Talanta xxx (xxxx) xxx

Contents lists available at ScienceDirect

Talanta

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

Short communication

A smartphone-assisted portable biosensor using laccase-mineral hybrid microflowers for colorimetric determination of epinephrine

Miaorong Zhang [a,*], Yan Zhang [b], Chuankai Yang [a], Chunyun Ma [a], Jianguo Tang [a]

[a] Institute of Hybrid Materials, National Center of International Joint Research for Hybrid Materials Technology, National Base of International Science & Technology Cooperation, College of Materials Science and Engineering, Qingdao University, Qingdao, 266071, People's Republic of China
[b] Institute for Chemical Biology & Biosensing, College of Life Sciences, Qingdao University, 308 Ningxia Road, Qingdao, 266071, People's Republic of China

ARTICLE INFO

Keywords:
Biomineralization
Smartphone
Colorimetric detection
Epinephrine

ABSTRACT

A novel smartphone-assisted portable biosensor based on laccase-mineral hybrid microflowers (La-HMFs) was applied for real-time, accurate and reliable quantification of epinephrine (EP). La-HMFs with flower-like hierarchical nanostructure was synthesized via biomineralization using $Cu_3(PO_4)_2{\cdot}3H_2O$ as the mineral. Characterization results revealed laccase molecules as the framework were immobilized within La-HMFs. Smartphone-assisted colorimetric assays showed wide linear detection range (1–400 μM) and favorable anti-interference ability for EP detection, and the detection limit was 0.6 μM. Further, due to the protective effect of $Cu_3(PO_4)_2{\cdot}3H_2O$, the immobilized laccase exhibited good stability and desirable reusability.

1. Introduction

Epinephrine (EP) as a kind of significant inhibitory neurotransmitters plays a key role in the message transfer of biological activities in vivo, so the EP level is an important clinical diagnostic indicator [1]. In addition, EP drugs have been widespread used to treat many diseases such as bronchial asthma and anaphylactic shock [2]. Therefore, the reliable, accurate and rapid quantification of EP have vitally practical value. Although various methods have been investigated for EP detection [3–6], compared to other detection strategies, colorimetric detection possesses advantages of simple analysis and usage, cost effective, easy operation and monitoring. However, there are few reports on colorimetric detection of EP due to the lack of nanomaterials with satisfied detection performance [7,8]. Therefore, the synthesis of novel nanomaterials, which can realize the sensitive and selective detection of EP, is a critical priority. Meanwhile, traditional colorimetric detection based on spectrophotometer in the lab can not realize on-site instant detection, so the development of real-time, mobile and portable testing device is vital for EP detection in some special circumstances.

Biomineralization as a powerful means can fabricate advanced hierarchical architectures comprising organic frameworks and minerals in aqueous environments under ambient conditions, and the formed biominerals possess fascinating unique biofunctionalities, excellent physicochemical and mechanical properties toward external harsh environments [9–13]. Recently, Ge et al. synthesized a novel kind of flower-like protein-mineral hybrid nanomaterials via biomineralization using metal phosphate as the mineral [14], moreover, recent reports have demonstrated the performance of these novel biominerals was significantly enhanced compared to corresponding free proteins [15–19]. Although protein-mineral hybrid nanomaterials have been used for the construction of biosensors, up to now, there is no detailed study of appling them to the quantitative determination of EP.

Smartphone as analytical tool can bypass the use of expensive and bulky instrumentation for routine tests, moreover, the ubiquitous availability and broad accessibility of smartphones make detection realizable in remote or underdeveloped regions [20–23]. Further, smartphone augmented with accessories can complete more complex applications [24]. Therefore, combined with our previous study, here in this work, smartphone-assisted colorimetric biosensor was established for real-time, accurate and reliable determination of EP. In this biosensor, laccase-mineral hybrid microflowers (La-HMFs) fabricated via biomineralization reacted with EP to generate a colored product. Subsequently, a smartphone captured the colorimetric image and processed the color parameters to realize accurate quantification of EP. Detection results demonstrated that this easy-to-operate biosensor system had high sensitivity, good selectivity, desirable stability and favorable reusability.

