**4-Acetoxyphenol as a substrate for acetylcholinesterase-based sensor and its application for As(III) determination**

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Abstract

4-Acetoxyphenol (CAS 3233-32-7) was used as the substrate for electrochemical assay of acetylcholinesterase (AchE). The enzyme activity was reported as the current generated by anodic oxidation of hydroquinone, the product from esterase-catalyzed hydrolysis of 4-Acetoxyphenol. This reaction sequence was applied to As3+ determination with an amperometric biosensor, which was prepared by immobilizing AchE on a carbon screen-printed electrode. A protocol was developed based on quasi-irreversible inhibition of AchE by As3+. The concentration of As3+ correlated to the decrease of anodic current in a logarithm relationship. The analysis had a dynamic range of 2-500 M As3+ with maximum current reduction of 70% and a lower detection limit of 2.2 M. The AchE sensor was stable for at least 150 days at 22 ± 2 °C. The analysis was free from the interference by Cu2+, a reversible inhibitor to AchE and interferrent in arsenic determination by stripping voltammetry.

1. **Introduction**

Acetylcholinesterase (AchE, EC 3.1.1.7) is the most important enzyme in the cholinergic system. It catalyzes the hydrolysis of acetylcholine to choline, thereby deactivating the neurotransmitter. With a catalytic efficiency (*k*cat/*K*m) > ×108 M-1s-1, AchE catalysis is essentially diffusion controlled (Quinn 1987).

Among all / serine hydrolases, AchE stands out with a unique structure that accounts for its high activity(Silman and Sussman 2008, Dvir, Silman et al. 2010). It has a deep and narrow gorge leading to the active site with many aromatic residues presenting along the wall of the active site gorge. Close to the rim of the gorge, there is a peripheral anionic site (PAS) on the surface of the protein. It is flexible, and involved in the allosteric modulation of catalysis. Deeper in the gorge, the aromatic moieties are part of the catalytic anionic site (CAS). These aromatic groups are low-affinity sites that provide aromatic guidance to transport the substrate. At the bottom, there is an oxyanion hole that binds to the carbonyl group to facilitate ester hydrolysis. Despite of the complicated mechanism to transport substrate, there were signs that AchE was promiscuous as it catalyzed the hydrolysis of 4-aminophenol acetate, an ester that does not resemble acetylcholine(Pariente, Hernandez et al. 1993).

AchE is inhibited by many natural products that exhibit neurotoxicity. Natural AchE inhibitors are structurally diversified compounds, such as Fasciculine(Taylor, Radic et al. 1995), Aflatoxin(Pohanka 2011), and Anatoxin(Araoz, Molgo et al. 2010). Not only they are important for toxicology study, but also cause great concerns as pollutants. AchE has long been the target for inhibitor development for toxicology or pharmacology applications. These AchE inhibitors have been used as insecticides, nerve agents, and medications for Alzheimer's disease, etc(Pohanka 2012). All the structural features of AchE have been used for inhibitor development(Greenblatt, Dvir et al. 2003). Organophosphates and carbamates are suicide inhibitors of AchE, as they irreversibly inhibit AchE by modifying the serine hydroxyl group in the catalytic cycle. Other inhibitors are developed based on the binding of PAS or CAS sites. These inhibitors can be mono- or bi-dentate, and are usually reversible. Their kinetic mechanism can either be competitive or non-competitive. In addition to the organic chemicals, AchE is inhibited by many inorganics. Depending on the biological source of an AchE, it can be inhibited by F-, As3+, Hg2+, Cu2+, Zn2+, Cd2+, Fe3+ and Pb2+ (Wilson and Silman 1977, Froede and Wilson 1985, Frasco, Colletier et al. 2007, de Lima, Roque et al. 2013)

For on-site analysis of the above chemicals in the environment, electrochemical biosensor based on AchE inhibition is a desirable tool(Pundir and Chauhan 2012, Stepankova and Vorcakova 2016). Amperometric biosensor with screen-printed electrode (SPE) is an attractive option(Li, Yu et al. 2015, Barton, Garcia et al. 2016) as it integrates the selectivity of enzyme inhibition with the sensitivity of electrochemical detection on a disposable transducer. By design, AchE is immobilized on the SPE. A reaction sequence is used to link enzyme catalysis with electrochemical reaction. The enzyme activity is reported as the current at a working potential. The correlation between inhibitor concentrations to the current decrease provides the basis for analysis.

The abundance of Arsenic in the continental crust of the earth is around 1.5-2 ppm, although it varies considerably among geographic regions(Woolson, Moore et al. 1977). Inorganic arsenic exists as arsenite (As3+) and arsenate (As5+) in in environment. They have caused serious health problems by polluting food, drinking water, surface water, and ground water. To monitor arsenic pollution, an on-site determination is critical. This need may be addressed with an amperometric biosensor (Ma, Sengupta et al. 2014, Kaur, Kumar et al. 2015), as it is inexpensive, does not require special expertise to operate, can be miniaturized for mass production, and provides quantitative near-real time results.

Several enzymes were used for arsenic biosensor development. These enzymes include arsenite oxidase for direct As3+ determination(Male, Hrapovic et al. 2007), acid phosphatase for inhibition-based determination of As5+(Cosnier, Mousty et al. 2006, Sanllorente-Mendez, Dominguez-Renedo et al. 2012), AchE and laccase for As3+ base on inhibition(Wang, Milton et al. 2016). Among these enzymes, AchE has several important advantages for biosensor development such as high efficiency, exceptional stability, and direct evidence for neurotoxicity.

Three reaction sequences have been used in the amperometric assay for AchE(Miao, He et al. 2010). The earliest reaction sequence involves three reactions: including acetylcholine hydrolysis by AchE, choline oxidation by choline oxidase, and electrochemical oxidation of H2O2. This sequence is not only complicated, but also requires high potential for H2O2 oxidation. The sequence was simplified by replacing acetylcholine to acetylthiocholine in the second sequence, as the thiocholine product could be selectively oxidized on anode. Among different salts of acetylthiocholine, acetylthiocholine iodide (ATCI) has been the most prevalent choice. However, amperometric assay of AchE with ATCI was not selective, as iodide oxidation also generated anodic current in a wide range of working potentials (Bucur, Bucur et al. 2013). For As(III) determination, iodide caused additional problems by promoting arsenite oxidation (Stoytcheva, Sharkova et al. 1998). These evidences cast strong doubts on the validity of the results employing ATCI as the substrate in As(III) determination (Stoytcheva, Sharkova et al. 1998, Sanllorente-Mendez, Dominguez-Renedo et al. 2010, del Torno-de Roman, Alonso-Lomillo et al. 2015). To eliminate the interference from iodide, perchlorate or chloride of thiocholine were used to replace ATCI (Bucur, Bucur et al. 2013). Another approach to simplify the assay employed 4-aminophenol acetate as the substrate(Pariente, Hernandez et al. 1993). The sequence involved AchE hydrolysis to give 4-aminophenol, which was oxidized at low working potential. For AchE electrode fabrication, the reaction sequence is still complicated as 4-benzoquinoneimine undergoes spontaneous hydrolysis to give benzoquinone and ammonia.

To simplify the amperometric assay of AchE for sensor development, we propose to use 4-acetoxyphenol as the substrate based on a reaction sequence outlined in Figure 1. We tested the hypotheses that 4-acetoxyphenol would be hydrolyzed by AchE (Reaction 1), and it would not interfere anodic oxidation of 1,4-dihydroquione (Reaction 2) in amperometric assay.



**Figure 1**. The reaction sequence for amperometric AchE sensor with 4-Acetoxyphenol as the substrate

Although all inhibitors cause enzyme activity reduction, specific measurement protocols are required for each of them based on their respective chemical and kinetic mechanisms (Arduini, Amine et al. 2009, Arduini and Amine 2014, Amine, Arduini et al. 2016). In reported work on As3+ determination with AchE electrodes, the protocols were all based on the rapid steady state assumption (Stoytcheva, Sharkova et al. 1998, Stoytcheva, Sharkova et al. 1998, Sanllorente-Mendez, Dominguez-Renedo et al. 2010). However, this is inconsistent with the inhibition kinetics of free AchE (Wilson and Silman 1977). The inhibition of AchE by As3+ was found to be pseudo-irreversible. The association rate constant was 1.3 × 102 min-1 M-1, and the dissociation rate constant was 1.9 × 10-3 min-1 in 0.05 M phosphate, pH 7.4. In addition, the rate was increased in the presence of certain quaternary ligands, such as pyridine-2-aldoxime methiodide (2-PAM). The equilibrium constant was1.5 ×10-5 M at pH 7.4, suggesting the detection limit of As3+ is around 1 M. In this work, we evaluated the inhibition kinetics of the immobilized AchE by As3+, and developed a protocol accordingly.

1. **Materials and Methods**
	1. *Chemicals and Materials*

Acetylcholinesterase (E.C. 3.1.1.7) Type VI-S from *Electrophorus electricus* (electric eel) with activity of 217 U mg-1 protein, was purchased from Sigma-Aldrich. 4-Acetoxyphenol was purchased from Combi-Blocks (San Diego, CA, USA). All other chemicals were purchased from Sigma-Aldrich (reagent grade), and used as received.

Carbon Screen Printed Electrodes (SPE), including those with small round working electrodes (2 mm OD, RRPE1001C) and large rectangular working electrodes (4 × 5 mm, RRPE1002C), were purchased from Pine Research Instrumentation (Durham, NC, USA).

* 1. *AchE immobilization on SPE*

A mixture of BSA and AchE (containing 0.50 g, or 0.11 U of the AchE, 8 g of BSA in 10 l of 0.06 M phosphate, pH 7.0) was spread on the working electrode of carbon SPE (RRPE1002C). The proteins were deposited on the electrode surface by air drying, and then crosslinked by adding 10 l of 0.0021% of glutaraldehyde solution in water. The electrode was air dried at room temperature for 16 h to complete the crosslinking. It was stored in 0.1 M Tris-HCl, pH 7.0 for > 24 h at room temperature before use.

* 1. *Electrochemical Measurements*

Electrochemical experiment was carried out in the low volume SPE cell (Pine Research Instrumentation, Durham, NC, USA), and the condition was controlled by a DY2013 Potentiostat with DY2000 software (Digi-Ivy, Inc. Austin, TX, USA). The working potential was set with the Ag/AgCl electrode on the SPE as the reference. For cyclic voltammetry study, the SPE with a small working electrode (2 mm ID round, RRPE1001C) was used. For chronoamperometry study and AchE electrode preparation, we used the SPE with a larger working electrode (4 × 5 mm rectangular RRPE1002C).

