MINI-REVIEW

Biomedical Application of Immobilized Enzymes

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ABSTRACT: Reports on chemical immobilization of proteins and enzymes first appeared in the 1960s. Since then, immobilized proteins and enzymes have been widely used in the processing of variety of products and increasingly used in the field of medicine. Here, we present a review of recent developments in immobilized enzyme use in medicine. Generally speaking, the use of immobilized enzyme in medicine can be divided into two major categories: biosensors and bioreactors. A brief overview of the evolution of the biosensor and bioreactor technology, of currently existing applications of immobilized enzymes, of problems that researchers encountered, and of possible future developments will be presented. © 2000 Wiley-Liss, Inc. and the American Pharmaceutical Association J Pharm Sci 89: 979–990, 2000

Keywords: enzyme; immobilization; biosensor; bioreactor

INTRODUCTION

Reports on chemical immobilization of proteins and enzymes first appeared ~30 years ago. Since then, immobilized proteins have been widely used for the processing of a variety of products spanning industries from food to environmental control. In addition to their use in processing, immobilized proteins and enzymes have also been found useful in many bioanalytical and biomedical applications. These applications include the use of immobilized antibodies or antigens in bioaffinity chromatography, of immobilized receptors or ligands in the study of their interactions, and of immobilized cells in biosensors. Although there have been a large number of articles discussing immobilization techniques of proteins and enzymes as well as their applications in certain fields,[1–3] a review on the applications of immobilized proteins and enzymes in medicine and particularly during the past decade (i.e., 1990s) is still lacking.

Presently, immobilized proteins/enzymes are used routinely in the medical field, such as in the diagnosis and treatment of various diseases. For example, immobilized antibodies, receptors, or enzymes are used in biosensors and ELISA for the detection of various bioactive substances in the diagnosis of disease states; encapsulated enzymes are also used in bioreactors for the removal of waste metabolites and correction of inborn metabolic deficiency. Moreover, the use of artificial cells as well as the development of controlled-release drug delivery systems to release encapsulated enzymes or proteins may also be considered a form of immobilized enzyme use. However, this article will concentrate only on the review of current applications of traditionally defined immobilized enzymes in medicine; specifically in the areas of biosensors and bioreactors.

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IMMOBILIZED ENZYME USED IN BIOSENSORS

Enzyme-based electrodes represent a major application of immobilized enzymes in medicine. The high specificity and reactivity of an enzyme towards its substrate are properties being exploited in biosensor technology. Biosensors possess advantages such as reliability, sensitivity, accuracy, ease of handling, and low-cost compared with conventional detection methods. These characteristics, in combination with the unique properties of an enzyme already mentioned, render an enzyme-based biosensor ideal for biomedical applications. A summary of some recent applications of enzyme-based sensors in clinical diagnosis is presented in Table 1. Our discussion on this subject will be classified into four different categories: enzyme immobilization, sensitivity and selectivity, optical biosensor, and implantable biosensor.

Enzyme Immobilization

An enzyme-based electrochemical sensor is formulated by immobilizing a thin layer of enzyme(s) on the surface of the membrane of an electrode. The analyte to be monitored diffuses into the enzyme layer where the catalytic reaction occurs, either consuming a substrate or generating a product that can be detected electrochemically. The electroactive species produced are monitored either potentiometrically or amperometrically, and the electrochemical signal can be correlated to the concentration of the analyte to be measured. The immobilization method may affect the activity of the immobilized enzyme, and thus it can contribute significantly to the sensitivity of the biosensor.

Immobilization methods that are currently being used include absorption, cross-linking, and self-assembly, whereas materials into which enzymes are incorporated include carbon paste, conducting or nonconducting polymers, and different types of gels. The ultimate aim of these efforts is to develop an inexpensive and easy-to-prepare method that can yield a stable matrix possessing the maximum retention of the biological activity of the immobilized enzyme. Generally speaking, physical entrapment of enzymes in polymeric membranes is one of the most advantageous methods because it is rapid and simple, the retained enzyme activity is usually quite high, plus no chemical reaction that may lead to the inactivation of the enzyme is required. The major limitation of physical entrapment is, however, that unlike chemical immobilization where all types of substrates, whether they are large or small, can all reach the immobilized enzyme for interaction, the physically entrapped enzyme is prohibited from interaction with large substrates that are unable to diffuse into the matrix. Therefore, for

Table 1. Application of Biosensor in Medicine

Sensor	Immobilized Enzyme	Linear Range	Response Time (min)	Stability (months)	Ref No.
Glucose	Glucose oxidase/glucose dehydrogenase	~50 mM	~5	~6	4–8
Lactate	Lactate oxidase	~27 mM	~1	~9	9, 10
Oxalate	Oxalate oxidase	~1 mM	2~40	~24	11, 12
Urea	Urease	~100 mM	~5	~6	13, 14
Glutamate	Glutamate oxidase	~200 μM	~1		15, 16
Carnitine	Carnitine dehydrogenase and diaphorase	~1 nM	~3	~3	17
Theophylline	Theophylline oxidase	~30 μM	~1		18
Creatine and creatinine	Creatininase, creatinase and sarcosine oxidase	~30 mM	~4		19, 20
Cholesterol	Cholesterol oxidase	~3 mM			21, 22
Amino acid	Amino acid oxidase	~10 mM	~3	~2	23
Acetylcholine and choline	Acetylcholine esterase and choline oxidase	~100 μM	~2	~6	24–26
Bilirubin	Hemoglobin and glucose oxidase				27, 28
γ-Aminobutyric acid	Catalase and Γ-glutamate oxidase	~10 nM			29

assaying a large substrate, chemically immobilized enzyme should be used.

