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## Acetylcholinesterase Biosensor Platform Based on BP2000 for the Detection of Carbaryl

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# 基于乙酰胆碱酯酶生物传感平台的西维因检测

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**摘要:** 为给农药西维因检测提供一种新方法, 根据西维因抑制乙酰胆碱酯酶活性的原理, 以黑珍珠 2000 (BP2000) 为乙酰胆碱酯酶的固定化材料, 采用滴涂电极法构建了基于乙酰胆碱酯酶的西维因生物传感平台. 结果表明, 固定在 BP2000 上的乙酰胆碱酯酶保持了对氯化乙酰胆碱的催化活性, 并且由于 BP2000 材料的引入, 提升了电极有效的电化学活性表面积, 而且电极上物质的电化学氧化拥有较低氧化电位 (0.630 V) 并伴随质子传输. 由 BP2000 搭建成功的乙酰胆碱酯酶生物传感平台对西维因检测的线性响应范围为  $2.0 \text{ ng} \cdot \text{mL}^{-1} \sim 12.5 \text{ ng} \cdot \text{mL}^{-1}$ , 检测限为  $3.15 \text{ ng} \cdot \text{mL}^{-1}$ . 本研究对酶生物传感平台和酶生物燃料电池体系中酶电极的构建提供了一种简单方法及高效载体.

**关键词:** 乙酰胆碱酯酶; 西维因; 酶生物传感器; 电化学; 黑珍珠 2000

**中图分类号:** O646

**文献标识码:** A

西维因 (1-萘基-N-甲基氨基甲酸酯) 是如今应用较广泛的杀虫剂与除草剂, 可以抑制胆碱酯酶活性, 对人体免疫系统造成严重危害, 诱发身体畸形和癌变<sup>[1]</sup>, 实现其快速及灵敏的检测将具有十分重要的意义. 目前, 已经有高效液相色谱、毛细管电泳、气相色谱、薄层色谱、色谱-质谱联用等多种检测技术可以实现对西维因的检测<sup>[2-6]</sup>, 但这些测试技术普遍存在仪器设备昂贵、样品前处理复杂、分析耗时长等问题. 而随着生物电化学技术的迅猛发展, 电化学酶生物传感平台由于其拥有灵敏度高、响应速度快、花费低、检测限低、操作简单等优点快速地进入了研究者视野, 并在氨基甲酸酯类农药分析中取得了众多有价值的研究成果<sup>[7-9]</sup>.

目前, 已有商品供应的乙酰胆碱酯酶 (AChE) 是生物神经传导中的一种关键性酶, 具有羧肽酶和氨肽酶的活性, 该酶能降解乙酰胆碱, 终止神经递质对突触后膜的兴奋作用, 保证神经信号在生物体内的正常传递, 因氨基甲酸酯类农药对该酶活性的抑制机理研究较为深入, 因此基于乙酰胆碱酯酶的生物传感器得以广泛研究<sup>[10-13]</sup>. 针对于提

升乙酰胆碱酯酶生物传感器的性能已有大量的研究工作展开, 研究主要集中于以下三方面: 第一, 选择一个合适的载体材料固定乙酰胆碱酯酶; 第二, 在乙酰胆碱酯酶与电极间构建快速的电子传输通道; 第三, 提升乙酰胆碱酯酶电极面积<sup>[14]</sup>. 截止目前, 蛋白质<sup>[15-16]</sup>及酶<sup>[17-18]</sup>已经用于构建生物传感平台. 其中, 乙酰胆碱酯酶被固载于多壁碳纳米管<sup>[19]</sup>、双金属纳米棒<sup>[20]</sup>、半导体金属氧化物材料<sup>[21]</sup>以及天然高分子材料<sup>[22]</sup>等多种材料中以制备成该酶的生物传感平台.

黑珍珠 2000 (BP2000) 是具有巨大表面积与孔体积、导电性能良好的碳材料, 而且理化性能稳定, 是固定酶的理想载体. 目前已有使用其制备酶生物传感平台的相关报道<sup>[23]</sup>, 但使用 BP2000 载体制备乙酰胆碱酯酶生物传感平台还鲜有报道. 鉴于此, 本文以滴涂法在玻碳电极表面以 BP2000 作为载体以乙酰胆碱酯酶为探针, 成功制备乙酰胆碱酯酶生物传感平台, 并对西维因进行检测, 以期获得制备方法简单、检出限低、灵敏度高的电化学生物传感器, 也希望能为乙酰胆碱酯酶生物传感平台的搭建提供实验参考和技术支持.