* Corresponding author.
E-mail address: mrzhang2018@qdu.edu.cn (M. Zhang).

https://doi.org/10.1016/j.talanta.2020.121840
Received 7 September 2020; Received in revised form 25 October 2020; Accepted 28 October 2020
Available online 30 October 2020

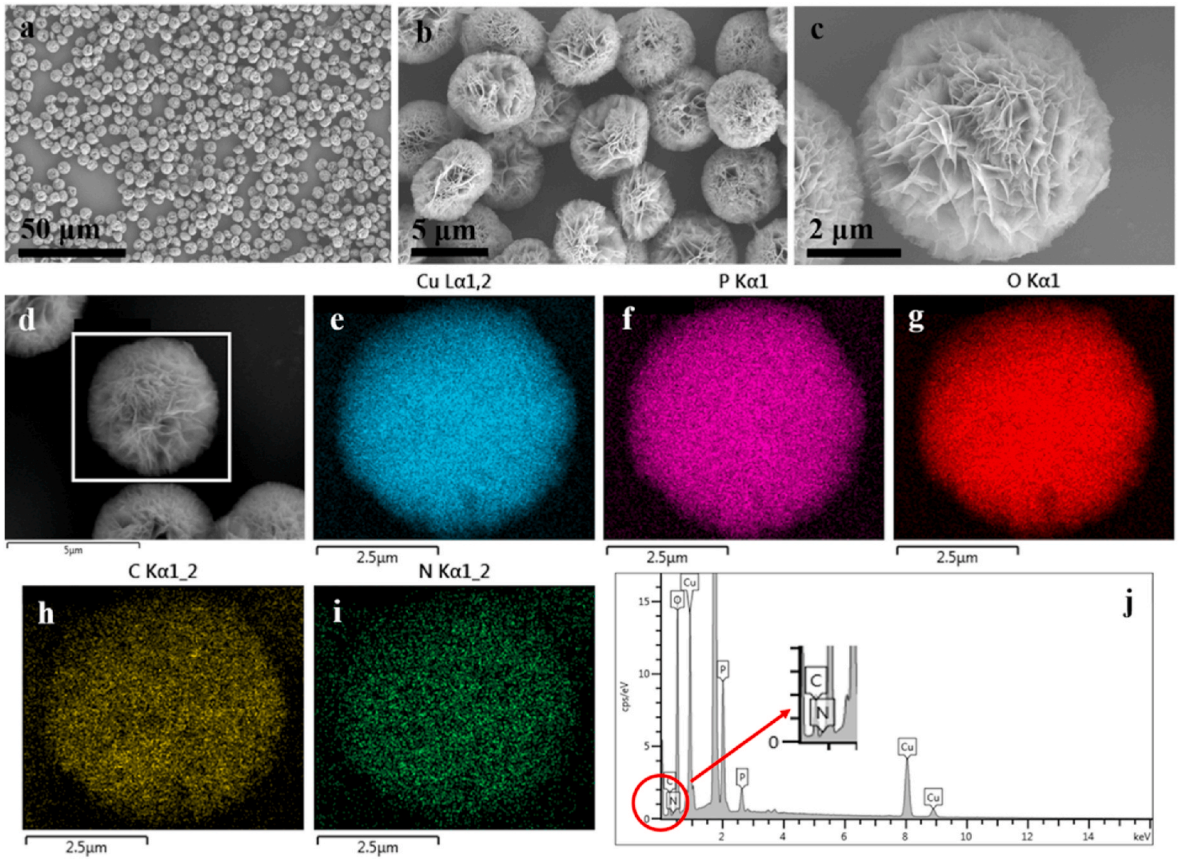

Fig. 1. (a–c) SEM images of La-HMFs. (e–i) Sensitive element distribution maps of the image d. (j) EDS of La-HMFs.

2. Experimental

2.1. Materials

Sodium dihydrogen phosphate dihydrate, disodium hydrogen phosphate dodecahydrate, sodium chloride, copper sulfate, epinephrine, ascorbic acid, citric acid, vitamin C, glucose, glycine, L-lysine and urea were purchased from Sinopharm Chemical Reagent Co., Ltd. Laccase (13.6 units mg^{-1}) from *Trametes versicolor* was purchased from Sigma-Aldrich Co., Ltd. All chemicals were of analytical grade and used without further purification. Deionized water was used in all the experiments.

2.2. Synthesis of La-HMFs

The synthesis of La-HMFs was similar to our previous report [25]. 40 μL of copper sulfate aqueous solution (120 mM) was dropwise added in 6 mL of 10 mM phosphate buffered saline (PBS) containing 0.1 mg mL^{-1} laccase under stirring (900 rpm), followed by incubation at 20 °C for three days. Then, the blue precipitate (La-HMFs) was obtained via filtration, and added with deionized water to prepare 3 mg mL^{-1} La-HMFs aqueous solution.

