Enzyme Electrodes for Medical Sensors

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Abstract: Enzyme electrodes for biosensors are discussed. Different methods to increase electron transfer between enzymes and electrodes are described. Results of encasing the enzymes in conducting polymers as well as in hydrogels are presented. The environment of the enzyme in the biosensor is compared to the natural environment of enzymes.

Keywords: Enzyme electrodes, electrochemistry, electron transfer, encapsulation, hydrogel, conducting polymers, cytosol, cytoplasm.

I) INTRODUCTION

Amperometric sensors have been used to sense or measure a chemical change in the human body or in the environment. These sensors convert a chemical signal into electricity and measure the change in current upon the change of signal. For a medical sensor the signal is usually electron transfer during an enzymatic reaction. This is measured by electrodes, which have the redox-active enzymes attached by direct either physical or chemical linkage. The linkage can also involve mediators, often coenzymes. Environmental factors affect the activity of the attached enzymes, changing therefore the measured electrical current. The most common biosensors detect glucose, lactate, and pyruvate. Glucose is important for the diagnosis of diabetes, lactate for respiratory deficiencies, and pyruvate for food quality.

This review focuses on the development of enzyme electrodes for biosensors. It discusses different enzyme attachments methods, and methods to increase the sensitivity of biosensors by optimizing electron transfer between the enzymes and the electrodes. The review also describes the encasing of the enzymes in conducting polymers, hydrogels, and the electrode itself. A recent review mentions some of the materials used for encasing enzymes onto and in electrodes [1]. Advances in enzyme electrodes used in organic solvents have been discussed in a recent review [2], but these electrodes are not used for medical applications and are not discussed here.

Sensitivity, detection limits, storage stability or shelf life, and operational stability of enzyme electrodes have been the limiting factors in the development of amperometric medical sensors. There has been considerable research conducted to increase the storage and operational stability of enzyme electrodes and this research is a focus of this review. The instability of enzymes is due to several factors: unfolding or denaturation, (e.g. by heat, pH, organic solvent, incompatible surface), loss of a cofactor, protein aggregation, irreversible inhibition (e.g. by binding of a small molecule), hydrolysis, presence of proteolytic enzymes or microorganisms, and chemical reactions (e.g. oxidation, reduction, nucleophilic substitution) [3]. Shelf lives up to one year have now been achieved [3]. In this review the environment of the enzymes in enzyme electrodes is contrasted with the environment of enzymes in cells; also the effect of the environment on storage and operational stability is discussed.

II) NATURAL ENVIRONMENT OF ENZYMES

Enzymes either reside in the cell membrane, the cell nucleus and mitochondrial matrix, or the cytoplasm. Enzymes in the cell membrane are in a hydrophobic alkyl environment, with at least one end projecting into the hydrophilic cytoplasm. One example of such an enzyme is cytochrome c oxidase, the final enzyme of the electron transfer chain in mitochondria.

The majority of the enzymes reside in the cytoplasm (prokaryotes) or cytosol (eukaryotes). Very little is known about the exact composition and structure of either cytoplasm or cytosol. In one standard biochemistry textbook it is written "the prokaryotic cytoplasm (cell contents) is by no means a homogeneous soup" [4]. In another textbook cytoplasm is described as "a fluid material containing many dissolved substances as well as sub-microscopic particles" [5]. The amount of proteins in the prokaryotic *E. coli* cell has been estimated [6]: a cell about 2.95 μm long and 0.64 μm wide contains about one million polypeptide chains in solution/suspension in the cytoplasm. The salt concentration in a typical cell is estimated using a theoretical model as 0.14 M [7]. This estimate does not include the high concentration of other small solute molecules. The composition of a buffer that "mimics the cytosol" is given as 150 mM sorbitol, 70 mM potassium gluconate, 5 mM $MgCl_2$, 35 mM HEPES, and 0.1 mM EGTA at pH 7.55 [8]. No explanation is given in this reference as to why this specific composition was chosen other than that it increased the reaction rate of glycolysis.

