CrossMark

ORIGINAL ARTICLE

Enhanced Sensitivity for Hydrogen Peroxide Detection: Polydiacetylene Vesicles with Phenylboronic Acid Head Group

Chen Jia[1] · Jie Tang[2] · Shengguo Lu[3] · Yuwang Han[2] · He Huang[3]

Abstract It was recently reported that, besides UV irradiated polymerization, polymerization of diacetylene compounds could also been initiated by radicals generated from enzyme catalyzed hydrogen peroxide (H_2O_2) decomposition. A new optical sensing method for H_2O_2 was proposed based on this phenomenon. However, the sensitivity of this method is relatively lower than existed ones. In the present work, phenylboronic acid (PBA) functionalized 10, 12-pentacosadiynoic acid (PDA-PBA) was synthesized and its vesicles were formed successfully as colorimetric sensor for H_2O_2 detection. It was found that color change during the polymerization of vesicles composed of the PBA modified monomer is much stronger than that of the non-modified one. The response of PDA-PBA vesicles to H_2O_2 is 16 times more sensitive than that of the PDA. The absorption of PDA-PBA at 650 nm is linearly related to the concentration of H_2O_2 and a detection limit of ~5 μM could be achieved.

✉ He Huang
biotech@njtech.edu.cn

[1] State Key Laboratory of Materials-Oriented Chemical Engineering, College of Chemistry and Chemical Engineering, Nanjing Tech University, Nanjing 211816, People's Republic of China

[2] College of Science, Nanjing Tech University, Nanjing 211816, People's Republic of China

[3] State Key Laboratory of Materials-Oriented Chemical Engineering, College of Pharmaceutical Sciences, Nanjing Tech University, Nanjing 211816, People's Republic of China

Introduction

Hydrogen peroxide (H_2O_2) has been widely used in bleaching, cleaning and disinfection [1]. It is also a by-product of oxidative metabolism [2]. The detection of H_2O_2 is of practical importance in food, clinical, modern medicine, environmental monitoring and various other industries [3, 4]. Many methods such as chemiluminescence [5, 6], electrochemical methods [7–9] and chromatography [10] have been developed for the detection of H_2O_2. However, these detection methods need either expensive instruments or reagents. Therefore, optical biosensors are of the significant interest for the detection of H_2O_2 due to their low cost and simple instruments [11].

Recently, conjugated polymer colorimetric biosensor has become popular due to its high sensitivity, selectivity and practicality [12–16]. We previously reported a free radical triggered polymerization of 10, 12-pentacosadiynoic acid (PCDA) vesicles as a colorimetric reagent for H_2O_2 detection [17]. The polymerization of well-packed diacetylene monomers can be induced by the hydroxyl radical produced from the horseradish peroxidase (HRP) catalyzed H_2O_2 decomposition reaction [18–20]. The color of the reaction mixture changes from colorless to blue during the polymerization. Under the optimal conditions, the colorimetric detection of H_2O_2 can be completed with 20 μg/mL HRP (pH = 7.0) at 30 °C in 30 min. However, the previously reported PCDA-based method showed a relatively higher detection limit of ca. 80 μM which has no advantage compared to other methods [17].

The topotactic polymerization of diacetylene is generally known to be acutely sensitive to the molecular order of molecular assemblies. So, the molecular assemblie of the diacetylene determines not only the ability to form stable vesicles but also its chromic behavior. Besides the total chain length and position of the diacetylenic unit within the chain,

Springer

chemical nature of the headgroup is a key structural factor for diacetylene compounds. Interactions between neighboring diacetylene molecules via their headgroups are especially important for signal amplification in sensors and this could be tailored by synthesis [21]. Niwa et al. prepared LB film of 10, 12-pentacosadiynamide phenylboronic acid (DA-PBA) and got a blue polymer film upon UV irradiation [22]. It is found that dehydration of boronic acid groups constrained in position on the surface of a two dimensional monolayer assembly would form cross-linked boronic anhydrides. Lee et al. also successfully prepared phenylboronic acids functionialized diacetylene network films on solid substrates [23]. In the present work, a polydiacetylene vesicle bearing phenylboronic acid group was prepared for the first time and used as a more sensitive colorimetric biosensor for H_2O_2 detection.