2.3. Characterization

The morphology and structure of La-HMFs were investigated by scanning electron microscopy (SEM, FEI Quanta 250 FEG) and transmission electron microscopy (TEM, FEI Talos F200i). Element distribution of La-HMFs was characterized via energy dispersive spectrometry (EDS). Characteristic groups of laccase, La-HMFs and $Cu_3(PO_4)_2{\cdot}3H_2O$ were studied by Fourier transform infrared spectra (FT-IR, MAGNA-IR 550). X-ray diffraction (XRD) pattern of La-HMFs was obtained via Bruker D8 advance X-ray powder diffractometer. The encapsulation yield and weight percentage of laccase within La-HMFs were tested according to the previously reported method [26].

2.4. Smartphone-assisted colorimetric determination of EP

150 μL of different concentration of EP was added in 2.85 mL of PBS containing 0.1 μg mL^{-1} La-HMFs. After reaction for 5 min, La-HMFs were recovered via centrifugation (10,000 r min^{-1}), and the colored supernatants were used for determination of EP concentration. First, the colorimetric image of supernatants was captured by a smartphone (iPhone X) within the self-designed detection accessory, which includes a dark box, a simulated sunlight source and sample stage. Subsequently, the captured image was analyzed by a ColorPicker app of iPhone X, which employs the RGB color model, which R, G and B stand for red, green and blue color channels, respectively, and P_R, P_G and P_B are defined as the pixel value of R, G and B. Due to the yellowish red-colored supernatants, the values of $P_R/(P_R + P_G + P_B)$ were used to distinguish the color difference of supernatants with different concentration of EP.

3. Results and discussion

3.1. Materials characterization

As shown in Fig. 1a–c, La-HMFs with flower-like hierarchical nanostructure comprised of interlaced nanosheets, and the size (~5 μm) of monodisperse La-HMFs was uniform. This unique morphology may induce adjacent laccase molecules to enerate synergistic effect. The element distribution maps of Fig. 1d are shown in Fig. 1e–i. Specifically, the element maps (Fig. 1h and i) of carbon and nitrogen indicated the existence of laccase moleculels within La-HMFs, and the copper, phosphorus and oxygen element maps (Fig. 1e–g) revealed the formation of

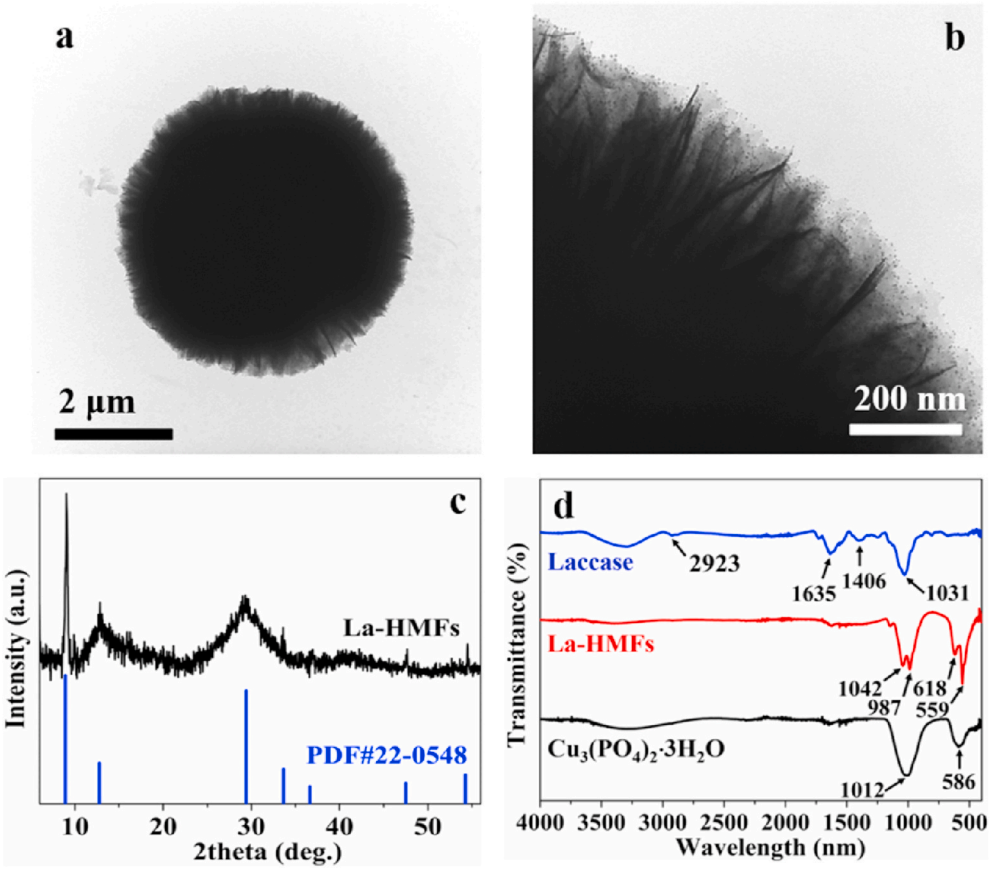

Fig. 2. (a–b) TEM images and (c) XRD pattern of La-HMFs. (d) FT-IR of laccase, La-HMFs and $Cu_3(PO_4)_2{\cdot}3H_2O$.