All experiments were carried out at 22 ± 2 °C. The current in amperometric determination was recorded for 150s. The current typically stabilized in < 60s. The average of the readings between 120s and 150s was used as the steady-state current. The collected data was exported to Excel for graph generation and analysis.

* 1. *Inhibition of immobilized AchE by Sodium Arsenite*

The inhibition of the immobilized AchE by As3+ was investigated for measurement protocol development. An AchE electrode was incubated in an NaAsO2 solution for a period of time before removal to determine the residual activity. The Inhibition was determined by the decrease of steady state current in amperometric measurement. In the plan, the concentrations of NaAsO2 were 5, 20 and 100 M, and the incubation times were between 1 min and 60 min. The data was fitted to a reversible pseudo-first order reaction model (Eq. 1) by a non-linear least-square method(Kemmer and Keller 2010).

 

 **Eq. 1**

2.5 [As3+] determination protocol

The concentration of As3+ was determined by its correlation to the inhibition of AchE. The AchE electrode was incubated with arsenite solution in 0.1 M Tris-HCl, pH 8.0 for 1 h at 22 ± 2 °C. The activity of the electrode was measured before and after the inhibition with an amperometric method based on the reaction sequence in Figure 1. The inhibition was calculated by Eq. 2, where *I* is inhibition, *i*0 is the steady state current in the absence of As(III), and *i*i is the steady state current of the electrode after inhibition.

 **Eq. 2**

1. **Results and Discussion**
	1. *Electrochemical characterization of the substrate and product*

The electrochemical reactions were first evaluated with 5 mM solutions of 4-acetoxyphenol or hydroquinone (QH2) in 0.1 M phosphate, pH 7.0. Cyclic voltammetry had initial potential at -0.8 V, scan rate at 50 mV s-1, and switching potential at 0.8 V.

Hydroquinone gave two well defined peaks at 195 and -275 mV in voltammogram. These peaks were reproducible in successive scans with the same electrode. The peak separation at 50 mV s-1 scan rate was 470 mV, indicating the interfacial kinetics was slow. Further investigation showed that the peak potential was a function of scanning rate. The peak potential separation increased from 420 to 672 mV as the scan rate increased from 20 to 1000 mV s-1. The peak current ratios were in the range between 0.85 and 1.09 (Supplement Material, Figure 1 and Table 1). The evidence showed that the electrode reaction of hydroquinone is quasi-reversible: the reaction was chemically reversible but the kinetics was not. Despite the non-ideal behavior of Reaction 2, the anodic peak height in CV was linearly correlated to the concentration of reactant in the range from 0.05 to 10 mM when scan rate was 1000 mV s-1 (Supplement Material, Figures 2 and 3).

**Figure 2**. Cyclic voltammograms of hydroquinone and 4-acetoxyphenol

The electrochemistry of quinone has been extensively studied and employed in many applications (Kim and Chung 2014). The interconversion between 1,4-benzoquinone (Q) and the corresponding hydroquinone (QH2) is quite complex as the reaction involves two electron transfer reactions and two protonation steps. The mechanism is dependent on pH, working potential, and reaction medium (Bailey, Ritchie et al. 1983). A nine-membered square scheme was used to analyze the pathways for intermediate interconversion via electron transfer and protonation (Quan, Sanchez et al. 2007). In phosphate buffer at pH 7.2, Reaction 2 gave two widely separated peaks (E = 334 mV) in cyclic voltammetry with a glassy carbon electrode. The pathway for Reaction 2 was postulated to involve a HeHe mechanism, although these four reactions could involve concerted steps.

The proposed AchE substrate 4-acetoxyphenol did not undergo any redox reactions in CV. This is consistent to an earlier report, indicating the oxidation took place at 1.1 V (vs Ag/AgCl) with a glassy carbon electrode. (Kakemoto, Murakami et al. 1991). The large difference in oxidation potentials between the substrate and product is highly desirable for selective determination of QH2. It is feasible to develop an amperometric method based on Reaction 2 (Figure 1) in a way similar to the reaction with 4-aminophenyl acetate as the substrate(Pariente, Hernandez et al. 1993, La Rosa, Pariente et al. 1994). The oxidation of 4-aminophenol is more complicated as it involves two electron transfer, and two protonation reactions to give *p*-iminoquinone as an intermediate; which is subsequently hydrolyzed to give Q as the final product(Wang, Li et al. 1999, Beiginejad, Nematollahi et al. 2013). In addition, 4-aminophenol acetate was less stable. It was oxidized when potential was above 0.4 V (with a sodium chloride saturated calomel electrode) in 0.1 M phosphate at pH 7.9 (La Rosa, Pariente et al. 1994). It also gave significant background in CV in 0.1 M phosphate, pH 7.5(Pariente, Hernandez et al. 1993). As the substrate for esterase sensor, 4-acetoxyphenol offers the advantages of simpler reaction mechanism, and cleaner background.

Voltammetry study with 2 mM QH2 shows that the oxidation started from 0 V. The steady state current increased linearly as the potential increased to 0.2 V, then leveled off at higher potential (Supplementary material, Figure 4). Therefore, we set working potential at 0.35 V for maximum response. Amperometric assay with working potential at 0.35 V gave a linear correlation between steady state current and [QH2] in the range between 0.05 to 10 mM. Regression analysis showed that the sensitivity was 8.76 A mM-1. For 4-acetoxyphenol, there was also a low yet appreciable current with a sensitivity of 0.150 A mM-1. Therefore, the sensitivity for the product was 57 folds higher than that for the substrate. Based on the result from CV study, 4-acetoxyphenol was unlikely oxidized at 0.35 V. However, it might undergo spontaneous hydrolysis to give QH2, which was oxidized to give the background current. Another plausible cause of the background current is from impurity, as the substrate was only 96% pure. These concerns are addressable by a study with purified 4-acetoxyphenol.

**Figure 3**. Standard curves for amperometric assay at 0.35 V for QH2 (blue) and 4-acetoxyphenol (red)

* 1. *Immobilization of AchE and hydrolysis of 4-acetoxyphenol*

We observed the formation of QH2 by HPLC (data not shown) when 4-acetoxyphenol was incubated with AchE in 0.1 M phosphate buffer, pH 7. The rate of QH2 formation depended on the amount of AchE and incubation time. Since no product was found in the control, we concluded that AchE could catalyze Reaction 1 (**Figure 1**).

We chose crosslinking AchE with glutaraldehyde (GA) for electrode fabrication. Ideally, an immobilization is efficient and precise, therefore allowing high surface functionalization on the working electrode. GA is known to be effective through reactions with accessible lysine residues on protein molecules. Bovine serum albumin can be added to change the probability of linkage formation between enzymes. However, GA does no distinguish lysines on proteins, therefore cannot create linkages between specific residues (Sassolas, Blum et al. 2012). Still, this method offers enormous flexibility as the underlying chemistry is adaptive for linking residues in different microenvironments(Walt and Agayn 1994, Migneault, Dartiguenave et al. 2004). In aqueous medium, GA may undergo intramolecular condensation to give tetrahydropyran or intermolecular condensation to give enal. The degree of polymerization is dependent on the condition. These molecules allow several types of reactions including nucleophilic addition, nucleophilic substitution, and Michael reaction. Indeed, several types of linkage were found in lysozyme crystal when treated with GA (Wine, Cohen-Hadar et al. 2007). Therefore, we believed GA crosslinking could be optimized to meet different objectives in electrode fabrication.

We used a two stage protocol in which the protein solution was deposited on the working electrode, then GA solution was applied for crosslinking. The variables in the optimization include the amount of AchE and BSA, the buffer, and the concentration of GA. For amperometric assay, the optimization involved maximizing the yield of steady state current as long as the electrode would generate reproducible results in 3 repeats (CV < 20%). The assay was carried out in 0.1 M phosphate, pH 7.0 because the reverse reaction in As3+ inhibition (**Eq. 1**) was slow in phosphate buffer(Wilson and Silman 1977). The concentration of 4-acetoxyphenol was at 20 mM.

For the first step, we investigated the impact of AchE in the range of 0.5 – 10 g, BSA in the range of 0 – 30 g in 0.1 M phosphate with pH between 6.5 – 7.5. For crosslinking step, we evaluated the GA concentration from 0.001% to 0.015%. Other than the pH value of phosphate, all other factors had strong impact on the performance of the electrode.

The yield of the current ranged from about 7 – 50 A g-1 of AchE in the scope of conditions. The concentration of GA was the most significant factor. For crosslinking of 5.3 g AchE with 10 g of BSA, increasing GA from 0.005 to 0.015% caused current decrease from 99.7 A to 68 A. BSA stabilized AchE in the immobilization. When GA was set at 0.01%, increasing BSA from 10 to 30 g caused current increase from 88.6 to 105.3 A. In general, the concentration of GA should be adjusted based on the total protein (Supplement Material, Table 2).

Higher concentration of GA also reduced the sensitivity to As3+ inhibition. For crosslinking of 5.3 g AchE with 20 g of BSA, increasing GA from 0.005 to 0.015% caused a reduction of maximum inhibition (measured with 1 mM As3+) from 69.3% to 65.7%. The similar trend was observed on other combinations of crosslinking conditions (Supplement Material, Table 2).

Importantly, the sensitivity to the inhibition had an inverse correlation with the amount of AchE in the range from 1 to 5 g (Figure 4). This trend was observed at both 0.1 mM and 0.5 mM of As3+. To finalize the optimization, we further reduced the amount of AchE to 0.5 g and adjusted the amount of BSA to 8 g. The protein mixture was immobilized by crosslinking with 10 l of 0.0021% GA.

**Figure 4**. Dependence of inhibition on the amount of immobilized AchE. The electrode was prepared by crosslinking 5 g (cycle) or 1 g (square) of AchE with 10 g of BSA by 10 l of 0.005% GA (n = 3)

**Figure 4** Dependence of steady state current on substrate concentration

There was a clear dependence of steady state current on the substrate concentration with the AchE electrode (Figure 4). Fitting the data to Michaelis-Menten equation gave a model with R2 = 0.9931, indicating the immobilized AchE exhibited similar kinetics to that in the free form, in which the reaction was diffusion controlled. The *V*max was 24.2 ± 1.1 A, corresponding to a current density of 121 + 5.25 A cm-2. The *K*M was 5.92 ± 0.65 mM. When substrate concentration was set at 20 mM, the reaction rate should be 77% of the *V*max.