Synthetic Routes

Purification of PCDA

The commercial PCDA was purified for further use. Briefly, PCDA solid powder was dissolved in methylene chloride and filtered through 0.45 μm membrane to remove the poly-PCDA. The methylene chloride was evaporated with a rotary evaporator and the resultant PCDA monomer was stored at −20 °C in the dark.

Synthesis of APCDA

Figure 1 shows the synthesis route for the mediate product 10, 12-pentacosadiynoic acid 2-aminoacetamide (APCDA). Briefly, 2.45 mmol purified PCDA monomer in 20 mL tetra-hydrofuran was mixed with 2.4 mmol N-hydroxysuccinimide and 2.45 mmol 1-ethyl-3(3-dimethyl ammonium propyl)-carbodiimide. The mixed solution was allowed to react for
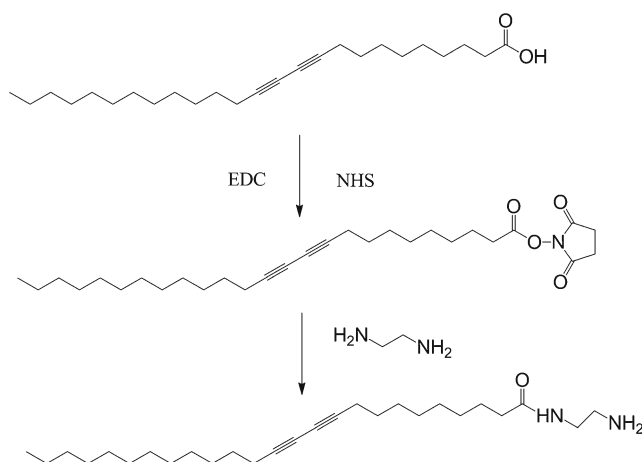

Fig. 1 The synthesis route of APCDA

24 h under 350 rpm mechanical stirring in the dark. The reaction solution was then evaporated to dryness with the rotary evaporator. The dried residue was re-dissolved in 30 mL chloroform and extracted with 20 mL saturated sodium chloride solution 6 times. The organic phase was collected and dried over anhydrous magnesium sulfate for 12 h. The product was dissolved in 50–70 mL chloroform. Ten milliliters ethylenediamine (4 mmol in dichloromethane) was added to the product solution dropwise. The pH of the solution was adjusted to 9.0 with triethylamine. The reaction was allowed to precede under 300 rpm mechanical at 30 °C in the dark. The reaction solution was then evaporated to dryness and the product, APCDA, was purified on a silica gel column with an eluant of chloroform: methyl alcohol (5:1). The yield of APCDA is 83–94 %. Figure 2 is the ^{1}HNMR of APCDA and data are as follows: ^{1}HNMR (300 MHz, CDCl3): δ(ppm) 0.78–0.81(t, J = 9, 3 H), 1.11–1.85 (m, 36 H), 2.14–2.16 (t, J = 6, 3 H), 2.24–2.26 (t, J = 6, 2 H), 3.31–3.34(dd, J = 9, 2 H), 7.19(brs, 1 H).

Synthesis of APCDA-PBA

The synthesis route of APCDA-PBA is present in Fig.3. Ten grams of 4-carboxyphenylboronic acid (PBA, 60.3 mmol) were added into a two-necked flask. Thionyl chloride (150 ml) was then added to the flask in a N_2 atmosphere. The mixture was magnetically stirred (350 rpm) for 24 h at 88 °C. The reaction mixture was then evaporated to dryness and the dry residue was re-dissolved in 60 ml anhydrous tetrahydrofuran. Chloroform (20 ml) was added dropwise to 3 mmol of APCDA in a N_2 atmosphere with an ice-bath. A drop of dimethyl formamide (DMF) was added after 30 min and kept in the ice-bath for another 2 h in the dark. After that, the obtained mixture was added dropwise to the previous prepared PBA solution and allowed to react for 20 h in the dark. The reaction mixture was evaporated to dryness with the rotary evaporator and the product was purified on a silica gel column with an eluant of chloroform: methyl alcohol (7:1). The yield of APCDA-PBA is between 91 and 94 %. Figure 4 is the ^{1}HNMR of APCDA-PBA and data are as follows: ^{1}HNMR (300 MHz, CDCl3): δ(ppm) 0.78–0.81(t, J = 9, 3 H), 1.18–1.58(m, 36 H), 2.14–2.17(t, J = 9, 4 H), 2.35–2.37(t, J = 6, 2 H), 2.87(s, 1 H), 2.94(s, 1 H), 7.05–7.06 (d, J = 3, 2 H), 7.19(brs, 2 H), 7.84–7.86 (d, J = 6, 2 H)..