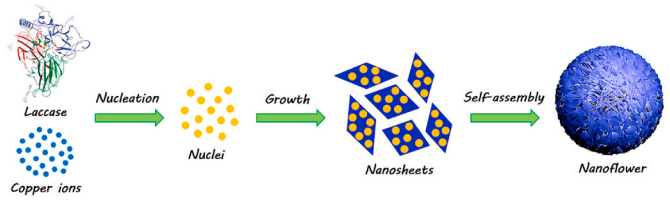

Fig. 3. Schematic representation of the formation of La-HMFs.

copper phosphate. In addition, EDS of La-HMFs also demonstrated the existence of above five elements (Fig. 1j). After calculation, the encapsulation yield and weight percentage of laccase within La-HMFs were 97% and 5%, respectively.

The TEM images of La-HMFs are shown in Fig. 2a–b. The interior structure of La-HMFs was solid and compact, and large amounts of spots on the edge of the nanosheets of La-HMFs could be the nuclei formed by laccase molecules and copper ions, which may induce the growth of nanosheets. XRD pattern of La-HMFs is shown in Fig. 2c. The diffraction peaks of La-HMFs were in accordance with $Cu_3(PO_4)_2{\cdot}3H_2O$ crystals (JCPDS card 22–0548), demonstrating the formation of high-crystalline $Cu_3(PO_4)_2{\cdot}3H_2O$ within La-HMFs [27,28]. FT-IR of laccase, La-HMFs and $Cu_3(PO_4)_2{\cdot}3H_2O$ are shown in Fig. 2d. The typical peaks of laccase at 1406 and 1635 cm^{-1} for –CONH and 2923 cm^{-1} for $–CH_2$ and $–CH_3$ were not observed in the spectrum of La-HMFs, suggesting most laccase molecules were immobilized within La-HMFs rather than on the surface of La-HMFs. In addition, the peaks at 586 (bending) and 1012 (stretching) cm^{-1} in the spectrum of $Cu_3(PO_4)_2{\cdot}3H_2O$ were attributed to P–O vibrations [29,30], by contrast, in the same wavelength region, the spectrum of La-HMFs had slight shift of peaks and new adsorption peaks (559, 618, 987 and 1042 cm^{-1}), indicating there existed covalent bonding between laccase molecules and $Cu_3(PO_4)_2{\cdot}3H_2O$.

Based on the above characteristic results and previous reports [14,31], the formation of La-HMFs was assumed to comprise three stages via biomineralization using laccase and $Cu_3(PO_4)_2{\cdot}3H_2O$ crystals as the organic framework and mineral, respectively, as shown in Fig. 3. First, numerous nuclei of primary $Cu_3(PO_4)_2{\cdot}3H_2O$ crystals were formed via the coordination between copper ions and amide groups of laccase molecules; second, primary $Cu_3(PO_4)_2{\cdot}3H_2O$ crystals kinetically grew into nanosheets; last, laccase molecules induced the self-assembly of nanosheets into nanoflowers.

3.2. Analytical performance

Fig. 4 shows the smartphone-assisted colorimetric assay based on La-HMFs. After colorimetric reaction, iPhone X captured the colorimetric image of supernatants with different concentration of EP, as shown in Fig. 4a. With the increase concentration of EP, the deepened color of supernatants was visible to the naked eye. Subsequently, a ColorPicker app randomly selected the color of supernatants containing different concentration of EP (Fig. 4b), then extracted the corresponding P_R, P_G and P_B parameters of different supernatants (Fig. 4c). According to the calculated $P_R/(P_R + P_G + P_B)$ value, the linear calibration curve between $P_R/(P_R + P_G + P_B)$ value and EP concentration was ploted in Fig. 4d (the error bars are the maximum positive and negative deviations of $P_R/(P_R + P_G + P_B)$ value of single sample relative to the arithmetic mean $P_R/(P_R + P_G + P_B)$ value of five samples). Under the EP concentration from 1 to 400 μM, the linear regression equation was $P_R/(P_R + P_G + P_B) = 0.376 + 0.000655C_{EP}$ (μM) with the linearity of $R^2 = 0.999$, and the limit of detection (LOD) was calculated to be 0.6 μM (LOD = 3s/k, where s and k are the relative standard deviation of five parallel controlled measurements and the slope of the linear calibration curve, respectively [32]).