The protocol was efficient to prepare AchE electrodes therefore met the need for electrode characterization. In an evaluation of operational stability, the initial steady sate currents were in the range between 25.0 – 30 .0 A, with an average of 27.6 ± 2.0 A (n = 6). The electrodes all underwent activity loss in 10 repeated uses. In each successive use, the steady state current was reduced by about 2 - 4% (Supplement Material, Figure 5). Therefore, all the measurements with the AchE electrode had the same repeats to minimize the impact from uses.

3.3 *Inhibition of immobilized AchE and measurement protocol*

It was known that Tris and high pH facilitated the binding of As3+ to free AchE(Wilson and Silman 1977), therefore the inhibition study was carried out by incubating the electrode in arsenite solution in 0.1 M Tris-HCl, pH 8.0. The concentration of As3+ was 5, 20, and 100 M, respectively; and incubation took place over the course of one hour.

The inhibition was found to be similar to the case with free AchE, which was dependent on both time and inhibitor concentration (**Figure 5**). Fitting the data to a model based on the reversible pseudo-first order mechanism (**Eq. 1**) gave a *k*1 of 2.84 × 103 M-1 min-1, and a *k*-1 of 1.75 × 10-1 M-1. Apparently, the association and dissociation for immobilized AchE were both faster than those for free AchE. The regression coefficient R2 was 0.7475 for this fitting, suggesting that the kinetics might not be adequately represented by the model. Nevertheless, it was clear that the protocol based on a steady state mechanism with rapid inhibitor biding was unsuitable as the equilibrium was not reached instantaneously. In addition, the mechanism showed that dissociation was still slow enough that the reaction could be treated as being irreversible in protocol development. Since the *K*i was 6.16 × 10-5 M, the lower detection limit should be in low M concentrations.

**Figure 5**. Inhibition of immobilized AchE by Arsenite

(cycles, 5 M, squares 20 M, triangles 100 M)

Based on the kinetic feature, our protocol involved a one-hour incubation of the electrode in arsenite solution in 0.1 M Tris-HCl, pH 8.0 followed by residual activity (*i*i) determination. The inhibition was calculated by **Eq. 2**. The inhibition increased rapidly in the concentration range of 2-20 M of As3+, then much slower as the concentrations were between 20 – 500 M. At concentration went higher, the inhibition did not increase significantly (**Figure 6A**). Over the wide dynamic range, the dependence of inhibition on [As3+] could be represented with a logarithm relationship. The *I*Max was typically 70-75%, much higher than those reported earlier(Stoytcheva, Sharkova et al. 1998, Sanllorente-Mendez, Dominguez-Renedo et al. 2010, del Torno-de Roman, Alonso-Lomillo et al. 2015).

A

B

**Figure 6** The correlation between *I*% and [As3+]

A focused evaluation at lower range of As3+ revealed more characteristics of the sensor (**Figure 6B**). Corresponding to 1 to 20 M As3+, the inhibition increased from 2.7 to 44.9 %. Least square regression gave a straight line with a R2 of 0.93. The line had an intercept (*I* %) of 5.3 ± 1.68, and a slope (*I* % M-1) of 2.27 ± 0.18. Therefore, the limit of detection (LOD) was 3 + intercept = 10.3% of uncorrected inhibition, corresponding to the concentration of As(III) at 2.2 M. This result was consistent with our kinetic data, and offered a LOD 15.3 folds lower than that (35.9 M) of the protocol based on rapid steady state in a more recent report(del Torno-de Roman, Alonso-Lomillo et al. 2015). The estimated inhibition at 20 M was 50.7 ± 5.7%. At higher inhibition, the logarithm relationship would be needed to correlate with the [As3+].

*3.4 Interference by selected contaminants*

Anodic stripping voltammetry (ASV) has been the most developed electrochemical analysis for arsenic determination. However, interference by common contaminants has been a major concern to use ASV in the field (Liu and Huang 2014, Luong, Lam et al. 2014, Antonova and Zakharova 2016).

Cu2+ is a common contaminant that may give false positive results for arsenic determination; because copper formed intermetallic alloy with arsenic in deposition step, and their peak potentials were too close in the stripping step(Yang, Guo et al. 2016). Cu2+ was also a reversible inhibitor to AchE(Frasco, Fournier et al. 2005). The impact of Cu2+ was tested by including 0 – 32 M CuSO4 in the 10 M As3+ solution for the inhibition step. The result showed that Cu2+ did not interfere with the assay as the inhibition was unchanged (**Figure 7**). Apparently, the new protocol eliminated the impact of Cu2+ by separating arsenic inhibition step from activity analysis. This feature is highly desirable because reversible inhibitor will not interfere the assay as long as they do not change the binding of As3+ to AchE.

Figure 7. The impact of Cu(II) on the biosensor assay of 10 M arsenite

Humic acid is a common contaminant known to interfere arsenic determination with ASV(Liu, Chen et al. 2014). We evaluated the impact of 0-40 mg L-1 humic acid on the assay of 10 M arsenite. Although there was no dose-inhibition correlation for humic acid, the inhibition was reduced from 28.3% to an average of 22.3 ± 1.8 % (**Figure 8**). This could be the consequence of electrode passivation as the polymeric humic acid might coat the carbon electrode surface.

**Figure 8**. The impact of humic acid on the biosensor assay of 10 M arsenite

3.5 *Storage* *Stability of the immobilized AchE*

Storage stability of an enzyme electrode is directly related to its shelf-life specification for commercial application. It is also important to have stable electrodes to work with in development, because many electrodes are needed to test hypothesis, scout options, improve or characterize performance.

 **Figure 9**. Storage stability of the AchE electrodes in 0.1 M Tris-HCl, pH 7.0 at 20-25 °C (n=5 for each time point)

The lifetimes of AchE electrodes were widely different: ranging from 2 days to 120 days, although it was not clear how fabrication method or storage condition contributed to the storage stability(Pundir and Chauhan 2012). We tested 6-day activity loss of AchE electrodes in buffers including phosphate or Tris-HCl with pH from 6 to 8, at temperatures from 4 – 25 °C. It was found the electrodes had essentially no activity loss in 0.1 M Tris-HCl, pH 7.0 under ambient temperature (22 ± 2 °C). Prolonged evaluation with AchE electrodes from the same batch showed that they were stable over 150 days (**Figure 9**).

1. **Concluding remarks**

This report describes a systematic approach to develop AchE biosensor to analyze As3+ in water. We proposed to use 4-acetoyxyphonol as the substrate to simplify amperometric assay for AchE sensor development. We found AchE could efficiently hydrolyze the substrate, and hydroquinone could be selectively determined in the presence of 4-acetoxyphenone. The reaction sequence under the optimal condition is efficient to report AchE activity on the carbon electrode.

Based on the inhibition kinetic mechanism, we separated the inhibition step from activity determination, thus eliminated the interference from Cu2+, a reversible inhibitor of AchE and a well-known interferrent in ASV for arsenic determination.

The fabrication of the electrode was optimized in terms of the composition of the proteins and crosslinking method. The dynamic range of the biosensor was from approximately 4 – 70%, resulting from the inhibition by 2-500 M As3+. The correlation could be represented by a logarithm relationship. In range of 10-50% inhibition, the correlation was linear with a sensitivity of 2.27 ± 0.18 *I* % M-1; and a LOD of 10.7% inhibition, corresponding to 2.2 M As3+. The electrode was exceptionally stable: it retained activity for 150 days in 0.1 M Tris-HCl, pH 7 under room temperature.

This prototypic biosensor appears to be a promising tool for arsenic field screening. However, the LOD of 2.2 M (165 ppb) is still much higher than the 10 ppb threshold set forth for drinking water by US EPA. We expect to increase the sensitivity, and reproducibility through precise immobilization and bioreceptor improvement (Schulze, Muench et al. 2005, Pundir and Chauhan 2012, Songa and Okonkwo 2016)

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1. **References**

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 Enzyme inhibitors like drugs and pollutants are closely correlated to human and environmental health, thus their monitoring is of paramount importance in anal. chem. Enzymic biosensors represent cost-effective, miniaturized and easy to use devices; particularly biosensors based on enzyme inhibition are useful anal. tools for fast screening and monitoring of inhibitors. The present review will highlight the research carried out in the last 9 years (2006-2014) on biosensors based on enzyme inhibition. We underpin the recent advances focused on the investigation in new theor. approachs and in the evaluation of biosensor performances for reversible and irreversible inhibitors. The use of nanomaterials and microfluidic systems as well as the applications of the various biosensors in real samples is critically reviewed, demonstrating that such biosensors allow the development of useful devices for a fast and reliable alarm system. [on SciFinder(R)]

Antonova, S. and E. Zakharova (2016). "Inorganic arsenic speciation by electroanalysis. From laboratory to field conditions: A mini-review." Electrochem. Commun. **70**: 33-38.

 The accurate on-site monitoring of arsenic in water is still an important issue for many countries. This review considers the most promising electrochem. methods for the anal. and speciation of the inorg. forms of arsenic under field conditions, with an emphasis on stripping voltammetry. [on SciFinder(R)]

Araoz, R., et al. (2010). "Neurotoxic cyanobacterial toxins." Toxicon **56**(5): 813-828.

 A review. Worldwide development of cyanobacterial blooms has significantly increased in marine and continental waters in the last century due to water eutrophication. This phenomenon is favored by the ability of planktonic cyanobacteria to synthesize gas vesicles that allow them to float in the water column. Besides, benthic cyanobacteria that proliferate at the bottom of lakes, rivers and costal waters form dense mats near the shore. Cyanobacterial massive proliferation is of public concern regarding the capacity of certain cyanobacterial strains to produce hepatotoxic and neurotoxic compds. that can affect public health, human activities and wild and stock animals. The cholinergic synapses and voltage-gated sodium channels constitute the targets of choice of cyanobacterial neurotoxins. Anatoxin-a and homoanatoxin-a are agonists of nicotinic acetylcholine receptors. Anatoxin-a(s) is an irreversible inhibitor of acetylcholinesterase. Saxitoxin, kalkitoxin and jamaicamide are blockers of voltage-gated sodium channels, whereas antillatoxin is an activator of such channels. Moreover the neurotoxic amino acid L-beta-N-methylamino-L-alanine was shown to be produced by diverse cyanobacterial taxa. Although controversial, increasing in vivo and in vitro evidence suggest a link between the ingestion of L-beta-N-methylamino-Lalanine and the development of amyotrophic lateral sclerosis/Parkinsonism-dementia complex, a neurodegenerative disease. This paper reviews the occurrence of cyanobacterial neurotoxins, their chem. properties, mode of action and biosynthetic pathways. [on SciFinder(R)]

Arduini, F. and A. Amine (2014). "Biosensors Based on Enzyme Inhibition." Adv. Biochem. Eng./Biotechnol. **140**(Biosensors based on Aptamers and Enzymes): 299-326.