It is known that enzyme reactions depend on the concentration of enzyme as well as the substrate. Many detailed kinetic studies have been carried out, usually to determine the mechanism of various enzyme catalysts. Usually these studies are carried out in dilute solutions of enzyme and substrate so as to be able to describe the kinetic data with Michaelis-Menten kinetics and to exclude effects of concentrated substrates on the three-dimensional structure and therefore the activity of the enzyme. One study, though, uses a concentrated sucrose solution to determine the effect

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Table 1. Attachment of Enzymes to Various Electrode Surfaces

Enzyme	Analyte	Mediator, SAM, crosslinking agent	Electrode	Detection Limit (M)	Storage Stability	Ref #
Enzymes physically absorbed on bare electrodes						
Cyt c reductase	NADH (**2**)		Gold		10% after 5d	[37]
AmOx	histamine		Graphite	2.7×10^{-6}		[38]
Enzymes covalently attached or crosslinked						
HRP	H_2O_2	MPA	Gold			[41]
HRP	H_2O_2	MPA	Gold	58×10^{-6}	87% after 14d	[42]
PutOx	Putrescine (**7**)	Glutaraldehyde (**4**), BSA	Gold	3×10^{-8}		[39]
AChE	ACh	Glutaraldehyde (**4**)	Sb	1×10^{-9}		[36]
Oxalox	Oxalate	Glutaraldehyde (**4**), BSA	Graphite, CrHCF	2.5×10^{-6}	>50% after 1 month	[40]
Electron transfer between enzyme and electrode via mediator						
HRP	NO	Quinone (**8**)	Glassy carbon	2.0×10^{-6}	70% after 1d	[54]
HRP	H_2O_2	Thionine (**5**), glutaraldehyde (**4**)	Glassy carbon	1.0×10^{-7}	70% after 1 month	[52]
ADH	EtOH	PQQ (**1**)	Carbon	5×10^{-6}		[55]
ADH	EtOH	PQQ (**1**), dialkyl sulfide, PPy	Gold		8d	[56]
CDH	Cellulobiose	MAP, FAD (**3**), heme	Gold	25×10^{-6}		[57]
GOx	Glucose	Aminoethylthiol, PQQ (**1**), FAD (**3**)	Gold			[50]
CYP101	camphor	Pdx^r	Tin oxide (Sb doped)		5hr (2,600 turnover)	[51]
Tyrosinase	Phenolic compounds	Morpho CDI (**9**)	RVC	2×10^{-6} µg/l	20d	[58]
NitRed	NO_3^-	MPA, Microperoxidase-11	Gold		>1hr	[59]
SAM and mediator						
GOx	Glucose	Alkanethiol, MPA, TTF (**6**)	Gold	3.5×10^{-6}	5d	[53]
Uricase	Uric acid	Alkanethiolate, POPC, MMP (**10**)	Gold		2hrs	[60]

of the amount of water on the stability of invertase [9], carefully excluding different mass transport properties due to the increased viscosity of the solution. It was observed that free water activity-increasing agents, such as the sugar sorbitol, increase enzyme stability in that case.

How much free water there is in a cell has been intensely debated. Estimates range from all water behaving like bulk water [10] to all water being structured [11]. Viscosities of the cytoplasm of different cells were given as 210 ± 140 Pa in J774 macrophages measured by microrheometer [12], 20-30 Pa in erythrocytes measured by electron spin resonance [13], and 4 Pa in mitochondria measured by ^{1}H NMR [14]. Explanations for the existence of structured water and higher viscosity than bulk water include the high concentration of ions [15-21], the interaction of water with proteins (all proteins or only cytoskeleton ones) [22-31], or simply the confinement of water in small spaces [32-35].

Little is known about the actual composition, structure, and viscosity of cytoplasm or cytosol, but it is probably a viscous liquid with a high concentration of dissolved ions and other solutes, that exhibits some structure, bordering membranes, proteins, and the cell skeleton. The exact composition is expected to be dependent on the cell type and growth state, and is also going to be different in various compartments of each cell. The inside of a cell might also be considered similar to a colloidal suspension, with the cell organelles as well as protein aggregates acting as colloidal particles.

III) ATTACHMENT OF ENZYMES TO VARIOUS ELECTRODE SURFACES

In general, it was found that the physical adsorption of enzymes onto metal electrodes leads to denaturing of the enzyme (e.g. ref. [36, 37]). The electron transfer rate is often not efficient either (e.g. ref. [38]). The enzyme also detaches from the electrode, which could be prevented by either crosslinking the enzyme [36, 39, 40] or covalently attaching it to the electrode [41, 42]. Therefore, there are two major