In search of suitable matrices for enzyme immobilization, there has been a growing interest in attaching the enzymes to the solid electrolyte Nafion. The Nafion membrane possesses a high surface adhesion property and a low swelling capability in aqueous media. Being a negatively charged polyelectrolyte matrix, Nafion exhibits a reduced permeability to negatively charged substances, therefore improving the sensor selectivity and virtually eliminating the interference of reductants such as ascorbate and acetaminophen. On the other hand, a Nafion film possesses a high ability to entrap positively charged redox-active compounds that can be used as mediators in bioelectrocatalytic reactions. In addition, the polyelectrolyte nature of the Nafion film can stabilize the ionic strength at the electrode surface, thereby preventing electrode fouling and increasing the dynamic range for analyte detection. Other polymer films, such as poly(vinyl alcohol),[30] polytetrafluoroethylene,[31] poly(vinyl alcohol) grafted 4-vinylpyridine,[32] poly(pyrrole-ammonium)/poly(pyrrole-lactobionamide),[33] polyaniline,[34] and poly(*o*-phenylenediamine)[35] have also been used recently in preparing biosensors because of their advantageous properties of high oxygen solubility, decent hydrophilicity, good biocompatibility, reasonable mechanical strength, and antifouling.[36] In addition, immobilization of heparin on artificial surfaces has also been extensively explored in an attempt to create a thromboresistant surface for the use of the biosensors *in vivo*.[37,38]

The low-temperature sol–gel process is another current attractive avenue for the immobilization of enzymes. Numerous sol–gel-based biosensors have been reported, and enzymes have been trapped in either inorganic or organic-modified inorganic materials such as silica,[39,40] alumina,[41] silane,[42] and silver[43] matrixes. Compared with membranes made of other inorganic materials, such as clays[43] and zeolites[44] that limit enzyme immobilization because of their molecular dimensions, the porous organic or inorganic sol–gel matrix possesses physical rigidity, chemical inertness, high biodegradable property, and thermal stability. In addition, the sol–gel matrix yields insignificant swelling in both aqueous and organic solutions.

Sensitivity and Selectivity

Amperometric biosensors based on oxidase enzymes that generate hydrogen peroxide are the most widely used biosensors. Glucose determination utilizing the enzyme glucose oxidase (GOD) is an example of such an amperometric biosensor, and the principle of such glucose electrodes is based on the following reactions:

$$\text{Immobilized layer: glucose} + O_2 \rightarrow \text{gluconic acid} + H_2O_2$$

$$\text{Electrode: } H_2O_2 \rightarrow O_2 + 2H^+ + 2e^-$$

Obviously, this type of sensor is based on either oxygen depletion (cathodic) or the electrochemical oxidation of H_2O_2 (anodic). However, direct oxidation of H_2O_2 requires a highly positive overpotential, and hence this type of sensor suffers from interferences due to the presence of easily oxidizable species in the biological fluids. Although the cathodic measurement of molecular oxygen concentration is perfectly suitable for glucose monitoring in solution, these devices also suffer potential difficulties when operating *in vivo* or in undiluted biological samples where the response is limited by physiological amounts of oxygen available. To solve the oxygen deficit problem, attempts have been made in glucose detection to use an additional catalase in recycling O_2 from H_2O_2,[45] or to develop approaches that allow O_2 to be delivered externally to the electrode surface, thereby producing an excess level of O_2 (compared with that of glucose) at the electrode surface.[46] This excess amount of O_2 will allow for better regulation of the reaction environment and, hence, oxygen (instead of glucose) does not become the limiting reagent in the reaction. However, incorporation of catalase is undesirable for *in vivo* use of microelectrodes because a variation in the O_2 level in the surrounding medium may cause fluctuation in electrode response and thus a second electrode would be needed to measure the actual O_2 level so that the accurate concentration of the substrate can be determined. The main concern associated with the use of an external O_2 delivery system, on the other hand, is the difficulty in regulating the oxygen generation or supply, leading to possible introduction of an inadequate amount of oxygen to the electrode membrane.[46]

Another approach to solve this oxygen deficiency problem is to place a diffusion-restriction membrane on top of the enzyme membrane. Such a diffusion-limiting membrane will yield high oxygen permeability but low glucose permeability, thereby providing a minimal concentration of

glucose within the enzyme layer and a relatively constant amount of oxygen.[30–35] Alternatively, some artificial electron acceptors or mediators, such as ferrocenes,[47] quinones,[48] diaminobenzenes,[49] and TTF,[50] have also been used to minimize the sensitivity to oxygen of the enzyme-based electrodes. These electron-transfer mediators, which are retained at the electrode surface using a membrane or entrapped in an electron-generated film at the surface, facilitate electron transfer between an electrode and a redox-active biological molecule, ensuring the attainment of a high signal-to-noise ratio. In addition to minimizing oxygen sensitivity, coupled mediated sensing systems, in which the mediator is attached to either the electrode or to the enzyme, could also provide a faster electron transfer, improved biosensor lifetime,[51] and reducing susceptibility to interferences from other electrochemically active species because of the low potential generally needed for oxidation of the mediators.[52]