 A review. The present chapter describes the use of biosensors based on enzyme inhibition as anal. tools. The parameters that affect biosensor sensitivity, such as the amt. of immobilized enzyme, incubation time, and immobilization type, were critically evaluated, highlighting how the knowledge of enzymic kinetics can help researchers optimize the biosensor in an easy and fast manner. The applications of these biosensors demonstrating their wide application have been reported. The objective of this survey is to give a crit. description of biosensors based on enzyme inhibition, of their assembly, and their application in the environmental, food, and pharmaceutical fields. [on SciFinder(R)]

Arduini, F., et al. (2009). "Reversible Enzyme Inhibition-Based Biosensors: Applications and Analytical Improvement Through Diagnostic Inhibition." Anal. Lett. **42**(9): 1258-1293.

 The present review reports the research carried out during past 9 years on biosensors based on reversible enzyme inhibition for detn. of drugs, pollutants, and toxic compds. Applications in food, environmental, and pharmaceutical fields are also reported. Special attention is paid to the optimization of parameters such as enzyme immobilization, substrate concn., and incubation time. On the basis of the studies reviewed here, it is our view that enzyme inhibition-based biosensors have been shown to be useful anal. tools. [on SciFinder(R)]

Bailey, S. I., et al. (1983). "The construction and use of potential-pH diagrams in organic oxidation-reduction reactions." J. Chem. Soc., Perkin Trans. 2(5): 645-652.

 Potential-pH diagrams were constructed from literature data for PhOH, 2,4,6-tri-tert-butylphenol, and hydroquinone. The relationship between chem. and electrochem. phenomena is described, and the significance of the results of chem. expts. discussed in relation to the potential-pH diagrams for the case of PhOH oxidn. [on SciFinder(R)]

Barton, J., et al. (2016). "Screen-printed electrodes for environmental monitoring of heavy metal ions: a review." Microchim. Acta **183**(2): 503-517.

 Heavy metals such as lead, mercury, cadmium, zinc and copper are among the most important pollutants because of their non-biodegradability and toxicity above certain thresholds. Here, we review methods for sensing heavy metal ions (HMI) in water samples using screen-printed electrodes (SPEs) as transducers. The review (with 107 refs.) starts with an introduction into the topic, and this is followed by sections on (a) mercury-coated SPEs, (b) bismuth-coated SPEs, (c) gold-coated SPEs (d) chem. modified and non-modified carbon SPEs, (e) enzyme inhibition-based SPEs, and (f) an overview of com. available electrochem. portable heavy metal analyzers. The review reveals the significance of SPEs in terms of decentralized and of in situ anal. of heavy metal ions in environmental monitoring. [Figure not available: see fulltext.]. [on SciFinder(R)]

Beiginejad, H., et al. (2013). "Electrochemical oxidation of some aminophenols in various pHs." J. Electrochem. Soc. **160**(1): H41-H46.

 Electrochem. oxidn. of ortho- and para- aminophenol derivs. (2-aminophenol, 2-methylaminophenol, 4-aminophenol, 4-methylaminophenol and acetaminophen) was studied in acidic solns. by cyclic voltammetry and controlled-potential coulometry. The oxidn. forms of para-aminophenols undergo hydrolysis by formation of p-benzoquinone as final product and the oxidn. of ortho-aminophenol derivs. is accompanied by dimerization reaction. The effect of different parameters such as substitute effect, pH, aminophenol concn. and time window of chosen electrochem. method is discussed on the reaction rate of dimerization and hydrolysis. The hydrolysis and dimerization reaction rates are completely dependent on the substituted group linked to N atom and position of amine group in the mol. [on SciFinder(R)]

Bucur, M.-P., et al. (2013). "Critical evaluation of acetylthiocholine iodide and acetylthiocholine chloride as substrates for amperometric biosensors based on acetylcholinesterase." Sensors **13**: 1603-1613.

 Numerous amperometric biosensors have been developed for the fast anal. of neurotoxic insecticides based on inhibition of cholinesterase (AChE). The anal. signal is quantified by the oxidn. of the thiocholine that is produced enzymically by the hydrolysis of the acetylthiocholine pseudosubstrate. The pseudosubstrate is a cation and it is assocd. with chloride or iodide as corresponding anion to form a salt. The iodide salt is cheaper, but it is electrochem. active and consequently more difficult to use in electrochem. anal. devices. The authors investigated the possibility of using acetylthiocholine iodide as pseudosubstrate for amperometric detection. Operational conditions for any amperometric biosensor that use acetylthiocholine iodide must be thoroughly optimized to avoid false anal. signals or a reduced sensitivity. The working overpotential detd. for different screen-printed electrodes was: carbon-nanotubes (360 mV), platinum (560 mV), gold (370 mV, based on a catalytic effect of iodide) or cobalt phthalocyanine (110 mV, but with a significant reduced sensitivity in the presence of iodide anions). [on SciFinder(R)]

Cosnier, S., et al. (2006). "Specific Determination of As(V) by an Acid Phosphatase-Polyphenol Oxidase Biosensor." Anal. Chem. **78**(14): 4985-4989.

 An original amperometric biosensor based on the simultaneous entrapment of acid phosphatase (AcP) and polyphenol oxidase (PPO) into anionic clays (layered double hydroxides) was developed for the specific detection of As(V). The functioning principle of the bienzyme electrode consisted of the successive hydrolysis of Ph phosphate into phenol by AcP, followed by the oxidn. of phenol into o-quinone by PPO. The Ph phosphate concn. was, thus, monitored by potentiostating the biosensor at -0.2 V vs. Ag/AgCl to detect amperometrically the generated quinone. The detection of As(V) was based on its inhibitory effect on AcP activity toward the hydrolysis of Ph phosphate into phenol. The As(V) can be specifically detd. in pH 6.0 acetate buffer without any interferences of As(III) or phosphate, the detection limit being 2 nM or 0.15 ppb after an incubation step for 20 min. [on SciFinder(R)]

de Lima, D., et al. (2013). "In vitro and in vivo inhibition of acetylcholinesterase and carboxylesterase by metals in zebrafish (Danio rerio)." Mar. Environ. Res. **91**: 45-51.

 Metals are natural components in ecosystems; however, if these elements are in excess, they can have adverse effects on living organisms. This study analyzes the interference of copper, lead, iron and cadmium in acetylcholinesterase (AChE) and carboxylesterase (CbE) activities in zebrafish. AChE was significantly inhibited in vitro by copper, iron, lead and cadmium at higher concns. (10 and 20 mmol/L), whereas CbE was inhibited only at a concn. of 20 mmol/L. In vivo, only lead and cadmium were able to cause AChE inhibition at higher concns., while iron didn't cause any changes, and copper promoted an increase in AChE activity at a concn. of 0.06 mg/L. CbE activity did not change at any of the times (two and seven days) and concns. tested, except in the case of copper exposure, which resulted in a decrease in CbE activity. Indeed, iodoacetamide treatment didn't changed AChE neither CbE activities, results which indicate that the metal inhibiting effect is probably not due to its biding to thiol groups close the active site of the enzyme. This outcome reveals that metals are important esterase inhibitors in zebrafish, and should be considered in environmental monitoring studies that use esterase inhibition as exposure biomarkers of organophosphate and carbamate pesticides. [on SciFinder(R)]

del Torno-de Roman, L., et al. (2015). "Dual Biosensing Device for the Speciation of Arsenic." Electroanalysis **27**(2): 302-308.

 Acetylcholinesterase (ACh) and acid phosphatase (AcP) have been simultaneously cross-linked onto an array of SPCEs, which was set up by a Ag/AgCl ref. electrode, a carbon counter electrode and two carbon working electrodes. The detection of As(III) and As(V) is based on their enzymic inhibitory effect. Different immobilization conditions, substrates, supporting electrolytes and chronoamperometric exptl. conditions have been studied for the sensitive and selective detection of both species of arsenic in complex matrixes, such as tap water and wine, at once. [on SciFinder(R)]

Dvir, H., et al. (2010). "Acetylcholinesterase: From 3D structure to function." Chem.-Biol. Interact. **187**(1-3): 10-22.

 A review. By rapid hydrolysis of the neurotransmitter, acetylcholine, acetylcholinesterase (AChE) terminates neurotransmission at cholinergic synapses. AChE is a very fast enzyme, functioning at a rate approaching that of a diffusion-controlled reaction. The powerful toxicity of organophosphate poisons is attributed primarily to their potent inhibition of AChE. AChE inhibitors are utilized in the treatment of various neurol. disorders, and are the principal drugs approved thus far by the FDA for management of Alzheimer's disease. Many organophosphates and carbamates serve as potent insecticides, by selectively inhibiting insect AChE. The detn. of the crystal structure of Torpedo californica AChE permitted visualization, for the 1st time, at at. resoln., of a binding pocket for acetylcholine. It also allowed identification of the active site of AChE, which, unexpectedly, is located at the bottom of a deep gorge lined largely by arom. residues. The crystal structure of recombinant human AChE in its apo-state is similar in its overall features to that of the Torpedo enzyme; however, the unique crystal packing reveals a novel peptide sequence which blocks access to the active site gorge. [on SciFinder(R)]

Frasco, M. F., et al. (2007). "Mechanisms of cholinesterase inhibition by inorganic mercury." FEBS J. **274**(7): 1849-1861.