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## 1 实 验

### 1.1 试剂与仪器

乙酰胆碱酯酶 (AChE, C3389-2KU)、西维因 (Carbaryl,  $100 \mu\text{g}\cdot\text{mL}^{-1}$ ) 和氯代乙酰胆碱 (ATCl) 购买于 Sigma 公司. BP2000 购买于 Cabot 公司亚太化学试剂分公司. 氯化钾、磷酸氢二钠、磷酸二氢钠、铁氰化钾均为分析纯, 购自北京化学试剂厂. 三电极体系的电化学工作站 (CHI 602A, 上海辰华), 对电极为铂丝电极, 饱和甘汞为参比电极, 制备的 AChE/BP2000/GC 电极 (直径 4 mm) 为工作电极.

### 1.2 AChE/BP2000/GC 电极、BP2000/GC 电极和 AChE//GC 电极的制备

首先, 取 10 mL 离心管分别加入 0.001 g BP2000 粉末和 5000  $\mu\text{L}$  去离子水, 超声分散配制成  $0.2 \text{ mg}\cdot\text{mL}^{-1}$  的 BP2000 溶液. 然后, 在 2 mL 离心管中分别加入 0.2 mg AChE 和 1000  $\mu\text{L}$   $0.1 \text{ mol}\cdot\text{L}^{-1}$  的磷酸盐缓冲溶液 (PBS, pH 7.0) 配制成  $0.2 \text{ mg}\cdot\text{mL}^{-1}$  AChE 的溶液, 并于  $4^\circ\text{C}$  冰箱中冷藏. 最后, 将上述超声均匀的 BP2000 溶液 500  $\mu\text{L}$  和 AChE 溶液 500  $\mu\text{L}$  于冰浴条件下磁力搅拌 24 h, 获得 BP2000 负载的 AChE 溶液, 移取 10  $\mu\text{L}$  该混合溶液滴涂于处理干净的玻碳电极表面制备成 AChE/BP2000/GC 电极, 于  $4^\circ\text{C}$  冰箱中冷藏干燥后以备常温下测试使用, 剩余的混合溶液则于冰箱中冷藏保存. 取 10  $\mu\text{L}$  超声均匀的  $0.2 \text{ mg}\cdot\text{L}^{-1}$  的 BP2000 溶液和 10  $\mu\text{L}$  AChE 溶液分别滴涂到处理干净的玻碳电极表面制备成 BP2000/GC 电极和 AChE//GC 电极, 以备室温下测试使用.

### 1.3 AChE 生物传感平台的工作原理

氯代乙酰胆碱在 AChE 催化下发生水解反应生成还原型胆碱, 该物质在 AChE/BP2000/GC 电极表面进行电化学氧化形成氧化型胆碱二聚体进而产生输出电流信号, 若西维因存在于体系中, AChE 的催化活性受其抑制, 使电极上生成的氧化型胆碱二聚体量减少, 输出电流信号减弱, 且这种抑制作用与西维因浓度成正比, 从而形成 AChE 生物传感平台, 示意图如图 1 所示.