Preparation of APCDA-PBA Vesicle

The produced APCDA-PBA (14.98 mg) was dissolved in 4 ml DMSO and 36 ml distilled water, heated to 70 °C and sonicated for 15 min. The solution was filtered and stored at 4 °C in the dark overnight.

Fig. 2 ¹HNMR of APCDA
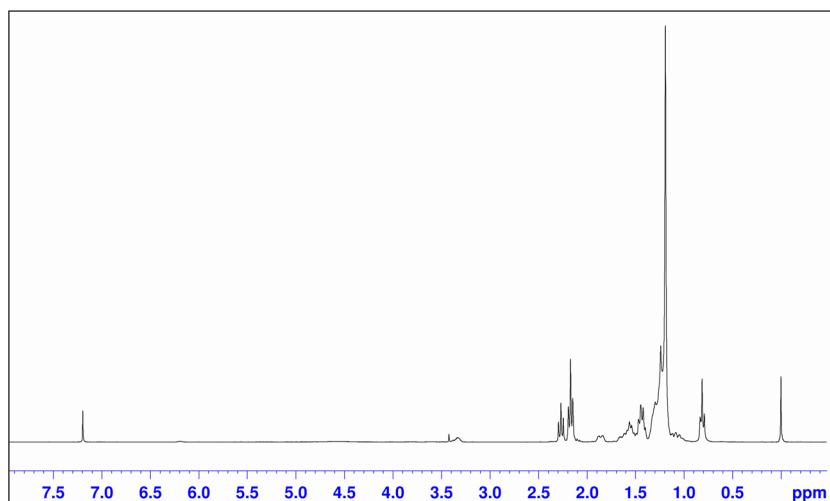
Results and Discussion

Characterization of APCDA-PBA

The reaction substrate (PCDA), mediate product (APCDA) and product (APCDA-PBA) were confirmed with FTIR (Fig.5). The asymmetric stretching vibrations of CH_2 groups of the diacetylene side chains can be found at 2928 cm^{-1} and 2852 cm^{-1}. The peak at 2349 cm^{-1} can be ascribed to the vibrations of the unsaturated double bond CH = CH in the diacetylene monomer. These feature FTIR peaks are shared by all three different diacetylene derivatives. APCDA showed an absorption peak at 3324 cm^{-1}, which was assigned to the stretching vibrations of N-H bond in amide. The absorption peaks at 1697 cm^{-1} and 1632 cm^{-1} can be assigned to the stretching vibrations of hydrogen-bonded carbonyl in carboxy bond and the stretching vibrations of hydrogen-bonded carbonyl in amide, respectively. The new peak at 1070 cm^{-1} is ascribed to the C-N bond in amide. These results indicate that the ethylene diamine monomer was successfully coupled with the PCDA. APCDA-PBA showed three new absorption peaks at 1810 cm^{-1}, 860 cm^{-1} and 1551 cm^{-1}, which were assigned to the stretching vibrations of C = C and stretching vibrations of C-H and B-O, respectively. These indicate that phenylboronic acid was coupled with the APCDA successfully.