 The poorly known mechanism of inhibition of cholinesterases by inorg. mercury (HgCl2) has been studied with a view to using these enzymes as biomarkers or as biol. components of biosensors to survey polluted areas. The inhibition of a variety of cholinesterases by HgCl2 was investigated by kinetic studies, x-ray crystallog., and dynamic light scattering. Our results show that when a free sensitive sulfhydryl group is present in the enzyme, as in Torpedo californica acetylcholinesterase, inhibition is irreversible and follows pseudo-first-order kinetics that are completed within 1 h in the micromolar range. When the free sulfhydryl group is not sensitive to mercury (Drosophila melanogaster acetylcholinesterase and human butyrylcholinesterase) or is otherwise absent (Electrophorus electricus acetylcholinesterase), then inhibition occurs in the millimolar range. Inhibition follows a slow binding model, with successive binding of two mercury ions to the enzyme surface. Binding of mercury ions has several consequences: reversible inhibition, enzyme denaturation, and protein aggregation, protecting the enzyme from denaturation. Mercury-induced inactivation of cholinesterases is thus a rather complex process. Our results indicate that among the various cholinesterases that we have studied, only Torpedo california acetylcholinesterase is suitable for mercury detection using biosensors, and that a careful study of cholinesterase inhibition in a species is a prerequisite before using it as a biomarker to survey mercury in the environment. [on SciFinder(R)]

Frasco, M. F., et al. (2005). "Do metals inhibit acetylcholinesterase (AChE)? Implementation of assay conditions for the use of AChE activity as a biomarker of metal toxicity." Biomarkers **10**(5): 360-375.

 The enzymic activity of acetylcholinesterase (AChE) has been shown to be altered by environmental contaminants such as metals. However, the available literature illustrates a background of contradictory results regarding these effects. Therefore, the main purpose of this study was to investigate the potential of 5 metal ions (nickel, copper, zinc, cadmium, and mercury) to inhibit AChE activity in vitro. First, to accomplish this objective, the possible interference of metals as test toxicants in the Ellman's assay, which is widely used to assess AChE activity, was studied. The potential influence of 2 different reaction buffers (phosphate and Tris) was also detd. The results suggest that the selected metals react with the products of this photometric technique. It is impossible to ascertain the artifactual contribution of the interaction of the metals with the technique when measuring AChE inhibition. This constitutes a major obstacle in obtaining accurate data. The presence of phosphate ions also makes enzymic inhibition difficult to analyze. Attending to this evidence, an assay using the substrate o-nitrophenyl acetate and Tris buffer was used to investigate the effects of metals on AChE activity. O-nitrophenyl acetate is also a substrate for esterases other than cholinesterases. It is therefore only possible to use it for the measurement of cholinesterase activity with purified enzymes or after a previous verification of the absence of other esterases in the sample tissue. Under these conditions, the results indicate that with the exception of nickel, all tested metals significantly inhibit AChE activity. [on SciFinder(R)]

Froede, H. C. and I. B. Wilson (1985). "The slow rate of inhibition of acetylcholinesterase by fluoride." Mol. Pharmacol. **27**(6): 630-633.

 The rate of reaction of F- with acetylcholinesterase was detd. with a stopped-flow app. for measurements on the millisecond time scale with PhOAc as a chromogenic substrate. The 2nd-order rate const. is 5 × 103 L/mol/s, which is very slow for a small sym. ion; it is 3-4 orders of magnitude smaller than for the substrates acetylcholine, acetylthiocholine, and PhOAc. The slowness of this reaction suggests that F- does not find a preexisting binding site but must create one, probably by breaking and reforming H bonds. With hydrolysis measurements made on the usual time scale, kcat = 7.5 × 105 min-1 and Km = 2.0 mM. Furthermore, F- enhances substrate inhibition, and with low PhOAc concn., the percentage of inhibition is independent of substrate concn. [on SciFinder(R)]

Greenblatt, H. M., et al. (2003). "Acetylcholinesterase. A multifaceted target for structure-based drug design of anticholinesterase agents for the treatment of Alzheimer's disease." J. Mol. Neurosci. **20**(3): 369-383.

 A review. The structure of Torpedo californica acetylcholinesterase is examd. in complex with several inhibitors that are either in use or under development for treating Alzheimer's disease. The noncovalent inhibitors vary greatly in their structures and bind to different sites of the enzyme, offering many different starting points for future drug design. [on SciFinder(R)]

Kakemoto, M., et al. (1991). "Determination of p-hydroxybenzoic acid esters by HPLC using a electrochemical detector." Bunseki Kagaku **40**(2): 83-87.

 A specific and sensitive HPLC method using an electrochem. detector and pretreatment method is described for the detn. of p-hydroxybenzoic acid esters that are used as preservatives in cosmetics. Seven p-hydroxybenzoic acid esters were sepd. using Chemcosorb 5-ODS-H (4.6 × 100 mm) as the stationary phase and 0.2 mmol/dm3 perchloric acid methanol soln.-0.05 mol/dm3 acetic acid soln. (pH 3)-pyridine (75:25:0.5 vol%) as the mobile phase. The order of elution was as follows: Me, Et, iso-Pr, n-Pr, sec-Bu, iso-Bu and n-Bu. The potential of the electrochem. detector was set at +1.2V vs. Ag/AgCl. The linear dynamic range was approx. 103 (10-10-10-7). The min. detectable amt. for the Me ester was found to be 50 pg and 50-150 pg for other esters. The electrochem. detection method was more sensitive than UV detection at 254 nm. This method was applied to detn. of p-hydroxybenzoic acid esters in cosmetics. [on SciFinder(R)]

Kaur, H., et al. (2015). "Advances in arsenic biosensor development - A comprehensive review." Biosens. Bioelectron. **63**: 533-545.

Kemmer, G. and S. Keller (2010). "Nonlinear least-squares data fitting in Excel spreadsheets." Nat. Protoc. **5**(2): 267-281.

 We describe an intuitive and rapid procedure for analyzing exptl. data by nonlinear least-squares fitting (NLSF) in the most widely used spreadsheet program. Exptl. data in x/y form and data calcd. from a regression equation are inputted and plotted in a Microsoft Excel worksheet, and the sum of squared residuals is computed and minimized using the Solver add-in to obtain the set of parameter values that best describes the exptl. data. The confidence of best-fit values is then visualized and assessed in a generally applicable and easily comprehensible way. Every user familiar with the most basic functions of Excel will be able to implement this protocol, without previous experience in data fitting or programming and without addnl. costs for specialist software. The application of this tool is exemplified using the well-known Michaelis-Menten equation characterizing simple enzyme kinetics. Only slight modifications are required to adapt the protocol to virtually any other kind of dataset or regression equation. The entire protocol takes ∼1 h. [on SciFinder(R)]

Kim, R. S. and T. D. Chung (2014). "The electrochemical reaction mechanism and applications of quinones." Bull. Korean Chem. Soc. **35**(11): 3143-3155.

 A review. This tutorial review provides a general account of the electrochem. behavior of quinones and their various applications. Quinone electrochem. has been investigated for a long time due to its complexity. A simple point of view is developed that considers the relative stability of the reduced quinone species and the values of the first and second redn. potentials. The 9-membered square scheme in buffered aq. solns. is explained and semiquinone radical stability is discussed in this context. Quinone redox reaction has also been employed in various studies. Diverse examples are presented under three broad categories defined by the roles of quinone: mol. tool for phys. chem., versatile electron mediator, and charge storage for energy conversion devices. [on SciFinder(R)]

La Rosa, C., et al. (1994). "Determination of organophosphorus and carbamic pesticides with an acetylcholinesterase amperometric biosensor using 4-aminophenyl acetate as substrate." Anal. Chim. Acta **295**(3): 273-282.

 Organophosphorus and carbamic pesticides have been detd. with an amperometric acetylcholinesterase-based 4-aminophenyl acetate biosensor. The glassy carbon enzyme membrane covered electrode poised at +250 mV (vs. sodium chloride SCE) oxidizes the 4-aminophenol formed in the hydrolysis of 4-aminophenyl acetate by acetylcholinesterase in the glutaraldehyde crosslinked layer. The activity of acetylcholinesterase is inhibited in the presence of pesticides. The decrease in activity of the enzyme is monitored by the 4-aminophenyl acetate sensor and is correlated to the concn. of pesticide present in soln. The influence of the acetylcholinesterase loading and the acetylcholinesterase to neutral protein (bovine serum albumin) ratio on the biosensor response was studied and the measuring conditions including pH, substrate concn., and others were optimized. Detection limits of 4.0 and 13.0 nmol L-1 for paraoxon and carbaryl, resp., were achieved with a 3-min preincubation time. [on SciFinder(R)]

Li, Z., et al. (2015). "The art of signal transforming: electrodes and their smart applications in electrochemical sensing." Anal. Methods **7**(23): 9732-9743.

 Electrochem. sensors provide some advantages over optical-based sensors because they can offer comparable instrumental sensitivity and are more amenable to miniaturization. Electrodes are a necessary and crit. component in the design of electrochem. biosensors. Herein, the applications of several novel electrodes are covered in this manuscript, including screen-printed electrodes (SPEs), interdigitated microelectrodes (IDμEs), paper microelectrodes and nanoelectrodes. Microelectrodes and nanoelectrodes are discussed in detail due to their unique properties. Various well-developed and recognized elements were utilized for biosensor construction, as is indicated in this review. Different immobilization methods can be utilized to improve the quality and level of detection. Many nanomaterials, such as carbon nanotubes (CNTs), graphene, metal-org. frameworks (MOFs), and hollow nanoparticles (HNPs), have been used, which have the potential to improve the sensitivity of the electrochem. biosensors. This manuscript highlights the effect of rapid advancement of nanotechnol. and microfluidics and their application to diverse fields of scientific research as well as addnl. applications, challenges, and perspectives. [on SciFinder(R)]

Liu, Z.-G., et al. (2014). "Role of Fe(III) in preventing humic interference during As(III) detection on gold electrode: Spectroscopic and voltammetric evidence." J. Hazard. Mater. **267**: 153-160.

 A drawback of As(III) detection using square wave anodic stripping voltammetry (SWASV) is that it is susceptible to interferences from various metals or org. compds., esp. in real sample water. This study attempts to understand the interference of co-existing of Fe(III) and humic acid (HA) mols. to the electrochem. detection of As(III) using Fourier transform IR (FTIR) spectrum and XPS. The electrochem. expts. include stripping of As(III) in the solns. contg. HA with different concns., cyclic voltammetry in 0.5 M H2SO4 in the presence of HA or Fe(III) with/without addn. of Fe(III) or HA, and stripping of As(III) in the presence of HA or Fe(III) with/without addn. of Fe(III) or HA. FTIR and XPS are employed to confirm the affinity of HA to Fe(III) or As(III) in acidic condition. [on SciFinder(R)]

Liu, Z.-G. and X.-J. Huang (2014). "Voltammetric determination of inorganic arsenic." TrAC, Trends Anal. Chem. **60**: 25-35.