## 2 结果与讨论

### 2.1 裸 GC 电极、BP2000/GC 电极和 AChE/BP2000/GC 电极的电化学

图 2 是裸 GC 电极 (a)、BP2000/GC 电极 (b) 和 AChE/BP2000/GC 电极 (c) 在扫速  $100 \text{ mV}\cdot\text{s}^{-1}$  时, 对  $1 \text{ mmol}\cdot\text{L}^{-1} \text{ K}_3[\text{Fe}(\text{CN})_6]$  和  $0.1 \text{ mol}\cdot\text{L}^{-1} \text{ KCl}$  混合溶液的电化学响应. 由图可知: 上述电极在  $-0.2 \sim 0.7 \text{ V}$  电位窗内均展现出一对稳定、可逆程度较好的氧化还原峰, 与裸 GC 电极 (曲线 a,  $\Delta E_p: 995 \text{ mV}$ ) 和 AChE/BP2000/GC 电极 (曲线 c,  $\Delta E_p: 1100 \text{ mV}$ ) 相比, BP2000/GC 电极 (曲线 b,  $\Delta E_p: 891 \text{ mV}$ ) 的峰电位差  $\Delta E_p$  最小, 氧化峰电流及还原峰电流最高, 这是由于 BP2000 优良的导电性能提升了电子在电极上的传输速率. 当 AChE 覆盖于 BP2000/GC 电极上时, 曲线 c 中的氧化峰电流与还原峰电流均有所下降, 也就是说非导电的 AChE 层阻碍了电子在电极上传递<sup>[24]</sup>. 作者又根据 Randles-Sevcik 方程计算得出裸 GC 电极、BP2000/GC 电极和 AChE/BP2000/GC 电极的有效电化学生活性表面积

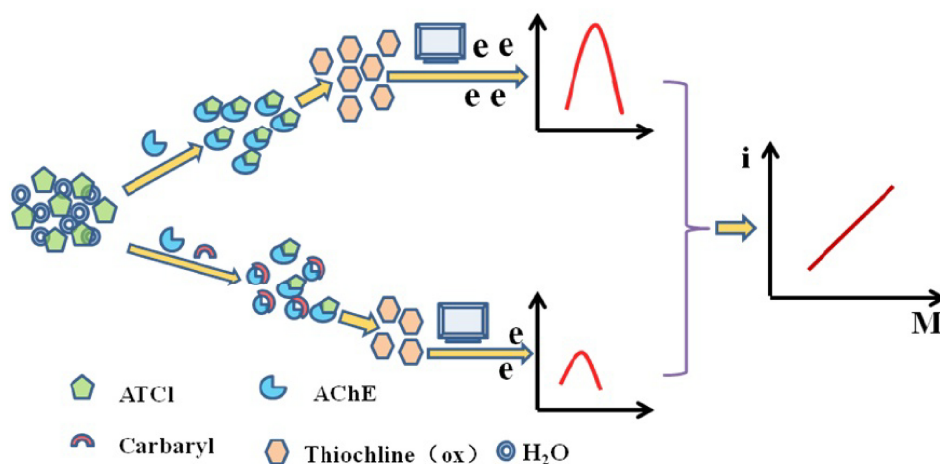


图 1 AChE 生物传感平台工作原理示意图

Fig. 1 Working principle diagram of the AChE biosensor platform

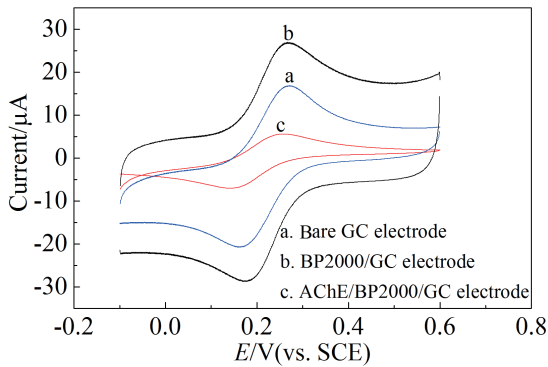


图2  $K_3[Fe(CN)_6]$ 和KCl溶液中,裸GC电极(a),BP2000/GC电极(b)和AChE/BP2000/GC电极(c)在扫速 $100\text{ mV}\cdot\text{s}^{-1}$ 时的循环伏安图

Fig. 2 Cyclic voltammograms of (a) bare GC electrode, (b) BP2000/GC electrode and (c) AChE/BP2000/GC electrode in the  $K_3[Fe(CN)_6]$  and KCl mixed solution, scan rate:  $100\text{ mV}\cdot\text{s}^{-1}$ .