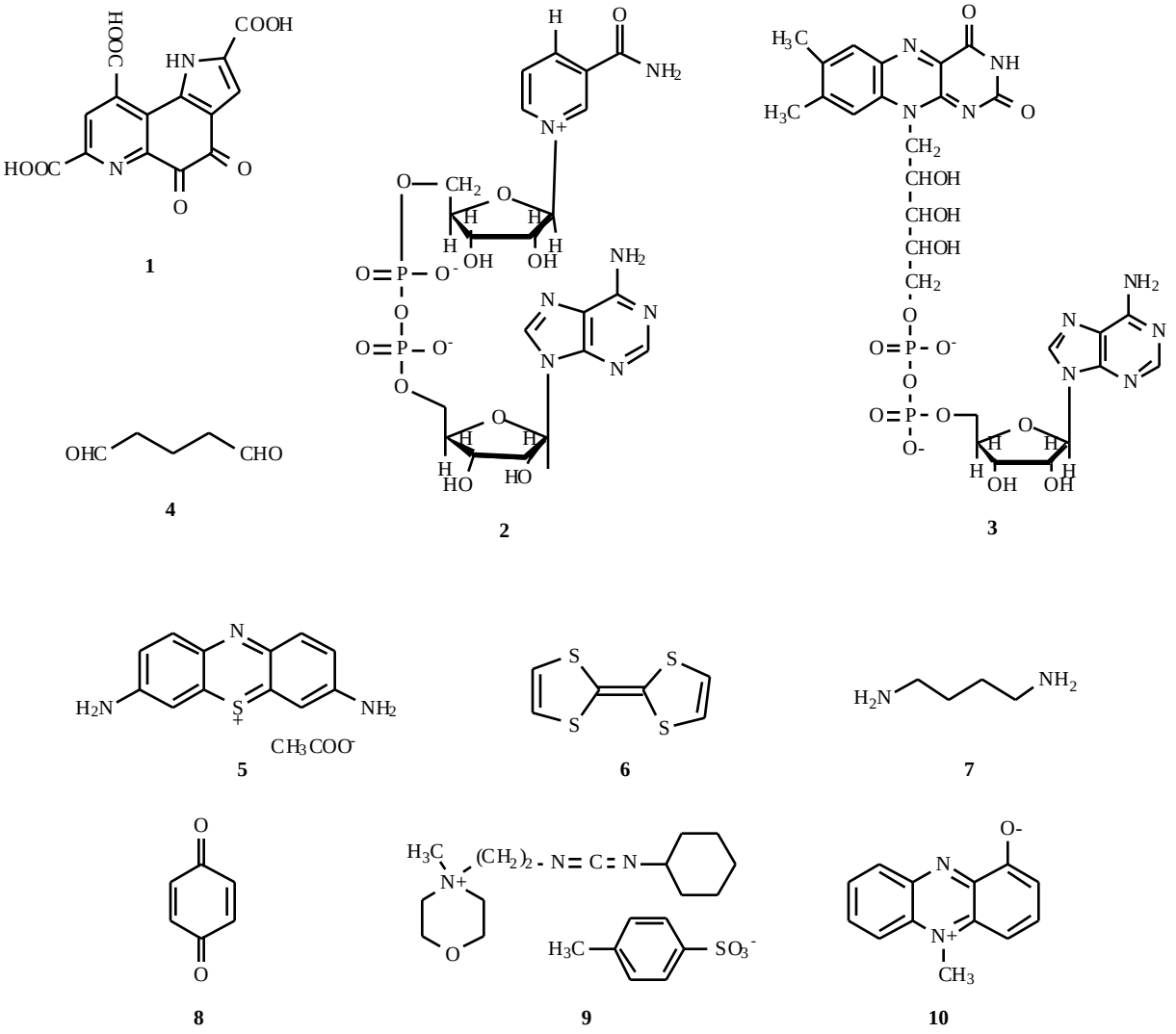

Fig. (1). Structures of compounds mentioned in (Table 1).

problems that need to be solved in the design of enzyme electrodes: the stability of the enzyme in the environment at the electrode needs to be improved, and electron transfer from the enzyme to the electrode needs to be increased via a mediator. The major strategies to deal with these two problems have been to encase the enzyme in a material that is supposed to stabilize the enzyme's structure and function, and to provide redox active compounds as mediators between the enzyme and the electrode.

The first method used to stabilize enzymes on electrodes involved self-assembled monolayers (SAM) of alkyl thiols to generate surfaces mimicking lipid bilayers. This is expected to provide the enzyme with an environment more similar to the environment in a cell, since cells have many cell organelles all surrounded by membranes. The techniques of SAM formation on electrodes [43] and their application in biosensors [41, 44] have been reviewed. The electron transfer rate depends on the length of the alkane thiol [45] and the exact environment of the enzyme [46]. The data shown in (Table **2)** seems to indicate that SAM, at least as used in combination with electron transfer mediators, stabilizes the enzymes, since electron transfer measurements after hours and days are repeatable. In fact, in some cases, electron transfer seems to peak after a few days only [47]. This is an indication that cell-like environments stabilize enzymes on electrodes. Detection limits and storage stabilities for these cases have not been reported.