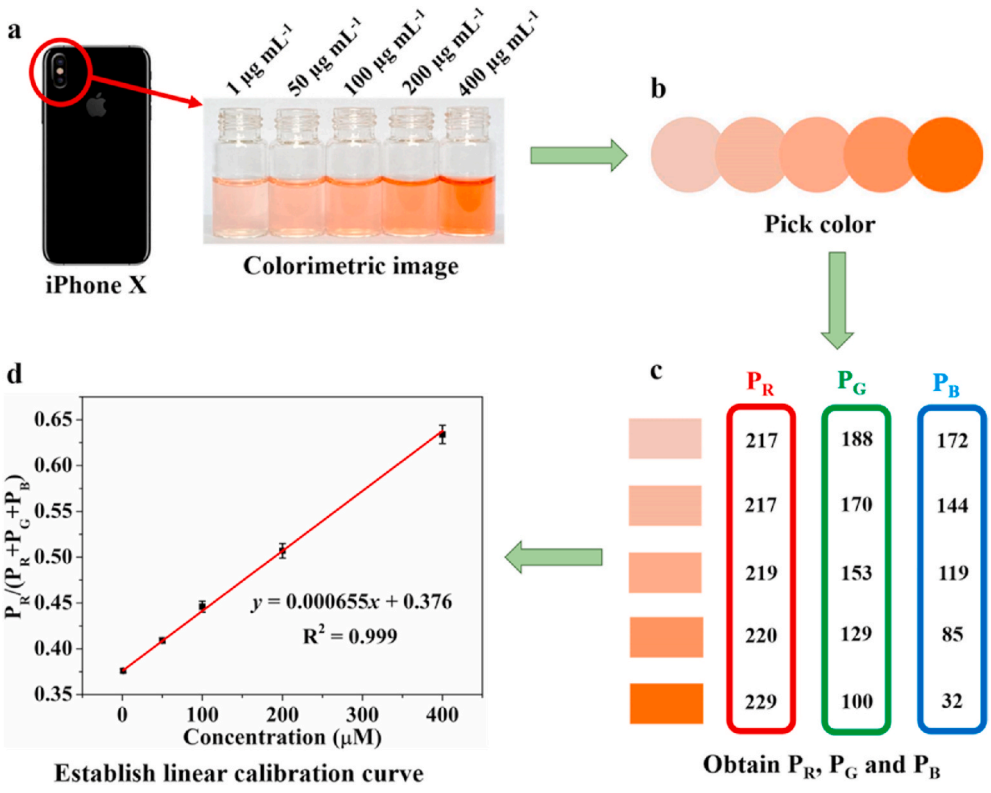

Fig. 4. Smartphone-assisted colorimetric assays using La-HMFs. (a) Colorimetric image captured by iPhone X camera; (b) pick color via ColorPicker app; (c) obtain P_R, P_G and P_B; (d) establish linear calibration curve.