分别为 $0.0792\text{ cm}^2$ 、 $0.1260\text{ cm}^2$ 和 $0.0253\text{ cm}^2$ ,即在BP2000的修饰作用下,GC电极的有效电化学活性表面积增加了0.6倍,这进一步说明BP2000对电极上电子运输有积极的促进作用,而且AChE已经被负载到电极的表面<sup>[14]</sup>。

## 2.2 AChE/BP2000/GC电极在PBS缓冲体系中的电化学

图3是AChE/BP2000/GC电极在饱和氮气条件、扫速 $10\text{ mV}\cdot\text{s}^{-1}$ 时, $0.1\text{ mol}\cdot\text{L}^{-1}$  PBS (pH 7.0)体系及含 $10\text{ mol}\cdot\text{L}^{-1}$  ATCl的 $0.1\text{ mol}\cdot\text{L}^{-1}$  PBS (pH 7.0)体系(曲线b)的循环伏安图。如图3所示,AChE/BP2000/GC电极在 $0.1\text{ mol}\cdot\text{L}^{-1}$  PBS (pH 7.0)缓冲测试体系中,没有任何氧化峰与还原峰出现(曲线a);而将AChE/BP2000/GC电极移入到含 $10\text{ mmol}\cdot\text{L}^{-1}$  ATCl的 $0.1\text{ mol}\cdot\text{L}^{-1}$  PBS (pH 7.0)体系时,在 $496\text{ mV}$ 处开始物质的电化学氧化,并于 $0.630\text{ V}$ 处达到最大峰电流(曲线b),这是由于电极表面上的AChE催化氯代乙酰胆碱水解生成还原型胆碱,其进一步于电极上氧化生成氧化型胆碱二聚体的缘故<sup>[25]</sup>,且该最大氧化峰电位比文献<sup>[26]</sup>报道的溶胶-凝胶AChE生物传感平台低 $20\text{ mV}$ 。

将AChE/BP2000/GC电极分别置于氮气饱和和pH为5.0、6.0、7.0的 $0.1\text{ mol}\cdot\text{L}^{-1}$  PBS(含 $10\text{ mmol}\cdot\text{L}^{-1}$  ATCl)体系中进行循环伏安测试,扫速为 $10\text{ mV}\cdot\text{s}^{-1}$ ,电位窗 $0\sim 1.2\text{ V}$ ,实验结果示于图4。由图4可知,随着体系pH值由5.0至7.0的增大,电化学氧化的最大峰电位分别位于 $0.807\text{ V}$ 、 $0.732\text{ V}$

及 $0.630\text{ V}$ 处并明显发生负移动,说明该电极的电化学过程伴随着质子的传输<sup>[25]</sup>,同时观察到体系pH为7.0时电化学氧化的峰电流最大,因此选择pH为7.0的 $0.1\text{ mol}\cdot\text{L}^{-1}$  PBS体系作为AChE生物传感平台的电解质溶液。

## 2.3 AChE生物传感平台对西维因浓度的响应

图5是AChE/BP2000/GC电极分别受西维因

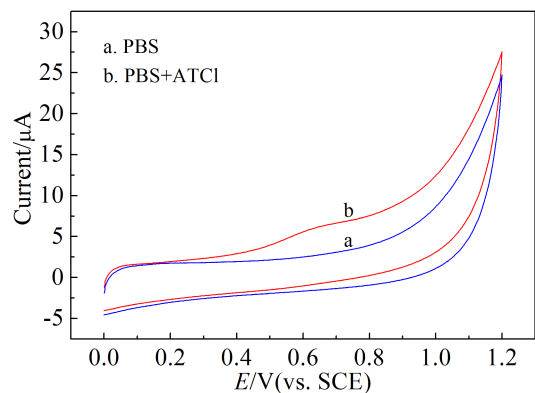


图3 AChE/BP2000/GC电极在扫速 $10\text{ mV}\cdot\text{s}^{-1}$ 、 $0.1\text{ mol}\cdot\text{L}^{-1}$  PBS缓冲溶液(pH 7.0)(曲线a)和含 $10\text{ mmol}\cdot\text{L}^{-1}$  ATCl的 $0.1\text{ mol}\cdot\text{L}^{-1}$  PBS (pH 7.0)(曲线b)中的循环伏安图

Fig. 3 Cyclic voltammograms of the AChE/BP2000/GC electrode in  $0.1\text{ mol}\cdot\text{L}^{-1}$  PBS (pH 7.0) (Curve a) and  $0.1\text{ mol}\cdot\text{L}^{-1}$  PBS (pH 7.0) with  $10\text{ mmol}\cdot\text{L}^{-1}$  ATCl (Curve b)