 Arsenic is a notorious poison and one of the world's greatest environmental hazards. Electrochem. techniques hold great promise for detecting or monitoring arsenic because they are highly sensitive, easy to perform, and low cost. We present and discuss the voltammetric detn. of inorg. arsenic. In combination with an effective preconcn. (or deposition) step, voltammetry, as one of the most powerful techniques in electroanal., achieves the sensitive measurement of trace arsenic. On the basis of org. and biol. mols. and inorg. nanomaterials, such as As(III)-specific ligands, enzymes, carbon nanotubes, graphene, and nanoparticles of noble metals (gold, silver and platinum), we comprehensively review electrochem. voltammetry for the detection of inorg. arsenic [As(III) and As(V)]. Also, we show how potential obstacles are overcome using chem.-modified electrodes. Finally, we cover future development and applications based on electrochem. techniques. [on SciFinder(R)]

Luong, J. H. T., et al. (2014). "Recent advances in electrochemical detection of arsenic in drinking and ground waters." Anal. Methods **6**(16): 6157-6169.

 A review. Anodic stripping voltammetry (ASV) using noble electrodes is based on the redn. of As3+ to As0, followed by its stripping or oxidn. to As3+ or As5+ species, the two predominant forms of arsenic in water. The rapid and convenient ASV method can detect As(III) at the low ppb level, but it is susceptible to interferences from various endogenous metals or org. compds. in waters. Electrode surface modification with metallic nanoparticles (NPs), carbonaceous nanomaterials (carbon nanotubes and graphene) and even enzymes (arsenite oxidase) can improve detection sensitivity and selectivity, while circumventing such interferences. All electrochem. methods aim for a detection limit below the World Health Organization guideline value of 10 ppb (133.3 nM). Despite numerous publications in this field during the last ten years with respect to novel electrode materials and electrolytes, reproducibility of electrochem. detection is still problematic and the anal. of arsenic in real ground-water samples is far from certainty and triviality. Considerable efforts are still needed to develop electrode materials and anal. procedures for reliable detection of arsenic with sub-ppb levels in the presence of endogenous toxic metals and orgs. in water matrixes. [on SciFinder(R)]

Ma, J., et al. (2014). "Speciation and detection of arsenic in aqueous samples: A review of recent progress in non-atomic spectrometric methods." Anal. Chim. Acta **831**: 1-23.

 A review is given. Inorg. As displays extreme toxicity and is a class A human carcinogen. It is of interest to both anal. chemists and environmental scientists. Facile and sensitive detn. of As and knowledge of the speciation of forms of As in aq. samples are vitally important. Nearly every nation has relevant official regulations on permissible limits of drinking water As content. The size of the literature on As is therefore formidable. The heart of this review consists of 2 tables: one is a compilation of principal official documents and major review articles, including the toxicol. and chem. of As. This includes comprehensive official compendia on As speciation, sample treatment, recommended procedures for the detn. of As in specific sample matrixes with specific anal. instrument(s), procedures for multi-element (including As) speciation and anal., and prior comprehensive reviews on arsenic anal. The 2nd table focuses on the recent literature (2005-2013, the coverage for 2013 is incomplete) on As measurement in aq. matrixes. Recent As speciation and anal. methods based on spectrometric and electrochem. methods, inductively coupled plasma-mass spectrometry, neutron activation anal. and biosensors are summarized. We deliberately excluded at. optical spectrometric techniques (at. absorption, at. fluorescence, inductively coupled plasma-optical emission spectrometry) not because they are not important (in fact the majority of arsenic detns. are possibly carried out by one of these techniques) but because these methods are sufficiently mature and little meaningful innovation has been made beyond what is in the officially prescribed compendia (which are included) and recent reviews are available. [on SciFinder(R)]

Male, K. B., et al. (2007). "Biosensor for Arsenite Using Arsenite Oxidase and Multiwalled Carbon Nanotube Modified Electrodes." Anal. Chem. (Washington, DC, U. S.) **79**(20): 7831-7837.

 A biosensor for arsenite was developed using Mo-contg. arsenite oxidase, prepd. from the chemolithoautotroph NT-26 that oxidizes arsenite to arsenate. The enzyme was galvanostatically deposited for 10 min at 10 μA onto the active surface of a multiwalled C nanotube modified glassy C electrode. The resulting biosensor enabled direct electron transfer, i.e., effecting redn. and then reoxidization of the enzyme without an artificial electron-transfer mediator. Arsenite was detected within 10 s at an applied potential of 0.3 V with linearity up to 500 ppb and a detection limit of 1 ppb. The biosensor exhibited excellent reproducibility, 2% at 95% confidence interval for 12 repeated analyses of 25 ppb arsenite. Cu, a severe interfering species commonly found in groundwater, did not interfere, and the biosensor was applicable for repeated anal. of spiked arsenite in tap H2O, river water, and a com. mineral H2O. [on SciFinder(R)]

Miao, Y., et al. (2010). "History and New Developments of Assays for Cholinesterase Activity and Inhibition." Chem. Rev. (Washington, DC, U. S.) **110**(9): 5216-5234.

 A review. Cholinesterases (ChEs) are key enzymes in a range of important areas such as neurobiol., toxicol. and pharmacol. Two major groups, acetylcholinesterase (AChE) and butyrylcholinesterase (BCE), play important roles in human and animal function and health. AChE is present mainly in the central nervous system. It is bound to cellular membranes of excitable tissues and involved in neurotransmission. It catalyzes the hydrolysis of neurotransmitter acetylcholine into choline. AChE is found in the membranes of red blood cells, where it is known as erythrocyte ChE. BChE, also known as pseudocholinesterase, plasma or serum ChE, is found in plasma, liver, and muscle tissues. Although this enzyme has a similar mol. structure to AChE, it is characterized by a different but overlapping spectrum of substrate specificities in which AChE preferentially hydrolyzes acetyl esters, whereas BChE hydrolyzes butyrylcholine. Assays for activity and inhibition are applicable to a very broad range fields. These assays can either be obsd. visually or measured by optical and electrochem. techniques or by piezoelec. devices. However, most of these reviews focused on biosensor areas. There is a great gap between traditional assays and newly developed. The more general assays are introduced for detn. of organophosphorus, organochlorine or toxic compd. for food and environmental application. New material science and biomimetic techniques have contributed to the development of assays. The assays appeared in the last 40 years of the 20th century, many new and important developments during the past 20 years are discussed. [on SciFinder(R)]

Migneault, I., et al. (2004). "Glutaraldehyde: Behavior in aqueous solution, reaction with proteins, and application to enzyme crosslinking." BioTechniques **37**(5): 790-796,798-802.

 A review. Glutaraldehyde possesses unique characteristics that render it one of the most effective protein crosslinking reagents. It can be present in at least 13 different forms depending on soln. conditions such as pH, concn., temp., etc. Substantial literature is found concerning the use of glutaraldehyde for protein immobilization, yet there is no agreement about the main reactive species that participates in the crosslinking process because monomeric and polymeric forms are in equil. Glutaraldehyde may react with proteins by several means such as aldol condensation or Michael-type addn., and we show here 8 different reactions for various aq. forms of this reagent. As a result of these discrepancies and the unique characteristics of each enzyme, crosslinking procedures using glutaraldehyde are largely developed through empirical observation. The choice of the enzyme-glutaraldehyde ratio, as well as their final concn., is crit. because insolubilization of the enzyme must result in minimal distortion of its structure in order to retain catalytic activity. The purpose of this paper is to give an overview of glutaraldehyde as a crosslinking reagent by describing its structure and chem. properties in aq. soln. in an attempt to explain its high reactivity toward proteins, particularly as applied to the prodn. of insol. enzymes. [on SciFinder(R)]

Pariente, F., et al. (1993). "4-Aminophenyl acetate as a substrate for amperometric esterase sensors." Anal. Chim. Acta **273**(1-2): 399-407.

 4-Aminophenyl acetate (PAPA) was applied as a new substrate for esterase activities. Acetylcholinesterase from elec. eel (EC 3.1.1.7) was detd. using PAPA as an improved substrate. Acetylcholinesterase was covalently bound to a nylon filter mesh using glutaraldehyde and bovine serum albumin as cross-linkers. The modified membrane was subsequently attached to the surface of a glassy carbon electrode. 4-Aminophenol generated by the enzymic reaction was detected by cyclic voltammetry and amperometry at an activated glassy carbon electrode. A linear current response, proportional to the PAPA concn., over the range 0.1 μM-0.5 mM was obtained. Optimization of various kinetic parameters of the biosensor is discussed. The electrochem. detection of PAP was also used for the detn. of acetylcholinesterase in whole human blood. [on SciFinder(R)]

Pohanka, M. (2011). "Cholinesterases, a target of pharmacology and toxicology." Biomed. Pap. **155**(3): 219-230.

 A review. Background. Cholinesterases are a group of serine hydrolases that split the neurotransmitter acetylcholine (ACh) and terminate its action. Of the two types, butyrylcholinesterase and acetylcholinesterase (AChE), AChE plays the key role in ending cholinergic neurotransmission. Cholinesterase inhibitors are substances, either natural or man-made that interfere with the break-down of ACh and prolong its action. Hence their relevance to toxicol. and pharmacol. Methods and Results. The present review summarizes current knowledge of the cholinesterases and their inhibition. Particular attention is paid to the toxicol. and pharmacol. of cholinesterase-related inhibitors such as nerve agents (e.g. sarin, soman, tabun, VX), pesticides (e.g. paraoxon, parathion, malathion, malaoxon, carbofuran), selected plants and fungal secondary metabolites (e.g. aflatoxins), drugs for Alzheimer's disease (e.g. huperzine, metrifonate, tacrine, donepezil) and Myasthenia gravis (e.g. pyridostigmine) treatment and other compds. (propidium, ethidium, decamethonium). Conclusions. The crucial role of the cholinesterases in neural transmission makes them a primary target of a large no. of cholinesterase-inhibiting drugs and toxins. In pharmacol., this has relevance to the treatment of neurodegenerative disorders. [on SciFinder(R)]

Pohanka, M. (2012). "Acetylcholinesterase inhibitors: a patent review (2008 - present)." Expert Opin. Ther. Pat. **22**(8): 871-886.