In addition, increased sensitivity and selectivity of a enzyme-based electrode can also be obtained by modifying the electrode surface with semiconducting films using electropolymerization of a redox monomer, such as phenazine,[53] phenoxazine and phenothiazine derivatives,[54] viologens,[55] and thionine.[56] Polymer-modified electrodes with a three-dimensional distribution of mediators are preferred over a monolayer distribution of mediators for the design of biosensors because a much larger catalytic response of the polymer coating will be obtained because of the volume effect. In general, the major obstacles that prevented rapid progress in the practical application of the semiconducting electrode in glucose sensing in the early of 1990s were: (1) physical instability and light sensitivity of the semiconductor structures; (2) dramatic decrease in the biosensor response to the change of buffer conditions and the ionic strength of the solution; and (3) limitation in the dynamic range of the biosensor response due to an insufficient concentration of dissolved oxygen in the measured samples.[57] However, with the advent of application of an additional semipermeable membrane on top of the enzyme layer and of enzyme field effect transistors,[58,59] the stability, sensitivity, and dynamic range of semiconducting sensors have recently been greatly improved.[60]

Because not all enzyme-catalyzed reactions are involved with transducer-active compounds (e.g., H^+, O_2, OH^-), only a limited number of substances can be determined by using a sensor consisting of a single enzyme. In this regard, the use of coupled enzyme reactions for analyte conversion provides a more favorable alternative. In such cases, the primary products produced by the reaction of the analyte with the first enzyme is further converted by a second enzyme to produce products detectable by a transducer.[61] Coupled enzyme reactions are also employed to filter out chemical signals by eliminating the interference on the enzyme and/or tranducer reaction caused by other constituents in the sample.[62,63] Alternatively, for certain measurements, an enzyme-based sensor can also be constructed by using the inhibition of the enzyme activity. In this case, the analyte is not a substrate but an inhibitor to the specific reaction of the enzyme. A reduction in the electrical signal can then be inversely correlated to the analyte concentration in the solution.[64,65]

Glucose dehydrogenase-based electrodes have also been developed to potentiometrically or amperometrically monitor glucose concentration. In most instances, glucose dehydrogenase systems require the presence of NAD or NADP. Requirements also depend on oxygen saturation of the sample and whether or not a redox mediator hasbeen incorporated[7,8] to enhance glucose detection.

Optical Biosensor

In addition to the electrochemical transduction systems, interest in optical biosensors based on immobilized enzymes has also been gradually growing. Immobilization techniques used in optical sensors include adsorption,[66] physical entrapment,[67] and photocontrolled polymerization.[68] The sol–gel method of entrapping enzymes in transparent silicate matrices is especially suitable in optical biosensors because it allows for the monitoring of chemical reactions of immobilized proteins by changes in the visible absorption spectra.[67] Fiberoptic biosensors for monitoring glucose,[69] cholesterol,[70] phospholipid,[71] and bilirubin[72] have been reported. Recently, the determination of analyte concentration is based on the measurement of the oxygen partial pressure in the analyte sample. In these devices, changes in the oxygen level were measured via quenching of the fluorescence of an oxygen-sensitive dye. In the case of the glucose sensor, the enzymatic depletion of oxygen, which is the result of transformation of the glucose by the immobilized enzyme glucose oxidase, leads to a decrease in the fluorescence quenching efficiency of the oxygen-sensitive dye element by O_2; as a result, the in-

crease in the fluorescence intensity of the sensor during the enzymatic reaction corresponds to the glucose concentration.[69] Alternatively, H_2O_2 produced in the oxidation of glucose by glucose oxidase can also be used for glucose detection through its further reaction with a dye precursor to produce a colored dye by the enzyme peroxidase. The rate of the colored dye formation is indicative of the glucose concentration.[73] The main drawback of this technology, however, lies in the rather slow response time for such fiberoptic biosensors, primarily because of the indirect nature for the analyte measurements. However, enzymes containing a flavin adeninedinucleotide (FAD) reaction site, such as glucose oxidase, cholesterol oxidase, and lactate monooxygenase, exhibit characteristic absorption spectrum with bands at 377 and 455 nm. On the addition of the analyte, the enzyme is almost completely decolorized; thus, the development of reagent-free sensor is also being explored.[67,74] These sensors offer the advantage of more rapid communication with the optrode for response. Recently, FAD- or ferrocene-reconstituted enzymes have been used in the enzymatic sensor and should offer some benefits in future development of the fiberoptic biosensing devices.[75,76]