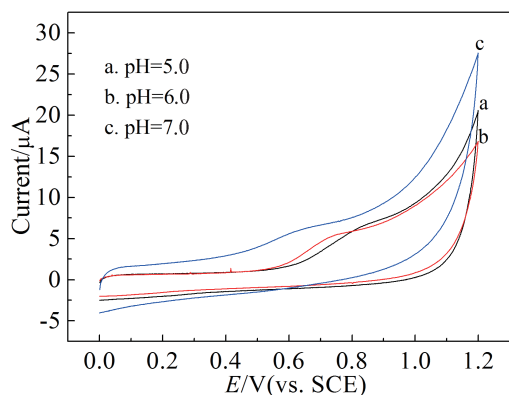


图4 AChE/BP2000/GC电极在含 $10\text{ mmol}\cdot\text{L}^{-1}$  ATCl的 $0.1\text{ mol}\cdot\text{L}^{-1}$  PBS体系中扫速 $10\text{ mV}\cdot\text{s}^{-1}$ 时,pH分别为5.0(a)、6.0(b)和7.0(c)时的循环伏安图

Fig. 4 Cyclic voltammograms of the AChE/BP2000/GC electrode in  $0.1\text{ mol}\cdot\text{L}^{-1}$  PBS and  $0.1\text{ mol}\cdot\text{L}^{-1}$  PBS with  $10\text{ mmol}\cdot\text{L}^{-1}$  ATCl at pH 5.0 (a), 6.0 (b) and 7.0 (c), scan rate:  $10\text{ mV}\cdot\text{s}^{-1}$

0 ng·mL<sup>-1</sup>、2.5 ng·mL<sup>-1</sup> 和 5.0 ng·mL<sup>-1</sup> 抑制后,在含 10 mmol·L<sup>-1</sup> ATCl 的 0.1 mol·L<sup>-1</sup> PBS(pH 7.0)体系中,于 0~1.2 V 电位窗内,扫速为 10 mV·s<sup>-1</sup> 时获得的循环伏安图.由图 5 可知,当 AChE/BP2000/GC 电极没有受到西维因抑制时,在电位 0.630 V 处有最大氧化峰电流出现(曲线 a),随着有抑制作用的西维因浓度由 2.5 ng·mL<sup>-1</sup> 至 5.0 ng·mL<sup>-1</sup> 缓慢增大,曲线 b 和曲线 c 在 0.630 V 处的氧化峰电流随之减小,表明基于 AChE/BP2000/GC 电极的 AChE 生物传感平台已被成功建立.

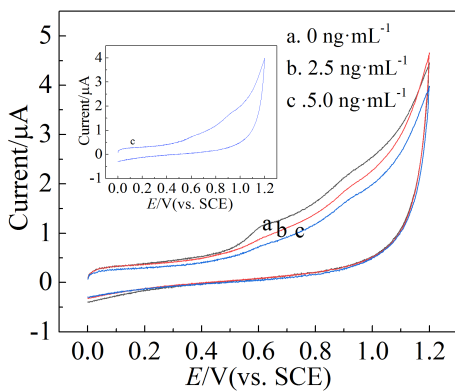


图 5 AChE/BP2000/GC 电极分别受(a) 0 ng·mL<sup>-1</sup>、(b) 2.5 ng·mL<sup>-1</sup> 和(c)5.0 ng·mL<sup>-1</sup> 西维因抑制后,于含 10 mmol·L<sup>-1</sup> ATCl 的 0.1 mol·L<sup>-1</sup> PBS(pH 7.0)体系中,扫速为 10 mV·s<sup>-1</sup> 时的循环伏安图

Fig. 5 Cyclic voltammograms of the AChE/BP2000/GC electrode inhibited by different concentrations of carbaryl (a) 0 ng·mL<sup>-1</sup>, (b) 2.5 ng·mL<sup>-1</sup> and (c) 5.0 ng·mL<sup>-1</sup>, in 0.1 mol·L<sup>-1</sup> PBS solution with 10 mmol·L<sup>-1</sup> ATCl (pH 7.0), scan rate: 10 mV·s<sup>-1</sup>.