 A review. Introduction: Both acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are present in the body in large amts. AChE is an important part of the cholinergic nervous system taking place in the central and peripheral nervous system. AChE is a target of several toxins such as insecticide carbofuran, nerve agents, sarin, soman, tabun and VX. Beside toxins, drugs for treatment of Alzheimer's disease and myasthenia gravis, such as galantamine, donepezil, rivastigmine, tacrine, huperzine, pyridostigmine and neostigmine, are known.Areas covered: The review gives an overview of the importance of the cholinergic nervous system, the biochem. of AChE and the role of AChE inhibitors. Current efforts to introduce potent drugs for Alzheimer's disease therapy and reduce toxicity, while keeping the maximal pharmacol. effect, are also discussed.Expert opinion: The current research effort into AChE inhibitors can be divided into two categories. First, new toxins useful for agricultural purposes and second, novel drugs that need to be prepd., although there is less interest in the new toxins. The research for drugs for Alzheimer's disease needs to focus on inhibitors that reduce the deposition of amyloid plaques, but do not initiate AChE expression. [on SciFinder(R)]

Pundir, C. S. and N. Chauhan (2012). "Acetylcholinesterase inhibition-based biosensors for pesticide determination: A review." Anal. Biochem. **429**(1): 19-31.

 A review. Pesticides released intentionally into the environment and through various processes contaminate the environment. Although pesticides are assocd. with many health hazards, there is a lack of monitoring of these contaminants. Traditional chromatog. methods-high-performance liq. chromatog., capillary electrophoresis, and mass spectrometry-are effective for the anal. of pesticides in the environment but have certain limitations such as complexity, time-consuming sample prepn., and the requirement of expensive app. and trained persons to operate. Over the past decades, acetylcholinesterase (AChE) inhibition-based biosensors have emerged as simple, rapid, and ultra-sensitive tools for pesticide anal. in environmental monitoring, food safety, and quality control. These biosensors have the potential to complement or replace the classical anal. methods by simplifying or eliminating sample prepn. and making field-testing easier and faster with significant decrease in cost per anal. This article reviews the recent developments in AChE inhibition-based biosensors, which include various immobilization methods, different strategies for biosensor construction, the advantages and roles of various matrixes used, anal. performance, and application methods for constructing AChE biosensors. These AChE biosensors exhibited detection limits and linearity in the ranges of 1.0 × 10-11 to 42.19 μM (detection limits) and 1.0 × 10-11-1.0 × 10-2 to 74.5-9.9 × 103 μM (linearity). These biosensors were stable for a period of 2 to 120 days. The future prospects for the development of better AChE biosensing systems are also discussed. [on SciFinder(R)]

Quan, M., et al. (2007). "Voltammetry of Quinones in Unbuffered Aqueous Solution: Reassessing the Roles of Proton Transfer and Hydrogen Bonding in the Aqueous Electrochemistry of Quinones." J. Am. Chem. Soc. **129**(42): 12847-12856.

 Cyclic voltammetry studies are reported for 2 representative quinones, benzoquinone and 2-anthraquinonesulfonate, in buffered and unbuffered aq. soln. at different pH's. While the redox reaction of quinones in buffered H2O is well described as an overall 2 e-, 2 H+ redn. to make the hydroquinone, a much better description of the overall reaction in unbuffered H2O is as a 2 e- redn. to make the strongly H-bonded quinone dianion, which will exist in H2O as an equil. mixt. of protonation states. This description helps to unify quinone electrochem. by bridging the apparent gap between the redox chem. of quinones in H2O and that in aprotic org. solvents, where quinones undergo 2 sequential 1 e- redns. to form the quinone dianion. [on SciFinder(R)]

Quinn, D. M. (1987). "Acetylcholinesterase: enzyme structure, reaction dynamics, and virtual transition states." Chem. Rev. **87**(5): 955-979.

 A review with 140 refs. of the sequence, conformation, quaternary structure, active site structure, reaction dynamics, and transition state structures of acetylcholinesterase. [on SciFinder(R)]

Sanllorente-Mendez, S., et al. (2010). "Immobilization of acetylcholinesterase on screen-printed electrodes. Application to the determination of arsenic(III)." Sensors **10**: 2119-2128.

 Enzymic amperometric procedures for measuring As, based on the inhibitive action of this metal on acetylcholinesterase enzyme activity, were developed. Screen-printed C electrodes (SPCEs) were used with acetylcholinesterase covalently bonded directly to its surface. The amperometric response of acetylcholinesterase was affected by the presence of As ions, which caused a decrease in the current intensity. The exptl. optimum working conditions of pH, substrate concn. and potential applied, were established. Under these conditions, repeatability and reproducibility of biosensors were detd., reaching values <4% in terms of relative std. deviation. The detection limit obtained for As was 1.1 × 10-8 M for Ach/SPCE biosensor. Anal. of the possible effect of the presence of foreign ions in the soln. was performed. The method was applied to det. levels of As in spiked tap H2O samples. [on SciFinder(R)]

Sanllorente-Mendez, S., et al. (2012). "Development of acid phosphatase based amperometric biosensors for the inhibitive determination of As(V)." Talanta **93**: 301-306.

 An enzymic amperometric procedure for the direct measurement of As(V) in the presence of As(III) was developed. The method is based on the inhibitive action of this species on acid phosphatase enzyme (AcP) activity. Screen-printed carbon electrodes (SPCEs) were used as support for the crosslinking immobilization of the enzyme AcP. 2-Phospho-L-ascorbic acid was used as a novel substrate, in arsenic detn., which amperometric response decreased by the presence of As(V) ions. The optimum working conditions were found using exptl. design methodol. Under these conditions, repeatability and reproducibility of the constructed biosensors were detd., reaching values below 8% in terms of RSD. The capability of detection obtained for As(V) was 0.11 μM for AcP/SPCE biosensors. Anal. of the possible effect of the presence of foreign ions in the soln. was performed. The method was successfully applied to the detn. of the As(V) content in a groundwater sample. [on SciFinder(R)]

Sassolas, A., et al. (2012). "Immobilization strategies to develop enzymatic biosensors." Biotechnol. Adv. **30**(3): 489-511.

 A review. Immobilization of enzymes on the transducer surface is a necessary and crit. step in the design of biosensors. An overview of the different immobilization techniques reported in the literature is given, dealing with classical adsorption, covalent bonds, entrapment, crosslinking or affinity as well as combination of them and focusing on new original methods as well as the recent introduction of promising nanomaterials such as conducting polymer nanowires, carbon nanotubes or nanoparticles. As indicated in this review, various immobilization methods have been used to develop optical, electrochem. or gravimetric enzymic biosensors. The choice of the immobilization method is shown to represent an important parameter that affects biosensor performances, mainly in terms of sensitivity, selectivity and stability, by influencing enzyme orientation, loading, mobility, stability, structure and biol. activity. [on SciFinder(R)]

Schulze, H., et al. (2005). "Insecticide detection through protein engineering of Nippostrongylus brasiliensis acetylcholinesterase B." Anal. Chem. **77**(18): 5823-5830.

 The sensitivity of acetylcholinesterase (AChE) biosensors for insecticide detection could be increased substantially by engineering AChE B of Nippostrongylus brasiliensis. The introduction of 10 single and 4 double mutations into the AChE peptide chain led to an increase in sensitivity to 10 of the 11 insecticides tested. The combination of 3 mutants with the wild-type enzyme in a multienzyme biosensor array enabled the detection of 11 out of the 14 most important organophosphates and carbamates at concns. below 10 μg/kg, the max. residue limit of infant food. The detection limit for pirimiphos Me could be reduced from 10 μg/L to a value as low as 1 ng/L (3.5 × 10-12 mol/L). The newly created biosensors exhibited an extraordinary high storage stability. There was no loss of sensitivity of N. There was no loss of sensitivity of N. brasiliensis AChE B, immobilized on screen-printed, disposable electrodes, even after 17-mo storage at room temp. [on SciFinder(R)]

Silman, I. and J. L. Sussman (2008). "Acetylcholinesterase: How is structure related to function?" Chem.-Biol. Interact. **175**(1-3): 3-10.

 A review. In accordance with its biol. role, termination of neurotransmission at cholinergic synapses by rapid hydrolysis of the neurotransmitter, acetylcholine, acetylcholinesterase (AChE) is one of nature's most efficient enzymes. The soln. of its 3-dimensional structure has revealed that its active site is located at the bottom of a deep and narrow gorge. Such an architecture was unanticipated in view of its high turnover no. The present review examines how the highly specialized structure of AChE, with its sequestered active site, contributes to its catalytic efficacy, and discusses how the traffic of substrate and products to and from the active site is controlled. [on SciFinder(R)]

Songa, E. A. and J. O. Okonkwo (2016). "Recent approaches to improving selectivity and sensitivity of enzyme-based biosensors for organophosphorus pesticides: A review." Talanta **155**: 289-304.

 A review. Pesticide detn. has attracted great attention due to the fact that they exhibit high acute toxicity and can cause long-term damage to the environment and human lives even at trace levels. Although classical anal. methods (including gas chromatog., high performance liq. chromatog., capillary electrophoresis and mass spectrometry) have been effectively used for anal. of pesticides in contaminated samples, they present certain limitations such as time-consuming sample prepn., complexity, and the requirement of expensive instrumentation and highly skilled personnel. For these reasons, there is an expanding need for anal. methods able to provide simple, rapid, sensitive, selective, low cost and reliable detection of pesticides at trace levels. Over the past decades, acetylcholinesterase (AChE) biosensors have emerged as simple, rapid and ultra-sensitive tools for toxicity detection of pesticides in the environment and food. These biosensors have the potential to complement or replace the classical anal. methods by simplifying or eliminating sample prepn. and making field-testing easier and faster with significant decrease in cost per anal. With the recent engineering of more sensitive AChE enzymes, the development of more reliable immobilization matrixes and the progress in the area of microelectronics, AChE biosensors could become competitive for multi-analyte screening and soon be used for the development of portable instrumentation for rapid toxicity testing of samples. The enzymes organophosphorus hydrolase (OPH) and organophosphorus acid anhydrolase (OPAA) have also shown considerable potential in OP biosensor applications and they have been used for direct detection of OPs. This review presents the recent advances in the fabrication of enzyme biosensors for organophosphorus pesticides (OPs) and their possible applications for toxicity monitoring of organophosphorus pesticide residues in real samples. The focus will be on the different strategies for the biosensor construction, the anal. performance of the biosensors and the advantages and disadvantages of these biosensor methods. The recent works done to improve the anal. performance, sensitivity and selectivity of these biosensors will also be discussed. [on SciFinder(R)]

Stepankova, S. and K. Vorcakova (2016). "Cholinesterase-based biosensors." J. Enzyme Inhib. Med. Chem. **31**(sup3): 180-193.