Electron transfer has been studied in detail for lactate dehydrogenase (LDH) [48, 49]. Electron transfer between the gold electrode and the redox enzyme is mediated in this case by pyrroloquinoline quinone (PQQ, ***1***) and nicotineamide dinucleotide (NAD^+, ***2***), the redox cofactor of the enzymes. Very efficient electron transfer was found with the careful building of the functionalized monolayer. A similar system was designed for glucose oxidase (GOx) with flavin adenine dinucleotide (FAD, ***3***) [50]. A high electron-transferturnover rate (number of substrate molecules reacted per enzyme per unit time) of 900 ± 150 s^{-1} has been achieved. The electron transfer rate does depend on the enzyme and its environment. The transfer rate for CYP101 was 0.017 s^{-1} [51], and for HRP with glutaraldehyde(***4***) and thionine(***5***) as mediators 0.097 s^{-1} [52] with less control over the three-dimensional arrangement of the enzyme on the electrode.

Mediators have also been combined with SAM as enzyme stabilizers: an electron transfer rate of 1.25 s^{-1} has

Table 2. Enzymes Encased by Sol-Gel Methods or Encased into Carbon Paste

Enzyme	Analyte	Enzyme Encased in (Method), Additive, Mediator	Electrode	Detection Limit (M)	Operational Stability	Ref #
Enzymes encased by sol-gel method.						
GOx	Glucose	Alumina (sol-gel), BSA, PPh layer	Aluminum	1.4×10^{-8}	> 60d	[64]
GOx	Glucose	Silica (sol-gel)	Silica		hours	[63]
LOx	Lactate	Silica (sol-gel)	Silica		minutes	[63]
GlyOx	Glycolate	Silica (sol-gel)	Silica		minutes	[63]
HRP	H_2O_2	Ormosil (sol-gel)	Silicate	1.0×10^{-7}	3 months	[69]
Enzymes mixed into carbon paste						
GOx	Glucose	Carbon paste, PDMS, DMF	Carbon	3×10^{-3}	15 hrs	[67]
Tyrosinase	Phenolic Compounds	Graphite, teflon	Carbon	6.7×10^{-5} to 3.1×10^{-8}	30d	[70]
Tyrosinase	Phenolic Compounds	Graphite-EPD	Carbon	8.0×10^{-6} to 5.4×10^{-9}	5d	[70]

been achieved with glucose oxidase (GOx) and tetrathiafulvalene (TTF, ***6***) as the mediator [53].

IV) ELECTRODES WITH ENCASED ENZYMES

Since it is assumed that denaturation occurs via the change of the enzyme's three-dimensional structure due to interactions with the electrode, another method used for the stabilization of enzymes on electrodes is to encase the enzyme into various materials. This also prevents the destruction of the enzyme by peptidases present in the body.

The enzyme can be encased into the electrode itself by sol-gel methods or by mixing it into the carbon paste of carbon paste electrodes (summarized in Table **2**). Since some of these methods use harsh conditions that often destroy enzyme activity, the more common method is to encase the enzyme in a polymer, which is placed on top of the electrode (summarized in Table **3**).

Few sol-gel methods for biosensors have been developed and recently reviewed [61]. Not only the acidity of the sol-gel preparation has to be controlled but also the porosity of the finished electrode [62]. In general, the encasing of the enzyme into the electrode via sol-gel methods seems to increase the enzyme electrode stability up to 200 times (from minutes to usually hours, sometimes months) [63, 64].

Oxidase biosensors commonly have the problem that they are sensitive to the amount of oxygen present, which makes their use in the body difficult. Carbon paste electrodes with oxidases incorporated into the carbon paste generally increase the stability of the oxidases in the presence of oxygen [65-68]. Carbon paste is more similar to the natural environment of an enzyme than silica or alumina. The operational stability of carbon electrodes is longer than the ones reported for silica. In the case of the alumina electrode in ref. [64], which has very good operational stability, the authors tried to reduce the effect of the non-biological metal environment by introducing the protein bovine serum albumin (BSA) into the sol-gel mixture. Similarly, the organically modified glass (ormosil, silica sol-gel modified with PEG) reaches an operational stability of three months [69].

