ni/Co LDHs was shown in Figure 2c. The absorbance at 417 nm always increased in 600 s and then remained unchanged for the next 300 s, which indicated the catalytic reaction was terminated without the adding terminating agent. And it also reduced the complexity of the experiment. Furthermore, at the beginning of 360 s, the rate of catalyze reaction was faster in deionized water than Tris-HCl buffer. Given all this, deionized water was selected as the optimal reaction medium in the catalyze reaction of Ni/Co LDHs.

To further characterize the peroxidase-like activity of the Ni/Co LDHs, other typical peroxidase substrates were used in place of ABTS including 3,3′,5,5′-tetramethylbenzidine (TMB) and o-phenylenediamine (OPD). As shown in Figure 2d-f, the oxidation of ABTS, TMB and OPD catalyzed by the Ni/Co LDHs formed green, blue and orange products and the maximum absorbance of the oxidation of ABTS, TMB and OPD is 417, 652 and 450 nm, respectively. And the absorbance was increased at 417, 652 and 450 nm, respectively with time in the presence of Ni/Co LDHs, while there was no absorbance in the absence of Ni/Co LDHs. All results confirmed that the as-prepared Ni/Co LDHs exhibited intrinsic peroxidase-like activity. Furthermore, Figure 2d-f also compares the catalytic activity of the reaction of ABTS, TMB and OPD with H_2O_2 using Ni/Co LDHs. Obviously, the reaction rate of ABTS with H_2O_2

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was faster than that of TMB and OPD. That is because ABTS contains negatively charged sulfonic groups, giving a strong electrostatic affinity toward the positively charged Ni/Co LDHs.[43] Therefore, ABTS, as the optimal peroxidase substrate and colorimetric indicator, was used to investigate the peroxidase-like activity of the Ni/Co LDHs and detect glucose and ACh.

Similarly to natural HRP, the catalytic activity of Ni/Co LDHs is also dependent on pH and temperature. Due that deionized water solution was selected as the reaction medium, we only examined the peroxidase-like relatively activity of Ni/Co LDHs varying the temperature from 10 °C to 65 °C. As shown in Figure S5, the catalytic performance of Ni/Co LDHs retained about above 80 % from 10 °C to 40 °C and attained its highest catalytic activity at 20 °C, Therefore, the Ni/Co LDHs exhibited high activity over the broad temperature range. However, in order to make the detection of glucose and ACh easy to carry out, 37 °C was set as the optimal temperature.

3.3. Kinetic study and mechanism of Ni/Co LDHs

To investigate the mechanism of the peroxidase-like activity of Ni/Co LDHs, the apparent steady-state kinetic parameters of the reaction of ABTS and H_2O_2 were determined by changing the concentration of H_2O_2 and ABTS, respectively (Figure S 6a and b). The enzyme kinetic parameters including Michaelis–Menten constant (K_m) and maximum initial velocity (V_{max}) were obtained according to the Lineweaver-Burk equation, the results was shown in Table S1. The apparent K_m value for the Ni/Co LDHs with H_2O_2 and ABTS, respectively, was all larger than that of HRP, suggesting that the Ni/Co LDHs had a lower affinity for H_2O_2 and ABTS than HRP. To further investigate the mechanism of Ni/Co LDHs catalysis, the peroxidase-like activity of Ni/Co LDHs was measured under standard reaction condition by varying concentration of H_2O_2 at a fixed concentration of ABTS or vice versa. The double reciprocal plots of initial velocity against the concentration of one substrate were obtained over a range of concentrations of the second substrate (Figure S6c and d). The slopes of the lines were parallel, which was characteristic of a ping-pong mechanism, as observed for HRP [44]. It indicated that Ni/Co LDHs bind and react with the first substrate, and then release the first product before reacting with the second substrate. The possible product in the present system might originate from Ni/Co LDHs' catalytic ability toward the decomposition of H_2O_2 to generate reactive oxygen species (ROS). Then, the electron spin resonance (ESR) spectroscopy as the direct analytical method was used for detection ROS. The signal of 5,5-dimethyl-1-pyrroline N-oxide (DMPO)/·OH was observed in the presence of Ni/Co LDHs (Figure 3a), which indicating Ni/Co LDHs could decompose H_2O_2 to generate ·OH radical, which could oxidize the peroxidase substrate ABTS. In addition, the signal of 2,2,6,6- tetramethyl-4-piperidine (TEMP)/$^1O_2$ was also observed in the presence Ni/Co LDHs (Figure 3b), which verified that $^1O_2$ was formulated via the addition of Ni/Co LDHs. Then, even in water solution, Ni/Co LDHs could catalyze the decomposition of H_2O_2 to generate ·OH and $^1O_2$, which resulted in the oxidation of ABTS.