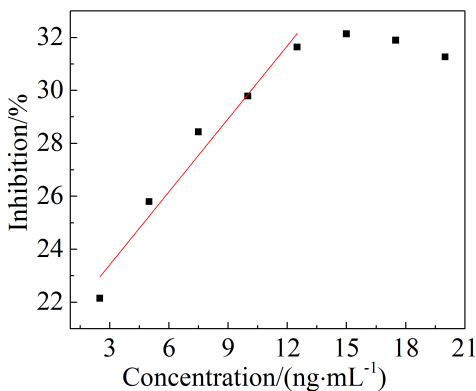


图 6 西维因抑制率与其浓度的关系图

Fig. 6 The plot showing relation between the inhibition of carbaryl and the concentration of carbaryl

表 1 AChE 生物传感平台和其它生物传感平台对西维因响应的对比

Tab. 1 Comparison between AChE biosensor platform and other biosensors platform when responding to carbaryl

Sensor	Linear range	Detection limit	Reference
AChE-G-PANI/GCE	38 ~ 194 ng·mL <sup>-1</sup>	20 ng·mL <sup>-1</sup>	[27]
ChE/carbon paste electrodes	1 ~ 15 μg·mL <sup>-1</sup>	0.40 μg·mL <sup>-1</sup>	[28]
GC/PANI/MWCNT/AChE	1.98 ~ 9.92 μg·mL <sup>-1</sup>	0.28 μg·mL <sup>-1</sup>	[2]
AChE/BP2000/GCE	2.5 ~ 12.5 ng·mL <sup>-1</sup>	3.15 ng·mL <sup>-1</sup>	This work

为进一步观察西维因的抑制作用,在上述 5.0 ng·mL<sup>-1</sup> 浓度的基础上,每次增加 2.5 ng·mL<sup>-1</sup> 直至西维因浓度达到 20.0 ng·mL<sup>-1</sup>,绘制西维因抑制率与其浓度的关系曲线如图 6.正如图 6 所示,在 2.5 ng·mL<sup>-1</sup> 至 12.5 ng·mL<sup>-1</sup> 浓度范围内,抑制率与西维因浓度呈线性关系,而在 12.5 ng·mL<sup>-1</sup> 至 20.00 ng·mL<sup>-1</sup> 较高浓度范围内,变化平缓,表明西维因与 AChE 的作用趋于饱和.将本 AChE 生物传感平台性能和其它生物传感平台性能进行比较(表 1),对比发现本生物传感平台有较低的检出限和较宽的线性范围.

### 2.4 AChE 生物传感平台的稳定性与重复性

AChE/BP2000/GC 电极在冰箱中 4 °C 下储存 15 天后,在上述的实验条件下,对 10 ng·mL<sup>-1</sup> 的西维因溶液进行测试,发现抑制峰电流降低了 4.10%,分析连续 9 次测量的电流响应值相对标准偏差为 3.38%,说明该电极有较好的稳定性与重复性,由此电极构成的 AChE 生物传感平台可运行性较高.

### 3 结 论

采用简单物理吸附法在 BP2000 载体上成功制备了基于乙酰胆碱酯酶的西维因生物传感平台,该生物传感平台之所以展现出较低检出限和较宽的线性范围,是由于 BP2000 有较高的导电性能与巨大的表面积,未来有望在酶生物传感器与酶生物燃料电池中得到广泛使用.