Implantable Biosensor

Biosensors, in addition to the benefits of simplifying existing biochemical measurements, offer the advantage of allowing continuous and real-time monitoring. In addition to their main function in monitoring the rapid changes in blood analyte concentrations, biosensors provide an essential element to the development of artificial biofeedback systems that are useful in blood glucose control, drug dosing and administration, and intravenous infusion of nutrients. In general, devices in clinical use can be classified into three categories based on their size and site for applications: bedside units, wearable modules, and implantable devices. The bedside unit is a rather large and complex device containing a miniaturized sensor system used in the hospitals or intensive care units. Wearable modules can be attached to certain parts of the patient's body and are presently used in research for short-term monitoring of certain clinical entities. Implantable devices offer the convenience of being used as either a monitoring or an alarm device for continuous detection of certain substance levels *in vivo*. However, issues such as biocompatibility, generation of immune response to the sensor, long-term fouling of the electrode, and the need for frequent calibration still remain as major concerns for the successful application of implantable biosensors *in vivo*. The current trend in sensor development leans towards miniaturization and integration of the device.[77–80] At the present time, enzyme-immobilized sensors capable of monitoring a variety of >10 different substances have already been commercialized, and a significant amount of effort is underway to further broaden the scope of applications.

IMMOBILIZED ENZYMES USED IN BIOREACTORS

The therapeutic application of immobilized enzyme in a bioreactor started as early as the 1960s. Since then, significant efforts have been made to apply this technology to the correction of inborn errors of metabolism, cancer treatment, blood detoxification, and removal of waste metabolites. Generally speaking, the enzyme(s) can be either immobilized on a solid support or encapsulated in sol–gel or an artificial cell (red blood cell or liposome) to construct the bioreactor. Although there are some reports on the immobilization of enzymes including phospholipase A_2 and heparinase [81–83] in bioreactors for blood detoxification, most efforts have been centered on the application of encapsulated enzymes for disease treatment. Compared with immobilized enzyme, enzymes entrapped in an artificial cell not only convert substrates that diffuse into the cell, but also possess low immunogenicity (due to the ability of artificial cells to mask their immune determinants) and prolonged blood clearance time. Initially, only a few enzymes, such as asparaginase and urease, had been used. Also, encapsulation of only single enzymes in the artificial cell had been attempted. At present, however, multiple enzymes are being encapsulated in one artificial cell, and such a multienzyme system has been used for the correction of metabolic imbalances.[84–87] Recent examples of utilizing immobilized enzymes for clinical applications include artificial cells containing enzymes such as SOD,[88] asparaginase,[89,90] urease,[91] lactate oxidase,[92] and alcohol oxidase[84,87] for replacing hereditary enzyme deficiency, suppressing the growth of lymphosarcoma, decreasing systemic urea and lactate concentrations, and treating alcohol poisoning, respectively. Most recently, there is a

tendency of utilizing cells that can self-express enzymes rather than encapsulating enzymes in the artificial cells.[93–95] Table 2 provides samples of bioreactors that have been extensively investigated during the last several years. Discussion of the most recent development in this field is classified into three categories: red blood cells as a carrier for enzymes, liposomes as a carrier for enzymes, and encapsulation of enzymes in sol–gel.

Red Blood Cell as A Carrier for Enzymes

As a new therapeutic approach, human and animal red blood cells (RBCs) have been used as the carrier vehicle for a number of exogenous enzyme drugs intended to be disseminated in an organism.[103] Because of their biocompatible nature, these cells will elicit little or virtually no immune response, thereby providing advantages of protecting the activity of encapsulated substances from rapid clearance and of avoiding toxic side effects.[104,105] In addition, RBCs can be readily obtained, and a large quantity of the drug can be entrapped into a rather small volume of the cell using certain well-defined methods.

Techniques for induction of exogenous substances into RBCs include endocytosis,[106] passive diffusion,[107] electroporation,[84] and hypotonic dialysis/isotonic resealing,[108] etc. Among them, hypotonic dialysis/isotonic resealing is currently the most widely used technique because it is simple to use, it provides an efficient encapsulation of a large amount of the protein, it is adaptable for scale-up purposes, and it provides a carrier cell possessing the same *in vivo* half-life as that of the normal RBC.[109]

Electroporation is another simple and efficient method for enzyme encapsulation. If the intensity of the electric field and the exposure time are maintained below certain critical values, the membrane rupture process required can be controlled to be completely reversible. Compared with hypotonic dialysis, electroporation provides several advantages, such as the preparation of the cell suspension can be achieved in an isotonic solution and the procedure can be manipulated easily and carried out routinely.[84] In addition to the loading techniques, the sources of RBCs can also affect the capacity for drug loading. Among different animal species from which the RBCs are obtained and treated with the hypotonic dialysis technique, the rat and rabbit have been reported to be the species that could present problems for enzyme encapsulation because these RBCs yield relatively low drug-loading capacity compared with human erythrocytes.[103,110]

Different methods employed in enzyme encapsulation could result in change in the properties of erythrocytes. A previous investigation has reported that erythrocytes to which the drug is loaded by the osmotic method function as the best drug carrier in systemic circulation because they are more stable than erythrocytes to which the drug is loaded by other methods.[111] On the other hand, chemically modified erythrocytes possess the benefit of targeting the organs of the mono-