The polymers used for encasing enzymes on top of electrodes are either conducting polymers, to ensure electron transfer from the enzyme to the electrode, or hydrogel polymers, which are expected to stabilize the three-dimensional structure of the enzyme by allowing for a sufficient amount of water to be present and by mimicking cytoplasm or cytosol properties. Sometimes, conducting particles are placed into hydrogels to maximize both electron transfer and enzyme stability. The best analyte detection limit has been achieved for a sol-gel enzyme electrode that immobilizes the protein BSA with the enzyme [64].

To decrease the oxygen sensitivity of oxidase electrodes, redox salts, dme(Os) (**11**), tyrosine derivatives, or ferrocene are incorporated into the polymer [71-75]. A coating of poly(o-phenylenediamine, **12**) proved effective to reduce interference of other electroactive species. [76]

Some of the longest lifetimes of enzyme electrodes are achieved by encapsulating the enzyme in a crosslinked protein or sugar-derivative layer [88-90, 95]. Since sugar and protein concentrations in the cells are high, this is a strong indication that a cell-like environment stabilizes enzymes. Only one of these studies [88-90, 95] reports a detection limit, which is in the μM range [89], about average for the summarized values. The sensitivity for one lactate biosensor has also been reported: 1.05 nA/μM for lactate oxidase encapsulated in the crosslinked protein fibrinogen [90]. Some of the best detection limits have been achieved by using polypyrrole (PPy, **13**) [74, 78], but that is highly dependent on the mediator (PQQ, **1**, seems to work the best). In the latter study the sensitivity of the biosensor is reported as 2.07 nA/mM [78]. Unfortunately, in both studies [74, 78] the stability has not been reported. The development of electrodes with encapsulated enzymes for *in vivo* applications has been reviewed [96]. Storage stabilities of 45% after two weeks have been reported for polymer-encased glucose sensors.

Table 3. Enzymes Encased in Polymers

Enzyme	Analyte	Enzyme Encased in (Method), Additive, Mediator	Electrode	Detection Limit (M)	Operational Stability	Storage Stability	Ref #
Enzymes encased in conducting polymers							
GOx	Glucose	PPy (**13**) (electropol.), Fc	Carbon paste, silicone oil	2.5×10^{-4}	650 meas., 15d		[76]
GOx	Glucose	PPy(**13**) (electropol.), TTF(**6**)/TCNQ(**14**) salt	PPy(**13**) TTF(**6**)/TCNQ(**14**) salt	3.0×10^{-4}	550 meas., 6d		[76]
GOx	Glucose	PPy (**13**) (electropol.)	Platinum	7.5×10^{-4}	600 meas., 12d		[76]
GOx	Glucose	PPy (**13**) (electropol.)	Platinum			40% after 3 weeks	[77]
GOx	Glucose	on top PPy (**13**)(electropol.), glutaraldehyde (**4**), BSA	Platinum			100% after 3 weeks	[77]
ADH	EtOH	PPy (**13**) (electropol.), PQQ (**1**), heme	Platinum	2.1×10^{-9}			[78]
ICDH	Isocitrate	PPy(**13**), $NADP^+$, Meldola's blue (**15**)	Pt-Ir	3.5×10^{-6}	100% after 30 meas.		[79]
PyOx	Pyruvate	Polytyramine (**16**) (electropol.)	Glassy Carbon		70% after 15 meas., 8d	74% after 50d	[80]
MalDH	Malate	Polytyramine (**16**) (electropol.)	Tungsten		95% after 32 meas., 10hrs	80% after 82d	[81]
GOx	Glucose	MPS, Os-PVP (**17**), multilayer	Au		> 100 meas.	90% after 3d	[82]
GOx	Glucose	PEDOT (**18**)	Platinum	5×10^{-6}		60% after 1 month	[83]
GOx	Glucose	PAB (**19**), Fc, TTF (**6**)	Silver			6d	[84]
LOx/LDH	Lactate	PANI (**20**)	PANI	5×10^{-5}		3 weeks	[85]
GDH	Glucose	Oxidized polyarbutin (**21**), PQQ (**1**)	Carbon		50% after 200 meas., 15d		[86]
HRP	H_2O_2	PMAS (**22**)/polylysine (**23**)	SnO_2			50% after 7d	[87]
Enzymes encased into hydrogels							
GOx	Glucose	DEAE-dextran (**24**)	Carbon			150d	[88]
LOx	Lactate	Gelatin (=protein)	Pt layer on polyanion-doped PPy (**13**)	5×10^{-6}	> 2months	2 years (-18 ∞C)	[89]
LOx	Lactate	Fibrinogen, glutaraldehyde (**4**)	Platinum		133 ± 26 hrs	60% after 20 weeks	[90]
LOx	Lactate	Fibrinogen, transglutaminase	Platinum		53 ± 9 hrs	100% after 20 weeks	[90]
Enzymes encased in hydrogels with conducting particles							
AmOx	Amines	PVI(**25**)-dme(Os) (**11**), PEG(400)	Graphite	2.20×10^{-6}	80% after 8h		[38]
GlOx, HRP	Glutamate	PVI(**25**)-dme(Os) (**11**), PEG(400)	Graphite		8 hrs		[91]
Laccase	Oxygen	PVI(**25**)-$Os(byp)_2$ClPyHCO (**26**), PEG(400)	Glassy Carbon			50% after 3d	[92]
GOx	Glucose	Chitosan (=glycoproteins), redox salts w/ $Ru(NH_3)_6^{3+}$	Glassy Carbon			50% after several weeks	[71]