The microstructures of poly-PCDA and poly-APCDA-PBA vesicles were imaged with a SEM (Fig.6). The poly-PCDA vesicles are composed of large spherical particles, while poly-APCDA-PBA vesicles composite of smaller rod-shaped and square particles. This can be explained that amphiphilic monomers with achiral headgroups usually form spherical liposomes and chiral amphiphiles form non-spherical structures such as helices and tubules [24]. Similar small particles can also be found in polydiacetylene-amino/1, 2-dimyristoyl-SN-glycero-3 -phosphocholine (PDA/DMPC) mixed vesicles, which can improve the UV-Vis absorption of the vesicles and enhance the color change [25]. The PDA/
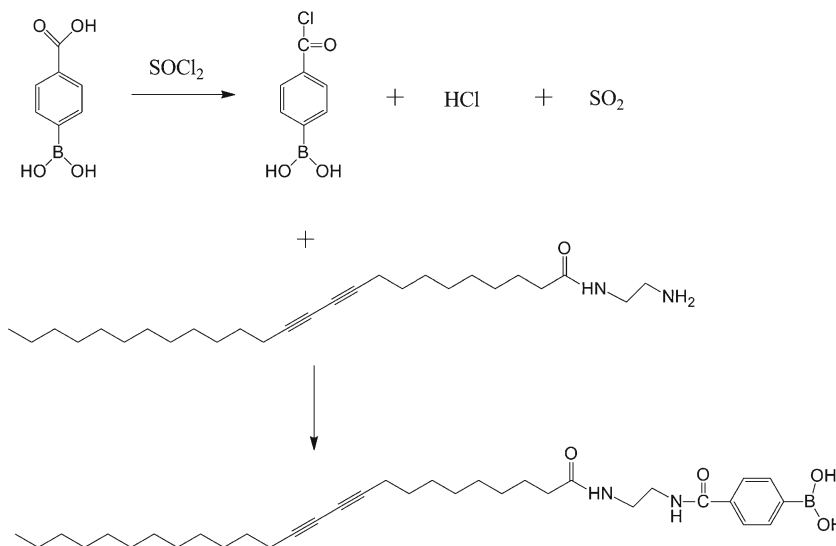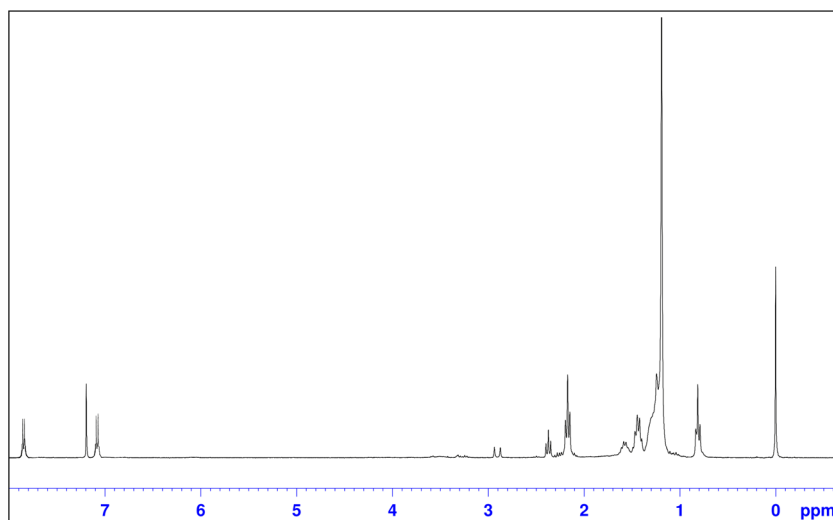
Fig. 3 The synthesis route of APCDA-PBA

Fig. 4 ^{1}HNMR of APCDA-PBA

DMPC mixed vesicle coupled with antigen has a flat shape, which can be attributed to its inherent variability and instability in preparation process [26]. Morphology change of vesicles can usually affect their detection sensitivities and stabilities [27–29]. The smaller vesicles provide more surface binding sites and thus improve the detection sensitivity of the colorimetric method [30].

UV Light Trigged Polymerization

The polymerization of diacetylene vesicles is initiated by UV light. The effects of irradiation time on the polymerization process of PCDA and APCDA-PBA were investigated. The absorption of the poly-PCDA at 650 nm (A_{650}) increased from 0.06 to 0.21 when the irradiation time was increased from 1 min to 2 min (Fig.7a). Meanwhile, A_{650} of poly-APCDA-PBA reached as high as 0.65 with 1 min irradiation. When irradiation time was increased to 2 min, the blue phase of poly-APCDA-PBA shifted to the purple phase and an absorption