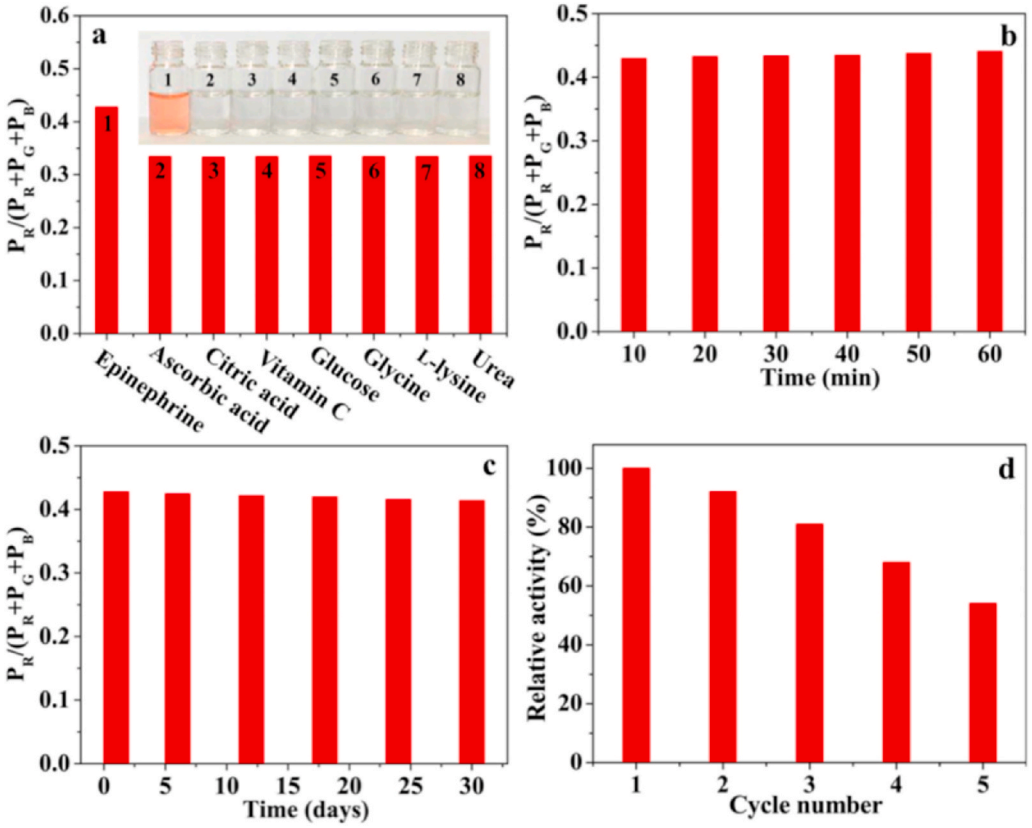

Fig. 5. (a) Colorimetric responses of smartphone-based colorimetric assays based on La-HMFs toward epinephrine and interferents including ascorbic acid, citric acid, vitamin C, glucose, glycine, L-lysine and urea. (b) Colorimetric stability, (c) storage stability and (d) reusability test of La-HMFs.

The anti-interference ability of La-HMFs was examined as shown in Fig. 5a. The supernatants with the addition of ascorbic acid, citric acid, vitamin C, glucose, glycine, L-lysine and urea had no color, and the calculated values of $P_R/(P_R + P_G + P_B)$ of above interferents were 0.333 (P_R=P_G=P_B), which was smaller than that ($P_R/(P_R + P_G + P_B) = 0.427$) of EP, which demonstrated La-HMFs have favorable selectivity for EP detection. In addition, the stability and reusability of La-HMFs were tested for EP detection. As shown in Fig. 5b, after colorimetric reaction for 5 min, the values of $P_R/(P_R + P_G + P_B)$ were almost unchanged during the next 60 min, indicating the good colorimetric stability of La-HMFs. Moreover, the values of $P_R/(P_R + P_G + P_B)$ only decreased 2% after stored for 30 days, which demonstrated the desirable storage stability of La-HMFs (Fig. 5c). Last, the reusability of La-HMFs was studied by reused for 5 cycles (Fig. 5d), the activiey of La-HMFs still retained 68% of their initial activity, demonstrating the good reusability of La-HMFs. Compared with the previously reported nanomaterials [33,34], La-HMFs exhibited good detection performance owing to the following two reasons. First, the high sensitivity of La-HMFs could be ascribed to the high activity of laccase; second, the unique specificity of laccase within La-HMFs ensured the good selectivity for EP detection.

4. Conclusions

In this work, La-HMFs were facilely synthesized via biomineralization using $Cu_3(PO_4)_2{\cdot}3H_2O$ as the mineral, then smartphone-assisted portable biosensor was constructed for colorimetric determination of EP. Element distribution maps and XRD pattern revealed the incorporated laccase molecules and $Cu_3(PO_4)_2{\cdot}3H_2O$ crystals within La-HMFs, moreover, FT-IR spectra demonstrated there existed covalent bonding between laccase molecules and $Cu_3(PO_4)_2{\cdot}3H_2O$ crystals. The detection results of smartphone-assisted colorimetric assay indicated La-HMFs exhibited wide detection range, favorable sensitivity, desirable selectivity, good stability and reusability for EP detection due to the high activity, unique hierarchical structure and specificity of La-HMFs.

Credit author statement

Miaorong Zhang: Conceptualization, Funding acquisition, Writing - original draft, Writing - review & editing. **Yan Zhang:** Writing - original draft & editing. **Chuankai Yang:** Investigation, Methodology. **Chunyun Ma:** Investigation, Methodology. **Jianguo Tang:** Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by Scientific Research Starting Foundation of Qingdao University, Qingdao Postdoctoral Applied Research Project and China Postdoctoral Science Foundation (2019M662298; 2019M652325).