### 参考文献(References):

[1] Zhang C, Cui H Y, Han Y F, et al. Development of a

- biomimetic enzyme-linked immunosorbent assay based on molecularly imprinted polymers on paper for the detection of carbaryl[J]. *Food Chemistry*, 2018, 240(1): 893-897.
- [2] Liu Z M, Zang X H, Liu W H, et al. Novel method for the determination of five carbamate pesticides in water samples by dispersive liquid-liquid microextraction combined with high performance liquid chromatography[J]. *Chinese Chemical Letters*, 2009, 20(2): 213-216.
- [3] Cheng X, Wang Q L, Zhang S, et al. Determination of four kinds of carbamate pesticides by capillary zone electrophoresis with amperometric detection at a polyamide-modified carbon paste electrode[J]. *Talanta*, 2007, 71(3): 1083-1087.
- [4] Oh-Shin Y S, Ko M, Shin H S, et al. Simultaneous quantification of insecticides including carbaryl in drinking water by gas chromatography using dual electron-capture and nitrogen-phosphorus detection[J]. *Journal of Chromatography A*, 1997, 769(2): 2285-291.
- [5] Patil V B, Shingare M S. Thin-layer chromatographic detection of carbaryl using phenylhydrazine hydrochloride [J]. *Journal of Chromatography A*, 1993, 653(1): 181-183.
- [6] Totti S, Fernández M, Ghini S, et al. Application of matrix solid phase dispersion to the determination of imidacloprid, carbaryl, aldicarb, and their main metabolites in honeybees by liquid chromatography-mass spectrometry detection[J]. *Talanta*, 2006, 69(3): 724-729.
- [7] Song Y H, Chen J G, Sun M, et al. A simple electrochemical biosensor based on AuNPs/MPS/Au electrode sensing layer for monitoring carbamate pesticides in real samples [J]. *Journal of Hazardous Materials*, 2016, 304 (3): 103-109.
- [8] Kestwal R M, Bagal-Kestwal D, Chiang B, et al. Fenu-greek hydrogel-agarose composite entrapped gold nanoparticles for acetylcholinesterase based biosensor for carbamates detection[J]. *Analytica Chimica Acta*, 2015, 886(7): 143-150.
- [9] Cesarino I, Moraes F C, Lanza M, et al. Electrochemical detection of carbamate pesticides in fruit and vegetables with a biosensor based on acetylcholinesterase immobilised on a composite of polyaniline-carbon nanotubes[J]. *Food Chemistry*, 2012, 135(3): 873-879.
- [10] Liu Q, Fei A, Huan J, et al. Effective amperometric biosensor for carbaryl detection based on covalent immobilization acetylcholinesterase on multiwall carbon nanotubes/graphene oxide nanoribbons nanostructure[J]. *Journal of Electroanalytical Chemistry*, 2015, 740(3): 8-13.
- [11] Hatefi-Mehrjardi A. Bienzyme self-assembled monolayer on gold electrode: An amperometric biosensor for carbaryl determination[J]. *Electrochimica Acta*, 2013, 114(12): 394-402.
- [12] Li Y, Shi L Y, Han G Y, et al. Electrochemical biosensing of carbaryl based on acetylcholinesterase immobilized onto electrochemically inducing porous graphene oxide network[J]. *Sensors and Actuators B: Chemical*, 2017, 238(1): 945-953.
- [13] Zhang Y, Arugula M A, Wales M, et al. A novel layer-by-layer assembled multi-enzyme/CNT biosensor for discriminative detection between organophosphorus and non-organophosphorus pesticides[J]. *Biosensors and Bioelectronics*, 2015, 67(5): 287-295.
- [14] Zhou L Y, Zhang X, Ma L, et al. Acetylcholinesterase/chitosan-transition metal carbides nanocomposites-based biosensor for the organophosphate pesticides detection[J]. *Biochemical Engineering Journal*, 2017, 128(12): 243-249.
- [15] Han X J, Huang W M, Jia J B, et al. Direct electrochemistry of hemoglobin in egg-phosphatidylcholine films and its catalysis to  $H_2O_2$  [J]. *Biosensors and Bioelectronics*, 2002, 17(9): 741-746.
- [16] Han X J, Cheng W L, Zhang Z L, et al. Direct electron transfer between hemoglobin and a glassy carbon electrode facilitated by lipid-protected gold nanoparticles[J]. *BBA-Bioenergetics*, 2002, 1556(2/3): 273-277.
- [17] Wang K Q, Yang H, Zhu L, et al. Direct electron transfers and electrocatalysis of glucose oxidase immobilized on glassy carbon electrode modified with Nafion and mesoporous carbon FDU-15[J]. *Electrochimica Acta*, 2009, 54 (20): 4626-4630.
- [18] Xu X(徐璇), Lu J S(卢菊生), Liu S Q(刘松琴). Fabrication and application of cytochrome P450 electrochemical biosensor in drug metabolism[J]. *Journal of Electrochemistry(电化学)*, 2015, 21(1): 45-52.
- [19] Chen D F, Liu Z N, Fu J Y, et al. Electrochemical acetylcholinesterase biosensor based on multi-walled carbon nanotubes/dicyclohexyl phthalate modified screen-printed electrode for detection of chlorpyrifos[J]. *Journal of Electroanalytical Chemistry*, 2017, 801(9): 185-191.
- [20] Lu X, Tao L, Song D D, et al. Bimetallic Pd@Au nanorods based ultrasensitive acetylcholinesterase biosensor for determination of organophosphate pesticides[J]. *Sensors and Actuators B: Chemical*, 2018, 255(2): 2575-2581.
- [21] Huang Q L, Wang Y, Lei L, et al. Photoelectrochemical biosensor for acetylcholinesterase activity study based on metal oxide semiconductor nanocomposites[J]. *Journal of Electroanalytical Chemistry*, 2016, 781(11): 377-382.
- [22] Cui H F, Wu W W, Li M M, et al. A highly stable acetylcholinesterase biosensor based on chitosan-TiO<sub>2</sub>-graphene