peak appeared at 550 nm. A_{650} did not show significantly change. Thus, 1 min UV irradiation time is enough for the polymerization of APCDA-PBA. These also indicate that the polymerization of APCDA-PBA is easier to be trigged
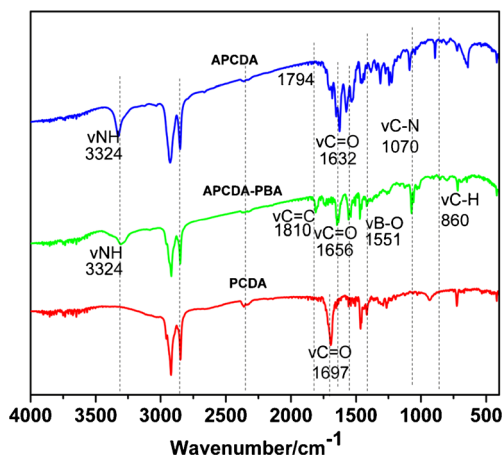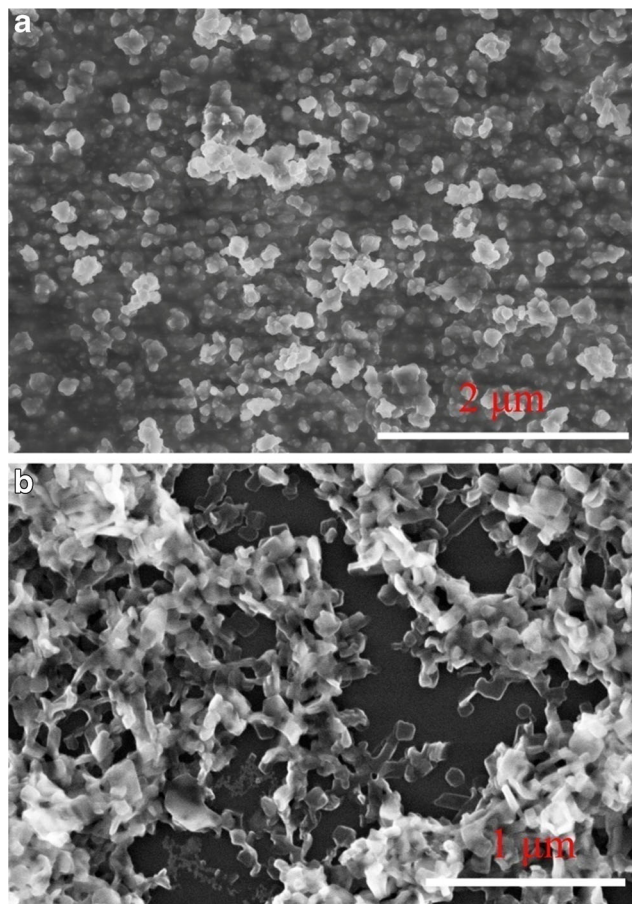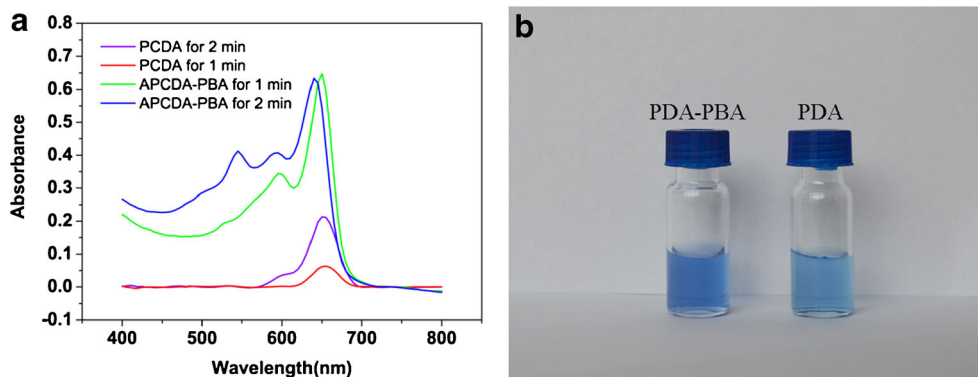

Fig. 6 SEM images of poly-PCDA vesicles (**a**) and poly-APCDA-PBA vesicles (**b**) The polymerization was initiated with 254 nm UV light at a distance of 30 cm