Table 2. Encapsulation of Enzymes in Artificial Cells

Enzyme (support)	Function	Potential Treatment	Ref No.
Phosphotriesterase (liposome and erythrocyte)	Degrading organophosphate	Organophosphate poison	96, 97
Alcohol dehydrogenase, acetaldehyde dehydrogenase (erythrocyte)	Converting alcohol to acetate	Alcohol poison	84, 87
Chloroperoxidase, lactoperoxidase, glucose oxidase (liposome)	Producing hydrogen peroxide and oxyacids	Bacterial infection	85, 86
Hydrogenase (erythrocyte)	Catalyzing the oxidation of H_2	Decompression sickness	98
Lactate oxidase (erythrocyte)	Converting lactate to acetate	Hyperlactatema	92
Γ-Asparaginase (liposome)	Degrading Γ-asparagine	Lymphoblastic leukemia	89–90
Superoxide dismutase (liposome)	Cleaning superoxide	Inflammation	99
DNA repair enzyme (liposome)	Repairing DNA damage	Skin aging and cancer	100, 101
Phospholipase A_2 (liposome)	Hydrolyzing of phospholipid	Hypercholesterolemia	102
Cytochrome P450 (cells encapsulated in cellulose sulfate formation)	Converting ifosfamide or oxazaphosphorine to tumoricidal metabolites	Tumor	93–95

nuclear phagocytic system because changes introduced in the membrane of the erythrocyte by the chemical method can be recognized by the macrophage cells. This recognition is shown by the fact that although the liver is the main target, the spleen provides the most selective removal of the drug-loaded RBCs. In contrast, crosslinking of Band 3, a prominent protein in the membrane that is involved in a number of functions of the erythrocyte, results in a more specific liver-targeting erythrocyte carrier.[111,112] Therefore, manipulation of the erythrocyte membrane by crosslinking can induce change in the distribution of the erythrocyte carrier. So far, crosslinking of erythrocytes with glutaraldehyde, dimethylsubrimidate, or toluene 2,4-diisocyanate have been most widely attempted.

Liposome as a Carrier for Enzymes

Like RBCs, liposomes can also fulfill the promise of a drug-carrier vehicle because of their ability to reduce toxicity and enhance efficacy of the agents that are encapsulated. However, therapeutic application of liposomes administered by the intravenous route has been limited, primarily because of a rapid clearance of liposomes from the bloodstream and their uptake by the macrophage cells in the liver and spleen.[113] Recently, liposomes with the ability to evade the rapid uptake by the reticuloendothelial system (RES) have been developed, and a significant improvement in the blood half-life has been achieved by using liposomes whose surfaces are modified with polyethyleneglycol (PEG).[114,115] The stabilization of the liposomes by PEG is due to the flexible dynamic motion of the PEG polymer chains that sterically prevent both electrostatic and hydrophobic interactions between the blood components and the liposome surface.[116] Because these liposomes exhibit dramatically different pharmacokinetics and biodistribution properties,[114,115] they present a new avenue with regard to therapeutic applications. Recent applications of the PEG-modified liposomes include use in enhancing efficacy of antineoplastic agents against tumors, increasing delivery of antiinflammatory agents to the sites of inflammation, and targeting the extravascular system using such liposomes that are further linked with the targeting ligands.[117]

Encapsulation of Enzymes in Sol–Gel

Sol–gel is a chemical synthesis technology employed in preparing gels, glasses, and ceramic powders. Synthesis of materials by the sol–gel process generally involves the use of metal alkoxides, mostly but not exclusively the SiO_2 materials, which undergo hydrolysis and condensation polymerization reactions to produce the gel. It is now a well-established knowledge for a wide variety of enzymes and proteins that their reactivity and function can be fully retained when they are being confined within the pores of the sol–gel matrix.[118,119] The porosity of the sol–gel glass allows small molecules to be diffused in, whereas the large enzyme macromolecule remains physically entrapped in the matrix. Sol–gel porous matrices in general, and doped matrices in particular, provide the following advantages: (1) they possess controllable surface area and relatively uniform pore size and distribution; (2) they are thermally stable; (3) they are able to enhance the stability of the encapsulated enzymes; and (4) they can prevent leaching of the entrapped enzyme. Recently, new classes of precursors, based on polyol silicates and polyol siloxanes and especially those derived from glycerol, have been under intense investigation.[120] These precursors can be distinguished from the traditional ones by properties such as being highly biocompatible and able to encapsulate proteins and cells under mild conditions in a reproducible manner.[120] Such characteristics enable the sol–gel carrier to address most of the problems encountered with traditional encapsulation methods. To this regard, like artificial cells, the sol–gel-entrapped enzymes may also be used in a bioreactor for the treatment of various enzyme-deficient diseases.[118,119]

CONCLUSION

This paper presents a brief review of recent (i.e., during the past decade) developments and medical applications of immobilized enzymes, particularly in the areas of biosensors and bioreactors. Although the progress in therapeutic use of immobilized enzymes is slow and somewhat staggered, because of the complexity of the human body system to be applied, the future prospect for application of immobilized enzymes in biosensing is promising. This promising future is particularly true because of the current advancement in technologies in microprocessing and microelectronic devices. Such technologies would allow the biosensors to be miniaturized during manufacturing, integrated with signal processing steps on a chip, and arrayed for more complicated substrate

analysis. In addition, the progress in optical transducing devices provides another promising avenue that can be incorporated in biosensor development.