(Table 3). contd.....

Enzyme	Analyte	Enzyme Encased in (Method), Additive, Mediator	Electrode	Detection Limit (M)	Operational Stability	Storage Stability	Ref #
GOx	Glucose	PAA(**27**)-Os(byp)$_2$ClPyHCO (**26**), PEG(400)	Gold		94% after 150min		[93]
LipDH	NADH	PVAB, FMN (**28**), H_2O_2, TEMED	Carbon paste		72% after 5d periodic use	90% after 1 month	[94]
GluRed	NADPH	PVAB, FMN (**28**), H_2O_2, TEMED	Carbon paste		67% after 5d periodic use	73% after 2 month	[94]
AChE	ACh	Gaquat (**29**)				1 year	[95]

V) ENZYMES IMMOBILIZED ON MEMBRANES

Amperometric enzyme membrane electrodes have been extensively reviewed [97]. They are the most common commercially available biosensors. Some of the materials for membranes are BSA, cellulose, gelatin, collagen, agarose, poly(acryl amide), polyphenylene (PPh), polycarbonate,

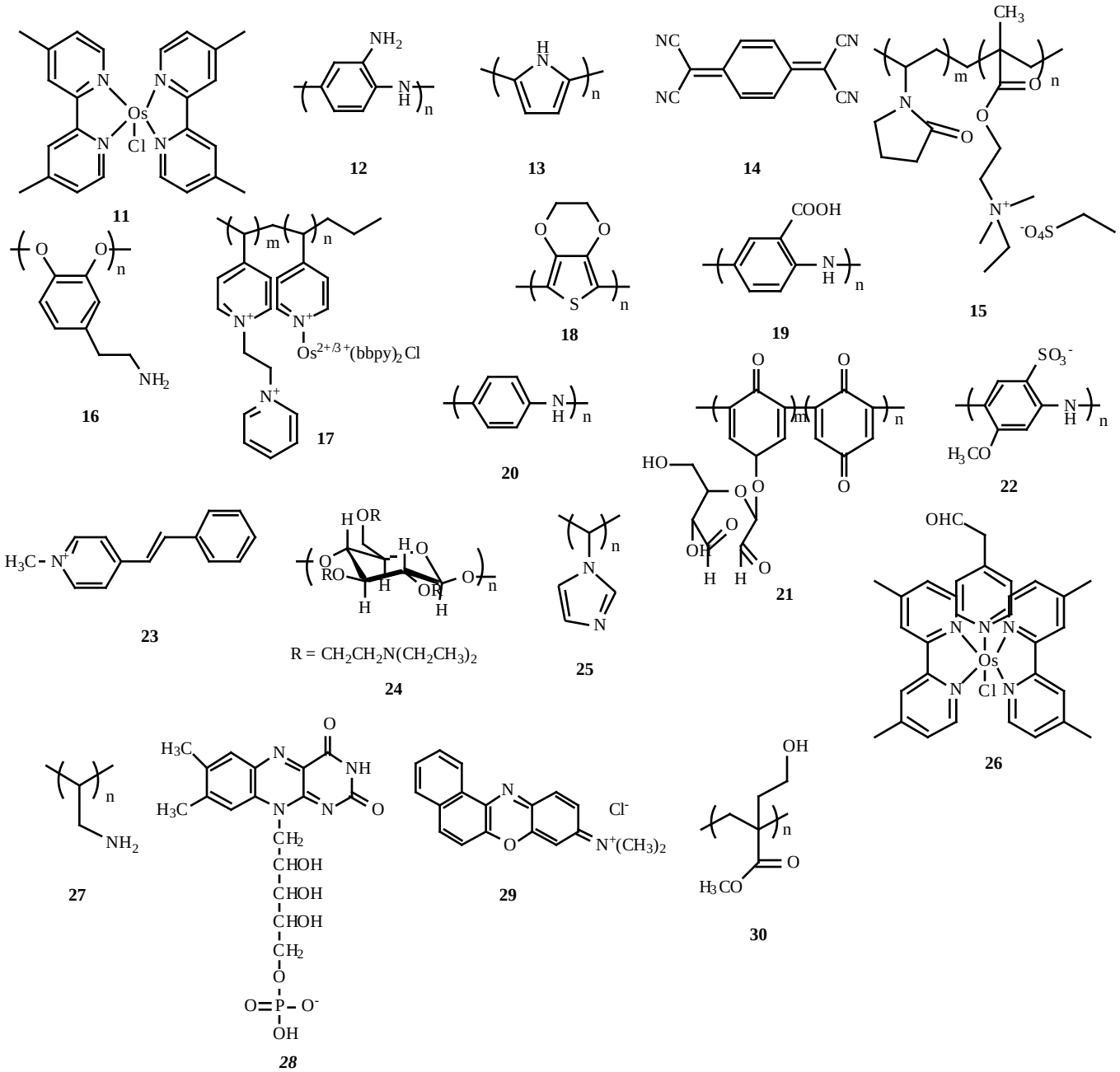

Fig. (2). Structures of compounds mentioned in (Table **3**) and text.

Table 4. Miniaturized Enzyme Electrodes

Enzyme	Analyte	Enzyme Encased in (Method), Additive, Mediator	Electrode	Detection Limit (M)	Stability	Ref #
GOx	Glucose	Polymer film with amine groups, glutaraldehyde (**4**)	Platinum		200 meas.	[105]
GOx	Glucose	Carbon ink (w/ prussian blue ($Fe_4[Fe(CN)_6]_3$), GOx)	Carbon ink		50% after 4hrs use	[106]
ADH	EtOH	Carbon ink (PVDC, carbon black), PQQ (**1**)	Carbon ink	1×10^{-6}	90% after 3d	[55]

carbon, nylon, teflon, and PVA. The longest stabilities reported in ref [97] are for collagen membranes, a natural fiber common in cells.

Membrane biosensors have been developed for implantation. For *in vivo* applications a large number of interference studies have to be conducted. For example, a glucose oxidase on polytyramine (**16**) electrode [98] and a urease on gelatin electrode [99] have been used to measure glucose in blood serum and it was found in both cases that few analytes interfere with glucose detection. To be implanted, though, the biosensor also has to be biocompatible, and if it is used in the blood stream, it also has to have anti-coagulation properties.