Fig. 5 FTIR spectra of PCDA (*red line*), APCDA (*blue line*) and APCDA-PBA (*green line*)

than that of PCDA alone. The much higher absorption of the poly-APCDA-PBA in blue region (Fig.7b) indicates the high sensitive of colorimetric method with APCDA-PBA vesicle.
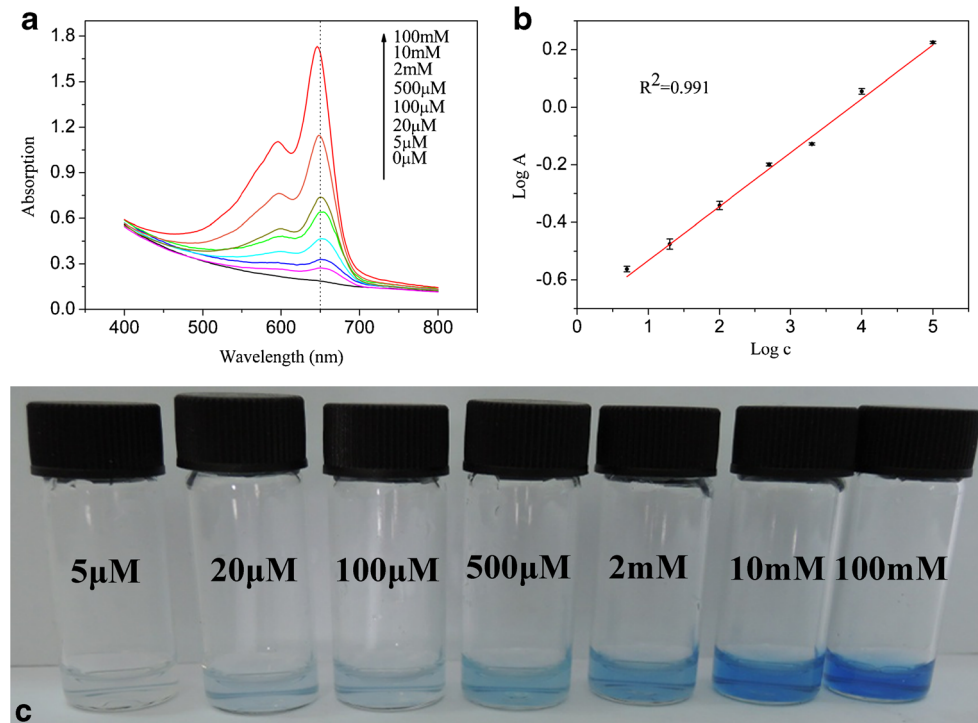
Detection of H$_2$O$_2$ with APCDA-PBA Vesicle

The potential application of APCDA-PBA vesicles in the quantification of H$_2$O$_2$ was explored. Figure 8a shows the UV-Vis absorption spectra of APCDA-PBA vesicles polymerized with various concentration of H$_2$O$_2$ ranging from 0 to 100 mM. It is clear the peak absorption at 650 nm increased with the increase of H$_2$O$_2$ concentration. APCDA-PBA vesicles can respond to as low as 5 μM H$_2$O$_2$, which is 16 times more sensitive than PCDA vesicles. In addition, the absorption of APCDA-PBA vesicles at 650 nm is significantly higher than that of PCDA vesicles [17] under same conditions, which

is consistent with the results of its UV-induced polymerization reaction (Fig.7b). Figure 8c shows the images of APCDA-PBA vesicles being polymerized with the different concentration of H$_2$O$_2$ from 5 μM to 100 mM. It is clear that the intensity of the blue color is significantly increased with the increase of the concentration of H$_2$O$_2$ and the color change of APCDA-PBA vesicles is more dramatic than that of PCDA vesicles [17] under same conditions.

The possibility of the quantification of H$_2$O$_2$ with APCDA-PBA vesicles was explored. The relationship between the absorption of poly-APCDA-PBA at 650 nm and the concentration of H$_2$O$_2$ is fitted in a linear regression log$A = -0.719 + 0.187$log$c$ ($R^2 = 0.991$, $n = 7$) (Fig.8b), where A is the absorbance of poly-APCDA-PBA at 650 nm and c is the concentration of H$_2$O$_2$ in μM. The lowest detectable concentration of H$_2$O$_2$ is ~5 μM.