3.4. One-step detection glucose using Ni/Co LDHs

Since the peroxidase-like activity of Ni/Co LDHs is H_2O_2 concentration dependent, and H_2O_2 is the main product of the glucose oxidase (GOx)-catalyzed reaction, a colorimetric detection of glucose could be developed using Ni/Co LDHs instead of HRP. Most works reported the glucose detection based on peroxidase mimetics most peroxidase mimetics possesses catalytic activity at acid

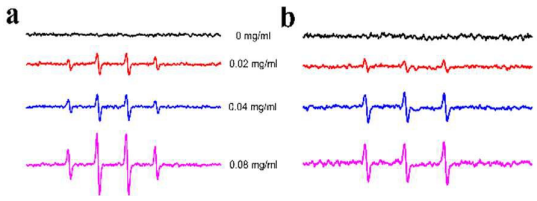

Figure 3 ESR spectra of the formation of ·OH (a) and $^1O_2$ (b) by the decomposition of H_2O_2 catalyzed using the different concentration of Ni/Co LDHs.

solution, however, GOx could be denatured at acid solution. Then, the two-step procedure was carried out in the following ways: GOx and glucose were incubated under neutral condition for about 30 min, and then peroxidase mimic and colorimetric substrate were added to the above solution and incubated under acid condition for about 20 min again. Despite the two-step method could avoid GOx denatured, the procedure was tedious, complex and time-consuming. Therefore, benefited from Ni/Co LDHs possessed peroxidase-like catalytic activity under physiological condition, the presented colorimetric assay for glucose was completed by one-step. The detail procedure of one-step method was described in experiment section 2.5. The results of detection glucose were shown in Figure 4. Figure 4b showed typical glucose concentration–response curves. The linear range for glucose was from 0.5 μM to 100 μM (Figure 4c) with a detection limit (DL) of 0.1 μM. This detection glucose method based on the Ni/Co LDHs system

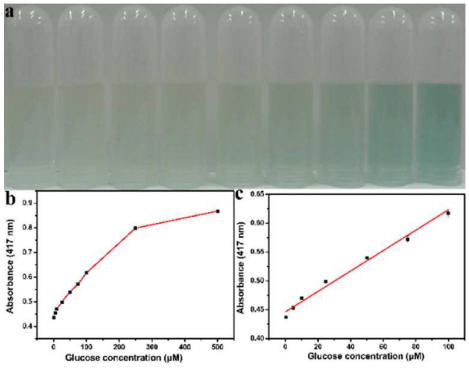

Figure 4 (a) The photograph of the colored products for different concentrations of glucose. (b) A dose–response curve for glucose detection at 417 nm by one-step method under physiological condition. (c) The linear calibration plot for glucose. The error bars represent the standard deviation of three measurements.

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not only gave a wider linear range and lower DL, but also showed easy reaction condition, rapid process and simple step compared with many other nanozymes-based glucose sensors (Table 1). To test the specificity of this method, the control experiment was conducted using fructose, lactose and maltose instead of glucose (Figure S7). As shown in Figure S7, the absorbance of glucose is obviously higher than the glucose analog, although the concentration of these analog are about 5-fold higher than that of glucose due to the specificity of GOx. Comparing to fructose and lactose, maltose has less interfere to the detection of glucose.[39, 45]

To confirm the practicability, the method was used in different human serum samples. According to the calibration curve, the results by our method were in good agreement with measured by the GOD-PAP biochemical analyzer in hospital (Table S2).

Table 1 Comparison of the present methods with other reported methods for the detection of glucose

Mimetic enzyme	Optimum condition	Method	Time	Linear range (μM)	LD (μM)	Ref.
C_{60}	pH 3.5 45 °C	two-step	2 h 30 min	1.0-40	0.5	25
WS_2 nanosheets	pH 6.9 60 °C	two-step	1h	5-300	2.9	31
Carbon nitrides	pH 3.0 60 °C	three- step	1h 55 min	5-100	1.0	27
CoFe LDHs	pH 4.0 30 °C	two-step	1h 30 min	1-10	0.6	46
Fe-MIL-88NH_2 MOFs	pH 4.0 45 °C	two-step	50 min	2-300	0.48	47
MoS_2/graphene oxide hybrid	pH 4.0 37 °C	two-step	1h 30 min	1-50	0.83	48
Au@Ag heterogeneous	pH 6.5 25 °C	one-step	30 min	50-20000	39	37
Ni/Co LDHs	pH 7.0 37 °C	one-step	30 min	0.5-100	0.1	This work