Some materials have been found to be biocompatsible. Glucose oxidase membrane electrodes made from poly(2-hydroxyethyl methacrylate) (pHEMA, **30**) [100] and poly(ethylene glycol) (PEG) [101] were found to be biocompatible. The pHEMA-covered electrode was stable for 50 hours. The PEG-electrode could not be monitored for longer than 5 hours due to difficulties with the experimental animal.

Heparin is the natural protein that prevents coagulation. Glucose oxidase and heparin have been co-immobilized on a poly(phenylene diamine) (PPD) membrane and used for a glucose sensor [102]. The performance in blood was improved in comparison to studies without heparin (no specific data given), but the sensitivity of the sensor decreased.

VI) MINIATURIZATION OF ENZYME ELECTRODES

Another requirement for implantation of a biosensor is small size. Two major advances have been made in the miniaturization of enzyme electrodes: screen printing the electrodes and using chip-manufacturing techniques for electrodes (summarized in Table **4**). FET-based sensors will not be discussed here, since they are not amperometric.

Biosensors based on screen-printed graphite electrodes have been reviewed recently [103]. Enzymes can either be contained in the carbon ink or be deposited on the electrode surface. Operational stability often is not important since a lot of these biosensors are designed for single use. The lowest detection limit was reported for an acetaldehyde sensor: a value of 1 μM was reported [104]. In this case, aldehyde dehydrogenase was deposited on the surface of a carbon electrode modified with Meldola blue (**15**).