Conclusions

In summary, the response of polydiacetylene vesicles to H_2O_2 can be effectively enhanced by the introduction of phenylboronic acid group. The absorption of PDA-PBA vesicles at 650 nm and the concentration of H_2O_2 are linearly related. The detection limit of the PDA-PBA vesicle based colorimetric method is ~5 μM H_2O_2, which is 16 times lower than that of the PDA vesicles based colorimetric method. The PDA-PBA vesicle is a promising tool for the detection of H_2O_2 in modern medicine, environmental and biotechnology monitoring.

References

1. Petlicki J, Palusova D, van de Ven TGM (2005) Physicochemical aspects of catalytic decomposition of hydrogen peroxide by manganese compounds. Ind Eng Chem Res 44:2002–2010. doi:10.1021/ie049595n

2. Root R, Metcalf J, Oshino N, Chance B (1975) H_2O_2 release from human granulocytes during phagocytosis. I. documentation, quantitation, and some regulating factors. J Clin Investig 55:945–955. doi:10.1172/JCI108024

3. Stuart A. G. Evans, Joanne M. Elliott, Lynn M. Andrews, Philip N. Bartlett et al (2002) Detection of hydrogen peroxide at mesoporous platinum microelectrodes. Anal Chem 74: 1322–1326. doi:10.1021/ac011052p

4. Lei C-X, Hu S-Q, Shen G-L, Yu R-Q (2003) Immobilization of horseradish peroxidase to a nano-Au monolayer modified chitosan-entrapped carbon paste electrode for the detection of hydrogen peroxide. Talanta 59:981–988. doi:10.1016/S0039-9140(02)00641-0

5. He S, Shi W, Zhang X, Li J, et al (2010) β-cyclodextrins-based inclusion complexes of $CoFe_2O_4$ magnetic nanoparticles as catalyst for the luminol chemiluminescence system and their applications in hydrogen peroxide detection. Talanta 82:377–383. doi:10.1016/j.talanta.2010.04.055

6. Han JH, Jang J, Kim BK, Choi HN, et al (2011) Detection of hydrogen peroxide with luminol electrogenerated chemiluminescence at mesoporous platinum electrode in neutral aqueous solution. J Electroanal Chem 660:101–107. doi:10.1016/j.jelechem.2011.06.012

7. Pajor-Swierzy A, Kolasinska-Sojka M, Warszynski P (2014) The electroactive multilayer films of polyelectrolytes and Prussian blue nanoparticles and their application for H_2O_2 sensors. Colloid Polym Sci 292:455–465. doi:10.1007/s00396-013-3091-x

8. Fan J, Bian X, Niu Y, Bai Y, et al (2013) Formation of three-dimensional Nano-porous silver films and application toward electrochemical detection of hydrogen peroxide. Appl Surf Sci 285: 185–189. doi:10.1016/j.apsusc.2013.08.034

9. Rismetov B, Ivandini TA, Saepudin E, Einaga Y (2014) Electrochemical detection of hydrogen peroxide at platinum-modified diamond electrodes for an application in melamine strip tests. Diam Relat Mater 48:88–95. doi:10.1016/j.diamond.2014.07.003

10. Nakashima K, Wada M, Kuroda N, Akiyama S, et al (1994) High-performance liquid chromatographic determination of hydrogen peroxide with peroxyoxalate chemiluminescence detection. J Liq Chromatogr 17:2111–2126. doi:10.1080/10826079408013535

11. Shiang YC, Huang CC, Chang HT (2009) Gold nanodot-based luminescent sensor for the detection of hydrogen peroxide and glucose. Chem Commun 23:3437–3439. doi:10.1039/b901916b

12. Kim JM, Lee YB, Yang DH, et al (2005) A polydiacetylene-based fluorescent sensor chip. J Am Chem Soc 127:17580–17581. doi:10.1021/ja0547275

13. Kolusheva S, Molt O, Herm M, et al (2005) Selective detection of catecholamines by synthetic receptors embedded in chromatic polydiacetylene vesicles. J Am Chem Soc 127:10000–10001. doi:10.1021/ja052436q