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REFERENCES

1. Ofagain C, Okennedy R.1991. Functionally-stabilized proteins- A review. Biotechnol Adv 9: 351–409.
2. Tischer W, Wedekind F. 1999. Immobilized enzyme: Methods and applications. Biocatalysis-From Discovery to Application 200:95–126.
3. Tyagi R, Gupta MN. 1998. Chemical modification and chemical cross-linking for protein/enzyme stabilization. Biochemistry 63:334–344.
4. Zhang YQ, Zhu J, Gu RA. 1998. Improved biosensor for glucose based on glucose oxidase-immobilized silk fibroin membrane. Appl Biochem Biotechnol 75:215–233.
5. Pandey PC, Upadhyay S, Pathak HC. 1999. A new glucose biosensor based on sandwich configuration of organically modified sol-gel glass. Electroanalysis11:59–64.
6. Zhou DM, Ju HX, Chen HY. 1997. A miniaturized glucose biosensor based on the coimmobilization of glucose oxidase and ferrocene perchlorate in nafion at a microdisk platinum electrode. Sens Actuators, B 40:89–94.
7. Markovarga G, Appelqvist R, Gorton L. 1986. A Glucose sensor based on glucose-dehydrogenase absorbed on a modified carbon electrode. Anal Chim Acta 179:371–379.
8. Appelqvist R, Markovarga G, Gorton L, et al. 1985. Enzymatic determination of glucose in a flow system by catalytic-oxidation of the nicotinamide coenzyme at a modified electrode. Anal Chim Acta169:237247.
9. de Keijzer MH, Brandts RW, Brans PGW. 1999. Evaluation of a biosensor for the measurement of lactate in whole blood. Clin Biochem 32:109–112.
10. Marzouk SAM, Cosofret VV, Buck RP, Yang H, Cascio WE, Hassan SSM.1997. A conducting salt-based amperometric biosensor for measurement of extracellular lactate accumulation in ischemic myocardium. Anal Biochem 69:2646–2652.
11. Reddy SM, Vadgama PM. 1997. Ion exchanger modified PVC membranes selectivity studies and response amplification of oxalate and lactate enzyme electrodes. Biosens Bioelectron 12:1003–1012.
12. Fernandes JR, Neto CD, Kubota LT, Tubino M. 1996. Use of sorghum seed tissue as a biocatalyst in a stirred reactor for oxalic acid determination. Anal Commun 33:397–399.
13. Eggenstein C, Borchardt M, Diekmann C, Grundig B, Dumschat C, Cammann K, Knoll M, Spener F. 1999. A disposable biosensor for urea determination in blood based on an ammonium-sensitive transducer. Biosens Bioelectron 14:33–41.
14. Liu DZ, Kai G, Kang C, Ni LH, Yao SZ. 1996. Sensitive specialization analysis of urea in human blood by surface acoustic wave urea sensor system. Microchem J 53:6–17.
15. Li QS, Zhang SL, Yu JT. 1996. Immobilization of L-glutamate oxidase and peroxidase for glutamate determination in flow injection analysis system. Appl Biochem Biotechnol 59:53–61.
16. Marzouk SAM, Cosofret VV, Buck RP, Yang H, Cascio WE, Hassan SSM. 1997. Amperometric monitoring of lactate accumulation in rabbit ischemic myocardium. Talanta 44:1527–1541.
17. Matsumoto, K., Yamada, Y., Takahashi, M., Todoroki, T., Mizoguchi, K., Misaki, H., Yuki, H. 1990. Fluorometric-determination of Carnination in serum with immobilized carnitine dehydrogenase and diaphorase. Clin Chem 36:2072–2076.
18. Wang, J. Dempsey, E., Ozsoz, M., Smyth, M.R. 1991. Amperometric enzyme electrode for therophylline. Analyst 116:997–999.
19. Kinoshita H, Torimura M, Kano K, Ikeda T. 1997. Peroxidase-based amperometric sensor of hydrogen peroxide generated in oxidase reaction: Application to creatinine and creatine assay. Electroanalysis 9:1234–1238.
20. Khan GF, Wernet W. 1997. A highly sensitive amperometric creatinine sensor. Anal Chim Acta 351:151–158.
21. Marazuela MD, Cuesta B, MorenoBondi MC, Quejido, A 1997. Free cholesterol fiber-optic biosensor for serum samples with simplex optimization. Biosens Bioelectron 12:233–240.
22. Gilmartin MAT, Hart JP. 1994. Fabrication and characterization of a screen-printed, disposable, amperometric cholesterol biosensor. Analyst 119: 2332–2336.
23. Lee YC, Huh MH. 1999. Development of a biosensor with immobilized D-amino acid oxidase for determination of L-amino acids. J Food Biochem 23: 173–185.
24. Horiuchi T, Torimitsu K, Yamamoto K, Niwa O. 1997. On-line flow sensor for measuring acetylcholine combined with microdialysis sampling probe. Electroanalysis 9:912–916.
25. Guerrieri A, Debenedetto GE, Palmisanso F, Zambonin F, Zambonin PG. 1995. Amperometric sensors for choline and acetylcholine based on a platinum-electrode modified by co-cross-linked bienzymic system. Analyst 120:2731–2736.