VII) CONCLUSIONS

Different compositions of enzyme electrodes have been reviewed. Simple enzyme electrodes with the enzymes adhering to a modified electrode surface have shown very good detection limits and sensitivities, but it has been difficult to find compositions with lifetimes that are useful for medical devices. To extend lifetimes, the enzyme has been encapsulated either in the electrode itself, or in polymers which are either conducting or various hydrogels (or both). Some advances have been made to design biocompatible membrane enzyme electrodes for *in vivo* use. Stabilities still need to be improved for effective use of enzyme electrodes in medical sensors. Miniaturization of enzyme electrodes has only been attempted in a few cases so far, another requirement for *in vivo* use. Acceptable stability has been achieved with screen-printed electrodes, but not yet with chips.

Although the exact composition and structure of the natural environment of enzymes (cytoplasm) is not known, it is known that it contains large amount of salts, sugars, and proteins dissolved in water. When these conditions are mimicked for an enzyme electrode by using peptides and polysaccharides as the direct environment of the enzyme, the stability, sensitivity, and detection limit of the enzyme electrodes generally improve.

LIST OF ABBREVIATIONS

ACh = Acetylcholine

AchE = Acetylcholine esterase

ADH = Alcohol dehydrogenase

AmOx = Amine oxidase

BSA = Bovine serum albumin

CDH = Cellulobiose dehydrogenase

CYP 101 = Cytochrome P450 camphor

Cyt c = Cytochrome c

DEAE-dextran = Diethylaminoethyl-dextran (**24**)

DMeO = [Osmium(4,4'-dimethyl bipyridine)$_2$Cl]$^{+/2+}$ (**11**)

DMF = Dimethyl formamide

FET = Field-effect transistor

FAD = Flavin adenine dinucleotide (**3**)

Fc = Ferrocene

FMN = Flavin mononucleotide (**28**)

Gaquat = Poly(vinyl pyrrolidone-co-dimethylaminoethyl methacrylate) – diethyl sulfate(**29**)

GDH = Glucose dehydrogenase

GlOx = Glutamate oxidase

GluRed = Glutathione reductase

GlyOx = Glycolate oxidase

GOx = Glucose oxidase

HEMA = 2-Hydroxyethyl methacrylate (**34**)

HRP = Horseradish peroxidase

ICDH = Isocitrate dehydrogenase

LipDH = Lipoate dehydrogenase

LOx = Lactate oxidase

MalDH = Malate dehydrogenase

Meas = Measurements

MMP = 1-Methoxy-5-methyl phenazonium ion (**10**)

Morpho CDI = N-Cyclohexyl-3-(2-morpho-lineethyl)-carbodiimide methyl-*p*-toluene sulfonate (**9**)

MPA = Mercaptopropionic acid

MPS = Mercaptopropane sulfonic acid

NAD = Nicotineamide dinucleotide (**2**)

NitRed = Nitrate reductase

Ormosil = Organically-modified silicate

Os-PVP = $Os(bpy)_2Cl$ – poly(4-vinyl pyridine) (**17**)

OxalOx = Oxalate oxidase

PAA = Poly(allyl amine) (**27**)

PAB = Poly(*o*-amino benzoic acid) (**19**)

PANI = Polyaniline (**20**)

PDMS = Poly(dimethyl siloxane)

Pdx^r = Putidaredoxin

PEDOT = Poly(3,4-ethylenedioxy thiophene) (**18**)

PEG = Poly(ethylene glycol)

PLL = Polylysine (**23**)

PMAS = Poly(2-methoxyaniline-5-sulfonic acid) (**22**)

POPC = Palmitoyl oleoyl phosphatidylcholine

POx = Peroxidase

PPD = Poly(phenylene diamine)

PPG = Poly(propylene glycol)

PPh = Polyphenol

PPy = Polypyrrole (**13**)

PQQ = Pyrroloquinoline quinone (**1**)

PSS = Poly(styrene sulfonate) (**24**)

PutOx = Putrescine oxidase

PVA = Poly(vinyl alcohol) (**22**)

PVAB = Poly(vinyl ferrocene-co-acrylamide),crosslinked with N,N'=methylene bisacrylamide

PVDC = Poly(vinyl dichloride)

PVI = Poly(vinyl imidazole) (**25**)

PyOx = Pyruvate oxidase

RVC = Reticulated vitreous carbon

SAM = Self-assembled monolayer

SbQ = Stilbazolium ion (**23**)

TCNQ = Tetracyanoquinodimethane (**14**)

TTF = Tetrathia fulvalene (**6**)

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