3.5. One-step detection ACh using Ni/Co LDHs

Furthermore, we also extended the one-step method to detect ACh. Since H_2O_2 could be generated in the presence of both AChE and ChOx in the solution containing ACh, and then ACh was also detected via the peroxidase mimic Ni/Co LDHs. Based on the peroxidase-like activity of Ni/Co LDHs under physiological condition, we employed one step process to detect ACh. That is because the optimum condition for the peroxidase-like activity of Ni/Co LDHs is agree with that of the activity of AChE and ChOx. In this one-step process, ACh was firstly converted to choline by AChE, and the ChOx could further catalytically oxidize choline to produce H_2O_2, which could oxidize ABTS in the presence of Ni/Co LDHs to produce the green-colored ox-ABTS. Therefore, the color change and the absorbance of ox-ABTS were employed to measure ACh content. Figure 5b showed typical ACh concentration−response curves. The linear range for ACh was from 10 μM to 150 μM with

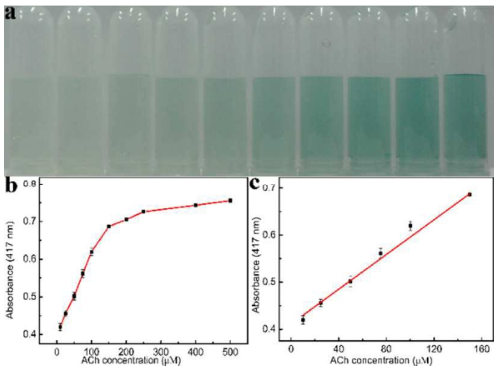

Figure 5 (a) The photograph of the colored products for different concentrations of ACh. (b) A dose−response curve for ACh detection at 417 nm by one-step method under physiological condition. (c) The linear calibration plot for ACh. The error bars represent the standard deviation of three measurements.

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the DL of 1.62 μM (Figure 5c). This one-step method based on Ni/Co LDHs gave a lower DL than the two-step method using platinum nanoparticles as catalyst (2.84 μM)[33]. The selectivity of the colorimetric sensing of ACh was investigated by detecting some interfering substances, including glycine, cystine, tryptophan, threonine and proline (Figure S8). As shown in Figure S8, ACh induced the more increase of the absorbance. And except that cysteine and proline induced less interference, all of the other substance showed lesser interference. This method was employed to detect ACh in milk in order to evaluate its applicability. The results of the determination and recovery are shown in Table S3. From Table S3, it can be seen that the average recoveries for ACh ranged from 90.2 to 104.8 %, indicating that the presented colorimetric assay has great potential in application of ACh detection on the food inspection.

Conclusions

In summary, by a facile one-step chemical coprecipitation method, we have obtained Ni/Co LDHs that exhibits peroxidase-like activity. Ni/Co LDHs, as a mimic peroxidase, exhibit several advantages. Firstly, compared to natural enzymes, Ni/Co LDHs was a promising candidate as enzyme mimics with advantages of ease of preparation, low-cost, and water-solubility. Secondly, Ni/Co LDHs showed high catalytic activity over a broad temperature range, from 10 °C to 40 °C. Last but it is also the most important is that Ni/Co LDHs had high catalytic activity at neutral pH. Because of the similar optimal condition (neutral pH, 37 °C) for the catalytic activity of Ni/Co LDHs with GOx, AChE and ChOx, we developed the one-step method for the detection of glucose and ACh in water, which showed shorter time, simple steps and ease of operation. Our work will facilitate the utilization of the intrinsic peroxidase activity of Ni/Co LDHs in biotechnology and bioassays, and make a great influence on the next generation of enzyme mimetics system.

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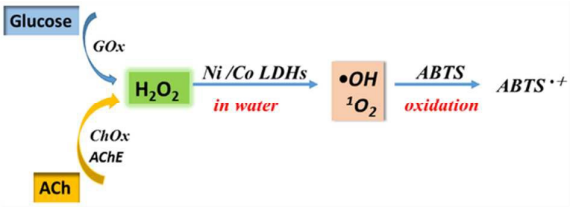

A one-step detection method of glucose and acetylcholine was developed based on the peroxidase-like activity of Ni/Co LDHs in water