26. Song ZH, Zhang ZJ, Fan WZ. 1998. A chemoluminescence biosensor for determination of acetylcholine and choline. Acta Chim Sinica 56:1207–1213.
27. Li XP, Rosenzweig Z. 1997. A fiber optic sensor for rapid analysis of bilirubin in serum. Anal Chim Acta 353:263–273.
28. Vidal MM, Gil MH, Delgadillo I, Alonso J. 1999. Study of the thermal stability and enzymatic activity of an immobilised enzymatic system for the bilirubin oxidation. Biomaterials 20:757–763.
29. Niwa O, Kurita R, Horiuchi T, Torimitsu K. 1998. Small-volume on-line sensor for continuous measurement of gamma-aminobutyric acid. Anal Biochem70:89–93.
30. Doretti L, Ferrara D, Gattolin P, Lora S, Schiavon F, Veronese FM. 1998. PEG-modified glucose oxidase immobilized on a PVA cryogel membrane for amperometric biosensor applications. Talanta 45: 891–898.
31. Turmanova S, Trifonov A, Kalaijiev O, Kostov G. 1997. Radiation grafting of acrylic acid onto polytetrafluoroethylene films for glucose oxidase immobilization and its application in membrane biosensor. J Membr Sci 127:1–7.
32. Deng Q, Li B, Dong S. 1998. Self-gelatinizable copolymer immobilized glucose biosensor based on Prussian Blue modified graphite electrode. Analyst 123:1995–1999.
33. Cosnier S, Lepellec A, Guidetti B, Rico-lattes I. 1998. A glucose biosensor based on enzyme entrapment within polypyrrole films electrodeposited on mesoporous titanium dioxide. J Electroanal Chem 449:165–171.
34. Hu SS, Luo JL, Cui D. 1999. An enzyme-chemically modified carbon paste electrode as a glucose sensor based on glucose oxidase immobilized in a polyaniline film. Anal Sci 15:585–588.
35. Myler S, Eaton S, Higson SPJ.1997. Poly(*o*-phenylenediamine) ultra-thin polymer-film composite membranes for enzyme electrodes. Anal Chim Acta 357:55–61.
36. Yacynych AM, Mark HB. 1976. The spectroelectrochemical study of the oxidation of 1,2-diaminobenzene:alone and in the presence of Ni (II). J Electrochem Soc 123:1346–1351.
37. Nakayama Y, Matsuda Y. 1992. Surface fixation of hydrogels heparin and glucose oxidase hydrogelated surfaces. ASAIO J M421–M424.
38. Yang Q, Atanasov P, Wilkins E. 1997. A needle-type sensor for monitoring glucose in whole blood. Biomed Instrum Technol January/February, 54–62.
39. Hartnett AM, Ingersoll CM, Baker GA, Bright FV. 1999. Kinetics and thermodynamics of free flavins and the flavin-based redox active site within glucose oxidase dissolved in solution or sequestered within a sol-gel-derived glass. Anal Chem 71:1215–1224.
40. Li J, Chia LS, Goh NK, Tan SN. 1999. Renewable silica sol-gel derived carbon composite based glucose biosensor. J Electroanal Chem 460:234–241.
41. Liu ZJ, Liu BH, Zhang M, Kong JL, Deng JQ. 1999. Al_2O_3 sol-gel derived amperometric biosensor for glucose. Anal Chim Acta 392:135–141.
42. Tang FQ, Shen JF, Zhang JF, Zhang GL. 1999. Enhancement of glucose biosensor sensitivity by addition of silver sols. Chem J Chin Univ 20: 634–636.
43. Shyu SC, Wang CM. 1998. Characterizations of iron-containing clay modified electrodes and their applications for glucose sensing. J. Electrochem Soc 145:154–158.
44. Liu BH, Yan F, Kong JL, Deng JQ. 1999. A reagentless amperometric biosensor based on the coimmobilization of horseradish peroxidase and methylene green in a modified zeolite matrix. Anal Chim Acta 386:31–39.
45. Lysenko V, Delhomme G, Soldatkin A, Strikha V, Dittmar A, JaffrezicRenault N, Martelet C. 1996. Adaptation of microthermal probes for the determination of biochemical species. Talanta 43: 1163–1169.
46. Huang T, Warsinke A, Koroljova-Skovobogat'ko OV, Makower A, Kuwana T, Scheller FW. 1999. A bienzyme carbon paste electrode for the sensitive detection of NADPH and the measurement of glucose-6-phosphate dehydrogenase. Electroanalysis 11:295–300.
47. Gasiorwski K, Brokos JB, Szyba K, Wozniak D, Fraser DM, Zakeeruddin SM, Graetzel M. 1999. Evaluation of genotoxic and immunotoxic activities of potential glucose biosensor components: Ferrocenes. Biometals 12:19–26.
48. Linsenmeier RA, McRipley MA. 1996. Fabrication of a mediated glucose oxidase recessed microelectrode for the amperometric determination of glucose. J Electroanal Chem 414:235–246.
49. Daly DJ, O'Sullivan CK, Guilbault GG. 1999. The use of polymers coupled with metallised electrodes to allow H_2O_2 detection in the presence of electrochemical interferences. Talanta 49:667–678.
50. Li QS, Ye BC, Liu BX, Zhong JJ. 1999. Improvement of the performance of H_2O_2 oxidation at low working potential by incorporating TTF-TCNQ into a platinum wire electrode for glucose determination. Biosens Bioelectron 14:327–334.
51. Li J, Chia LS, Goh NK, Tan SN. 1999. Renewable silica sol-gel derived carbon composite based glucose biosensor. J Electroanal Chem 460:234–241.
52. Popescu IC, Cosnier S, Labbe P. 1997. Peroxidase-glucose oxidase-poly(amphiphilic pyrrole) bioelectrode for selectively mediated amperometric detection of glucose, Electroanalysis 9:998–1004.