References

[1] W. Shimizu, T. Noda, H. Takaki, N. Nagaya, K. Satomi, T. Kurita, K. Suyama, N. Aihara, K. Sunagawa, S. Echigo, Y. Miyamoto, Y. Yoshimasa, K. Nakamura, T. Ohe, J.A. Towbin, S.G. Priori, S. Kamakura, Diagnostic value of epinephrine test for genotyping LQT1, LQT2, and LQT3 forms of congenital long QT syndrome, Heart Rhythm 3 (2004) 276–283.

[2] S.F. Kemp, R.F. Lockey, F.E.R. Simons, Epinephrine: the drug of choice for anaphylaxis. A statement of the world allergy organization, Allergy 63 (2008) 1061–1070.

[3] C.R.S. Matos, H.O. Souza, T.B.S. Santana, L.P.M. Candido, F.G.C. Cunha, E. M. Sussuchi, I.F. Gimenez, $Cd_{1-x}Mg_xTe$ semiconductor nanocrystal alloys: synthesis, preparation of nanocomposites with graphene-based materials, and electrochemical detection of lidocaine and epinephrine, Microchim. Acta 184 (2017) 1755–1764.

[4] B. Zhou, X. Li, X. Tang, P. Li, L. Yang, J. Liu, Highly selective and repeatable surface-enhanced resonance Raman scattering detection for epinephrine in serum based on interface self -assembled 2D nanoparticles arrays, ACS Appl. Mater. Interfaces 9 (2017) 7772–7779.

[5] T.H. Le, D.H. Lee, J.H. Kim, S.J. Park, Polypyrrole/graphene quantum dot composites as a sensor media for epinephrine, J. Nanosci. Nanotechnol. 20 (2020) 4005–4010.

[6] A. Roychoudhury, K.A. Francis, J. Patel, S.K. Jha, S. Basu, A decoupler-free simple paper microchip capillary electrophoresis device for simultaneous detection of dopamine, epinephrine and serotonin, RSC Adv. 10 (2020) 25487–25495.

[7] F. Zhang, M. Wang, D. Zeng, H. Zhang, Y. Li, X. Su, A molybdenum disulfide quantum dots-based ratiometric fluorescence strategy for sensitive detection of epinephrine and ascorbic acid, Anal. Chim. Acta 1089 (2019) 123–130.

[8] N. Saraf, A. Bosak, A. Willenberg, S. Das, B.J. Willenberg, S. Seal, Colorimetric detection of epinephrine using an optimized paper-based aptasensor, RSC Adv. 7 (2017) 49133–49143.

[9] A.-W. Xu, Y. Ma, H. Cölfen, Biomimetic mineralization, J. Mater. Chem. 17 (2007) 415–449.

[10] J.S. Evans, Composite materials design: biomineralization proteins and the guided assembly and organization of biomineral nanoparticles, Materials 12 (2019) 581.

[11] N.K. Dhami, M.S. Reddy, A. Mukherjee, Biomineralization of calcium carbonates and their engineered applications: a review, Front. Microbiol. 4 (2013) 314.

[12] B. Liu, Y. Cao, Z. Huang, Y. Duan, S. Che, Silica biomineralization via the self -assembly of helical biomolecules, Adv. Mater. 27 (2015) 479–497.

[13] D.M. Pereira, P. Habibovic, Biomineralization-inspired material design for bone regeneration, Adv. Healthcare Mater. 7 (2018) 1800700.

[14] J. Ge, J. Lei, R.N. Zare, Protein-inorganic hybrid nanoflowers, Nat. Nanotechnol. 7 (2012) 428–432.

[15] J. Han, P. Luo, L. Wang, J. Wu, C. Li, Y. Wang, Construction of a multienzymatic cascade reaction system of coimmobilized hybrid nanoflowers for efficient conversion of starch into gluconic acid, ACS Appl. Mater. Interfaces 12 (2020) 15023–15033.

[16] H. Wen, L. Zhang, Y. Du, Z. Wang, Y. Jiang, H. Bian, J. Cui, S. Jia, Bimetal based inorganic-carbonic anhydrase hybrid hydrogel membrane for CO_2 capture, J. CO_2 Util. 39 (2020) 101171.

[17] R.W. Jadhav, D.D. La, V.G. More, H.T. Vo, D.A. Nguyen, D.L. Tran, S.V. Bhosale, Self-assembled kanamycin antibiotic-inorganic microflowers and their application as a photocatalyst for the removal of organic dyes, Sci. Rep. 10 (2020) 154.