14. Wu A, Yuan G, Tian H, Federici JF, et al (2014) Effect of alkyl chain length on chemical sensing of polydiacetylene and polydiacetylene/ZnO nanocomposites. Colloid Polym Sci 292: 3137–3146. doi:10.1007/s00396-014-3365-y

15. Pecher J, Mecking S (2010) Nanoparticles of conjugated polymers. Chem Rev 110:6260–6279. doi:10.1021/cr100132y

16. Song YJ, Wei WL, Qu XG (2011) Colorimetric biosensing using smart materials. Adv Mater 23:4215–4236. doi:10.1002/adma.201101853

17. Lu S, Jia C, Duan X, Zhang X, et al (2014) Polydiacetylene vesicles for hydrogen peroxide detection. Colloid Surf A 443:488–491. doi:10.1016/j.colsurfa.2013.11.029

18. Wen F, Dong YH, Feng L, Wang S, et al (2011) Horseradish peroxidase functionalized fluorescent gold nanoclusters for hydrogen peroxide sensing. Anal Chem 83:1193–1196. doi:10.1021/ac1031447

19. Wu P, Cai Z, Chen J, Zhang H, et al (2011) Electrochemical measurement of the flux of hydrogen peroxide releasing from RAW 264.7 macrophage cells based on enzyme-attapulgite clay nanohybrids. Biosens Bioelectron 26:4012–4017. doi:10.1016/j.bios.2011.03.018

20. Tahir MN, André R, Sahoo JK, Jochum FD, et al (2011) Hydrogen peroxide sensors for cellular imaging based on horse radish peroxidase reconstituted on polymer-functionalized TiO_2 nanorods. Nanoscale 3:3907–3914. doi:10.1039/c1nr10587f

21. Jonas U, Shah K, Norvez S, et al (1999) Reversible color switching and unusual solution polymerization of hydrazide-modified diacetylene lipids. J Am Chem Soc 121:4580–4588. doi:10.1021/ja984190d

22. Niwa M, Shibahara S, Higashi N (2000) Diacetylenic monolayers containing a boronic acid moiety form a chemically and thermally stable poly(diacetylene) film on water. J Mater Chem 10:2647–2651. doi:10.1039/b004371k

23. Lee J, Yarimaga O, Lee CH, Choi YK, et al (2011) Network polydiacetylene films: preparation, patterning, and sensor applications. Adv Funct Mater 21:1032–1039. doi:10.1002/adfm.201002042

24. Reppy MA, Pindzola BA (2007) Biosensing with polydiacetylene materials: structures, optical properties and applications. Chem Commun 14(42):4317–4338. doi:10.1039/b703691d

25. Evrard D, Touitou E, Kolusheva S, Fishov Y, et al (2001) A new colorimetric assay for studying and rapid screening of membrane penetration enhancers. Pharm Res 18:943–949. doi:10.1023/A:1010980009823

26. Su Y, Li J, Jiang L (2004) Effect of amphiphilic molecules upon chromatic transitions of polydiacetylene vesicles in aqueous solutions. Colloids Surf B: Biointerfaces 39:113–118. doi:10.1016/j.colsurfb.2003.12.005

27. Lee J, Jeong EJ, Kim J (2011) Selective and sensitive detection of melamine by intra/inter liposomal interaction of polydiacetylene liposomes. Chem Commun 47:358–360. doi:10.1039/c0cc02183k

28. Lee SW, Kang CD, Yang DH, et al (2007) The development of a generic bioanalytical matrix using polydiacetylenes. Adv Funct Mater 17:2038–2044. doi:10.1002/adfm.200600398

29. Guo CX, Boullanger P, Liu T, Jiang L (2005) Size effect of polydiacetylene vesicles functionalized with glycolipids on their colorimetric detection ability. J Phys Chem B 109:18765–18771. doi:10.1021/jp052580y

30. Schneider HJ, Tianjun L, Lomadze N (2004) Sensitivity increase in molecular recognition by decrease of the sensing particle size and by increase of the receptor binding site - a case with chemomechanical polymers. Chem Commun 21:2436–2437. doi:10.1039/b409331c