- nanocomposites for detection of organophosphate pesticides[J]. *Biosensors and Bioelectronics*, 2018, 99(1): 223-229.
- [23] Wang K, Tang J, Zhang Z, et al. Laccase on Black Pearl 2000 modified glassy carbon electrode: Characterization of direct electron transfer and biological sensing properties for pyrocatechol[J]. *Electrochimica Acta*, 2012, 70(5): 112-117.
- [24] Chen D, Zhang W, Liu D, et al. Covalent coupling of organophosphorus hydrolase loaded quantum dots to carbon nanotube/Aunanocomposite for enhanced detection of methyl parathion[J]. *Biosensors and Bioelectronics*, 2010, 25(6): 1370-1375.
- [25] Shamagsumova R V, Shurpik D N, Padnya P L, et al. Acetylcholinesterase biosensor for inhibit or measurements based on glassy carbon electrode modified with carbon black and pillar[5]arene[J]. *Talanta*, 2015, 144(11): 559-568.
- [26] Raghu P, Kumara Swamy B E, Madhusudana Reddy T, et al. Sol-gel immobilized biosensor for the detection of organophosphorous pesticides: A voltammetric method [J]. *Bioelectrochemistry*, 2012, 83(2): 19-24.
- [27] Li Y P, Zhang Y Y, Han G Y, et al. An acetylcholinesterase biosensor based on graphene/polyaniline composite film for detection of pesticides[J]. *Chinese Journal of Chemistry*, 2016, 34(1): 82-88.
- [28] Caetano J, Machado S A S. Determination of carbaryl in tomato in natura using an amperometric biosensor based on the inhibition of acetylcholinesterase activity[J]. *Sensors and Actuators B: Chemical*, 2008, 129(1): 40-46.

## Acetylcholinesterase Biosensor Platform Based on BP2000 for the Detection of Carbaryl

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**Abstract:** With the purpose of providing a new method for carbaryl (a pesticide) detection, on the basis of the principle that acetylcholinesterase (AChE) activity can be restrained by carbaryl, an AChE biosensor platform based on BP2000 (as a fixation) was constructed by dropping method. As a result, it revealed that AChE immobilized on BP2000 maintained its catalytic activity for acetylcholine (ATCl), and due to the introduction of the BP2000 material, the effective electrochemical surface area of the modified electrode was enlarged. In addition, the electrochemical oxidation at the modified electrode occurred at low potential (0.630 V) accompanied by proton transmission. The AChE biosensor platform based on BP2000 matrix for carbaryl detection was able to reflect a linear response in the range of  $2.0 \text{ ng} \cdot \text{mL}^{-1} \sim 12.5 \text{ ng} \cdot \text{mL}^{-1}$  with the detection limit of  $3.15 \text{ ng} \cdot \text{mL}^{-1}$ . At last, this work will provide a simple method and an efficient matrix in establishing an enzyme electrode of enzymatic biosensor platform and enzymatic fuel cell.

**Key words:** acetylcholinesterase; carbaryl; enzyme-based biosensor; electrochemistry; black pearl 2000