[18] K. Wu, Y. Zhang, Q. Sun, Y. Chai, Q. He, X. Zhou, X. He, H. Ji, Preparation of cytochrome P450 enzyme-cobalt phosphate hybrid nanoflowers for oxidative coupling of benzylamine, Enzym. Microb. Technol. 131 (2019) 109386.

[19] W.-Y. Xie, F. Song, X.-L. Wang, Y.-Z. Wang, Development of copper phosphate nanoflowers on soy protein toward a superhydrophobic and self-cleaning film, ACS Sustain. Chem. Eng. 5 (2017) 869–875.

[20] A. Roda, E. Michelini, M. Zangheri, M.D. Fusco, D. Calabria, P. Simoni, Smartphone-based biosensors: a critical review and perspectives, Trends Anal. Chem. 79 (2016) 317–325.

[21] D. Zhang, Q. Liu, Biosensors and bioelectronics on smartphone for portable biochemical detection, Biosens. Bioelectron. 75 (2016) 273–284.

[22] L. Shen, J.A. Hagen, I. Papautsky, Point-of-care colorimetric detection with a smartphone, Lab Chip 12 (2012) 4240–4243.

[23] Y. Liu, X. Lin, X. Ji, Z. Hao, Z. Tao, Smartphone-based enzyme-free fluorescence sensing of organophosphate DDVP, Microchim. Acta 187 (2020) 419.

[24] D. Chang, K.T. Kim, E. Lindberg, N. Winssinger, Smartphone DNA or RNA sensing using semisynthetic luciferase-based logic device, ACS Sens. 5 (2020) 807–813.

[25] M. Zhang, N. Yang, Y. Liu, J. Tang, Synthesis of catalase-inorganic hybrid nanoflowers via sonication for colorimetric detection of hydrogen peroxide, Enzym. Microb. Technol. 128 (2019) 22–25.

[26] Y. Li, X. Fei, L. Liang, J. Tian, L. Xu, X. Wang, Y. Wang, The influence of synthesis conditions on enzymatic activity of enzyme-inorganic hybrid nanoflowers, J. Mol. Catal. B Enzym. 133 (2016) 92–97.

[27] M. Li, M. Luo, F. Li, W. Wang, K. Liu, Q. Liu, Y. Wang, Z. Lu, D. Wang, Biomimetic copper-based inorganic-protein nanoflower assembly constructed on the nanoscale fibrous membrane with enhanced stability and durability, J. Phys. Chem. C 120 (2016) 17348–17356.

[28] W. Ren, X. Fei, J. Tian, Y. Li, M. Jing, H. Fang, L. Xu, Y. Wang, Multiscale immobilized lipase for rapid separation and continuous catalysis, New J. Chem. 42 (2018) 13471–13478.

[29] K. Li, J. Wang, Y. He, M.A. Abdulrazaq, Y. Yan, Carbon nanotube-lipase hybrid nanoflowers with enhanced enzyme activity and enantioselectivity, J. Biotechnol. 281 (2018) 87–98.

[30] C. Wang, R. Tan, Q. Wang, One-step synthesized flower-like materials used for sensitivity detecting amyloid precursor protein, Anal. Bioanal. Chem. 410 (2018) 6901–6909.

[31] Y. Song, J. Gao, Y. He, L. Zhou, L. Ma, Z. Huang, Y. Jiang, Preparation of a flowerlike nanobiocatalyst system via biomimetic mineralization of cobalt phosphate with enzyme, Ind. Eng. Chem. Res. 56 (2017) 14923–14930.

[32] Y. Ding, B. Yang, H. Liu, Z. Liu, X. Zhang, X. Zheng, Q. Liu, FePt-Au ternary metallic nanoparticles with the enhanced peroxidase-like activity for ultrafast colorimetric detection of H_2O_2, Sensor. Actuator. B Chem. 259 (2018) 775–783.

[33] N. Alizadeh, S. Ghasemi, A. Salimi, T.-K. Sham, R. Hallaj, CuO nanorods as a laccase mimicking enzyme for highly sensitive colorimetric and electrochemical dual biosensor: application in living cell epinephrine analysis, Colloids Surf. B Biointerfaces 195 (2020) 111228.

[34] M. Guan, M. Wang, W. Qi, R. Su, Z. He, Biomineralization-inspired copper-cystine nanoleaves capable of laccase-like catalysis for the colorimetric detection of epinephrine, Front. Chem. Sci. Eng. (2020), https://doi.org/10.1007/s11705-020-1940-y.