 A review. Recently, cholinesterase-based biosensors are widely used for assaying anticholinergic compds. Primarily biosensors based on enzyme inhibition are useful anal. tools for fast screening of inhibitors, such as organophosphates and carbamates. The present review is aimed at compilation of the most important facts about cholinesterase based biosensors, types of physico-chem. transduction, immobilization strategies and practical applications. [on SciFinder(R)]

Stoytcheva, M., et al. (1998). "Electrochemical approach in studying the inactivation of immobilized acetylcholinesterase by arsenate(III)." Electroanalysis **10**(14): 994-998.

 An acetylcholinesterase amperometric sensor is used to register the current of oxidn. of thiocholine, the product of the acetylcholinesterase catalyzed hydrolysis of acetylthiocholine. The decrease of the value of the signal obtained in presence of arsenate(III) is interpreted as due to the diminution of the quantity of the generated thiocholine because of the inhibition of the immobilized acetylcholinesterase on the surface of a graphite electrode. The expts. were performed varying the concns. of the inhibitor and of the substrate, as well as pH. The kinetics of the inactivation of the immobilized acetylcholinesterase by arsenate(III) was investigated. Obtained results could be employed for the quant. detn. of AsO33- based on the inhibition of the immobilized acetylcholinesterase on the surface of a graphite electrode, using an electrochem. approach. [on SciFinder(R)]

Stoytcheva, M., et al. (1998). "Electrochemical approach in studying the inhibition of acetylcholinesterase by arsenate(III): analytical characterization and application for arsenic determination." Anal. Chim. Acta **364**(1-3): 195-201.

 The inhibition action of arsenate(III) on the activity of the enzyme acetylcholinesterase immobilized on the surface of a graphite electrode was studied, using an electrochem. approach. The value of the registered amperometric signal depends on the AsO33- concn. Parameters useful for the quant. arsenate(III) detn. (dynamic range, detection limit, sensitivity) are evaluated. PH dependencies and kinetic phenomena are discussed. A distinction between As(III) and As(V) species is made. The method is applied for estg. the As contents in industrial waters after Cu2+ and Fe3+ elimination as hydroxides. [on SciFinder(R)]

Taylor, P., et al. (1995). "Structural bases for the specificity of cholinesterase catalysis and inhibition." Toxicol. Lett. **82/83**(1-6): 453-458.

 A review, with 20 refs. The availability of a crystal structure and comparative sequences of the cholinesterases has provided templates suitable for analyzing the mol. bases of specificity of reversible inhibitors, carbamoylating agents and organophosphates. Site-specific mutagenesis enables one to modify the structures of both the binding site and peptide ligand as well as create chimeras reflecting one type of esterase substituted in the template of another. Herein we define the bases for substrate specificity of carboxylesters, the stereospecificity of enantiomeric alkylphosphonates and the selectivity of tricyclic arom. compds. in the active center of cholinesterase. We also describe the binding loci of the peripheral site and changes in catalytic parameters induced by peripheral site ligands, using the peptide fasciculin. [on SciFinder(R)]

Walt, D. R. and V. I. Agayn (1994). "The chemistry of enzyme and protein immobilization with glutaraldehyde." TrAC, Trends Anal. Chem. **13**(10): 425-430.

 A review, with 33 refs. Immobilization of proteins to solid matrixes has been performed for the last thirty years and has provided numerous examples of successful prepns. with use in enzyme reactors, sensor prepn. and immunodiagnostics. Among the arsenal of coupling reagents and procedures, glutaraldehyde plays a crit. role due to its reliability and ease of use. It displays a complex chem. that is transparent to most practitioners of immobilization. In this article we detail the structure and reactivity of glutaraldehyde protein immobilization. [on SciFinder(R)]

Wang, T., et al. (2016). "Laccase Inhibition by Arsenite/Arsenate: Determination of Inhibition Mechanism and Preliminary Application to a Self-Powered Biosensor." Anal. Chem. (Washington, DC, U. S.) **88**(6): 3243-3248.

 The reversible inhibition of laccase by arsenite (As3+) and arsenate (As5+) is reported for the first time. Oxygen-reducing laccase bioelectrodes were found to be inhibited by both arsenic species for direct electron-transfer bioelectrodes (using anthracene functionalities for enzymic orientation) and for mediated electron-transfer bioelectrodes [using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) as an electron mediator]. Both arsenic species were detd. to behave via a mixed inhibition model (behaving closely to that of uncompetitive inhibitors) when evaluated spectrophotometrically using ABTS as the electron donor. Finally, laccase bioelectrodes were employed within an enzymic fuel cell, yielding a self-powered biosensor for arsenite and arsenate. This conceptual self-powered arsenic biosensor demonstrated limits of detection (LODs) of 13 μM for arsenite and 132 μM for arsenate. Further, this device possessed sensitivities of 0.91 ± 0.07 mV/mM for arsenite and 0.98 ± 0.02 mV/mM for arsenate. [on SciFinder(R)]

Wang, Z., et al. (1999). "Spectroelectrochemistry for a coupled chemical reaction in the channel cell Part II. Kinetics of hydrolysis and the absorption spectrum of p-benzoquinoneimine." J. Electroanal. Chem. **464**(2): 181-186.

 The kinetics of the hydrolysis of p-benzoquinoneimine (BQI) as an intermediate of the electrooxidn. of p-aminophenol (PAP) was studied in a spectroelectrochem. channel flow cell. The electrooxidn. of p-aminophenol (PAP) as a typical EC reaction was applied in the development of the channel-type spectroelectrochem. cell designed for conducting in situ transmission UV-visible spectroscopic measurements. Monitoring the change of exptl. spectra as a function of flow rates and considering the contribution of all species in the reaction (PAP, BQI, benzoquinone (BQ)) to spectra, a method is presented to resolve composite exptl. spectra, using the theory developed in the previous paper (W., et al (1999)), and to det. the rate const. of BQI hydrolysis and the molar absorptivity of BQI. [on SciFinder(R)]

Wilson, I. B. and I. Silman (1977). "Effects of quaternary ligands on the inhibition of acetylcholinesterase by arsenite." Biochemistry **16**(12): 2701-2708.

 Arsenite inhibits acetylcholinesterase in a 2nd-order reaction. The rate and equil. consts. depend on pH and have values on the order of 102M-1 min-1 and 10-5M (dissocn.), resp. Some quaternary ammoniumligands completely block the arsenite inhibition of the enzyme, others decrease the rate of the reaction and some, notably pyridine-2-aldoxime methiodide (2-PAM), greatly accelerate the rate of the reaction. Accelerators may bind at a sep. enzyme site distinct from the anionic site involved in substrate binding. Although the kinetic data are consistent with a covalent reaction between arsenite and acetylcholinesterase, chem. evidence excludes the involvement of SH groups which are usually implicated in arsenite inhibition. [on SciFinder(R)]

Wine, Y., et al. (2007). "Elucidation of the mechanism and end products of glutaraldehyde crosslinking reaction by X-ray structure analysis." Biotechnol. Bioeng. **98**(3): 711-718.

 Glutaraldehyde has been used for several decades as an effective crosslinking agent for many applications including sample fixation for microscopy, enzyme and cell immobilization, and stabilization of protein crystals. Despite of its common use as a crosslinking agent, the mechanism and chem. involved in glutaraldehyde crosslinking reaction is not yet fully understood. Here we describe feasibility study and results obtained from a new approach to investigate the process of protein crystals stabilization by glutaraldehyde crosslinking. It involves exposure of a model protein crystal (Lysozyme) to glutaraldehyde in alk. or acidic pH for different incubation periods and reaction arrest by medium exchange with crystn. medium to remove unbound glutaraldehyde. The crystals were subsequently incubated in dild. buffer affecting dissoln. of un-crosslinked crystals. Samples from the resulting soln. were subjected to protein compn. anal. by gel electrophoresis and mass spectroscopy while crosslinked, dissoln. resistant crystals were subjected to high resoln. X-ray structural anal. Data from gel electrophoresis indicated that the crosslinking process starts at specific preferable crosslinking site by lysozyme dimer formation, for both acidic and alk. pH values. These dimer formations were followed by trimer and tetramer formations leading eventually to dissoln. resistant crystals. The crosslinking initiation site and the end products obtained from glutaraldehyde crosslinking in both pH ranges resulted from reactions between lysine residues of neighboring protein mols. and the polymeric form of glutaraldehyde. Reaction rate was much faster at alk. pH. Different reaction end products, indicating different reaction mechanisms, were identified for crosslinking taking place under alk. or acidic conditions. [on SciFinder(R)]

Woolson, E. A., et al. (1977). Medical and Biologic Effects of Environmental Pollutants: Arsenic, National Academy of Science.

Yang, M., et al. (2016). "Electrochemical determination of arsenic(III) with ultra-high anti-interference performance using Au-Cu bimetallic nanoparticles." Sens. Actuators, B **231**: 70-78.

 Bimetallic nanoparticles provide a new opportunity for enhancing electrocatalytic activity because of some possible synergetic effects. In this study, different compns. of Au-Cu bimetallic nanoparticles are prepd. via a simple hydrothermal method. The structure of Au-Cu bimetallic nanoparticles is characterized by using X-ray absorption fine structure (XAFS) techniques, X-ray diffraction (XRD) and XPS. The correlations between electrochem. performance and the compns. of Au-Cu bimetallic nanoparticles on the detn. of arsenic(III) are investigated through square wave anodic stripping voltammetry (SWASV). It is found that the amt. of copper (Cu) in Au-Cu bimetallic nanoparticles is crit. to the detection of arsenic(III). The XAFS results indicated that the Au-Au bond length (RAu-Au) can be influenced by the Cu concn. in the materials. Different RAu-Au of Au-Cu nanoparticles lead to the different electrochem. catalytic activity toward arsenic(III), further revealing the different electrochem. behavior. Compared with Au nanoparticles and com. Au electrode, the Au-Cu bimetallic nanoparticles showed enhanced electrochem. performance with high sensitivity at ppb level, low detection limit. Moreover, the Au89Cu11 bimetallic nanoparticles exhibited ultra-high anti-interference performance on the detection of arsenic(III). [on SciFinder(R)]