53. Li HH, Yan SH, Qi DY, Liu HY. 1998. Biosensors and clinic application based on immobilization of enzymes with beta-cyclodextrin. Proc Biochem Biophys 25:162–166.
54. Pern B, Lee HS, Gorton L, Skotheim T, Bartlett P. 1995. Redox polymers for electrocatalytic oxidation of NADH- a random block methyl-siloxane polymer containing meldola blue. Electroanalysis 7:935–940.
55. Choi JW, Min JH, Jung JW, Rhee HW, Lee WH. 1997. LB film containing acetylcholinesterase for fiber-optic organophosphorus sensor. Mol Cryst Liq Cryst Sci Technol, Sect A 294:451–454.
56. Ruan CM, Yang R, Chen XH, Deng JQ. 1998. A reagentless amperometric hydrogen peroxide biosensor based on covalently binding horseradish peroxidase and thionine using a thiol-modified gold electrode. J Electroanal Chem 455:121–125.
57. Dzyadevich SV, Korpan YI, Arkhipova VN, Alesina MY, Martelet C, El'Skaya AV, Soldatkin AP. 1999. Application of enzyme field-effect transistors for determination of glucose concentrations in blood serum. Biosens Bioelectron 14:283–287.
58. Pijanowska DG, Torbicz W. 1997. pH-ISFET based urea biosensor. Sens Actuators, B 44: 370–376.
59. Gorchkov DV, Soldatkin AP, Poyard S, Jaffrezic-Renault N, Martelet C. 1997. Application of charged polymeric materials as additional permselective membranes for improvement of the performance characteristics of urea-sensitive enzymatic field effect transistors .1. Determination of urea in model solutions. Mater Sci Eng, C 5: 23–28.
60. Karyakin AA, Karyakina EE, Gorton L. 1999. On the mechanism of H_2O_2 reduction at Prussian Blue modified electrodes. Electrochem Commun 1:78–82.
61. Sekine Y, Hall EAH. 1998. A lactulose sensor based on coupled enzyme reactions with a ring electrode fabricated from tetrathiafulvalen-tetracyanoquinodimethane. Biosens Bioelectron 13: 995–1005.
62. Zhang W, Chang HD, Rechnitz GA. 1997. Dual-enzyme fiber optic biosensor for pyruvate. Anal Chim Acta 350:59–65.
63. Volotovsky V, Kim N. 1998. EDTA determination by urease-based inhibition biosensor. Electroanalysis 10:61–63.
64. Chung MS, Lee YT, Lee HS. 1998. Flow injection biosensor for the detection of anti-cholinesterases. J Biochem Mol Biol 31:296–302.
65. Gyurcsanyi RE, Vagfoldi Z, Toth K, Nagy G. 1999. Fast response potentiometric acetylcholine biosensor. Electroanalysis 11:712–718.
66. Moreno-Bondi MC, Wolfbeis O. 1990. Oxygen Optrode for use in a fiber-optic glucose biosensor. Anal Chem 62:2377–2380.
67. Shtelzer S, Braun S. 1994. An optical biosensor based upon glucose immobilized in sol-gel silicate matrix. Biotechnol Appl Biochem 19:293–305.
68. Rosenweig Z, Kopelman R. 1996. Analytical properties and sensor size effects of a micrometer-sized optical fiber glucose biosensor. Anal Chem 68:1408–1413.
69. Tolosa L, Malak H, Raob G, Lakowicz JR. 1997. Optical assay for glucose based on the luminescence decay time of the long wavelength dye Cy5 (TM). Sens Actuators, B 45:93–99.
70. Marazuela MD, Cuesta B, MorenoBondi MC, Quejido A. 1997. Free cholesterol fiber-optic biosensor for serum samples with simplex optimization. Biosens Bioelectron 12:233–240.
71. Li XP, Rosenzweig Z. 1997. A fiber optic sensor for rapid analysis of bilirubin in serum. Anal Chim Acta 353:263–273.
72. Marazuela MD, Moreno-Bondi MC. 1998. Determination of choline-containing phospholipids in serum with a fiber-optic biosensor. Anal Chim Acta 374:19–29.
73. Yamanaka SA, Nishida F, Ellerby LM, Nishida CR, Dunn B, Valentine JS, Zink JI. 1992. Enzymatic activity of glucose oxidase encapsulated in transparent glass by sol-gel method. Chem Mater 4:497–500.
74. Trettnak W, Wolfbeis OS. 1989. Fully reversible fibre-optic glucose biosensor based on the intrinsic fluorescence of glucose-oxidase. Anal Chim Acta 221:195–203.
75. Katz E, Riklin A, Heleg-Shabtai V, Willner I, Buckmann AF. 1999. Glucose oxidase electrodes via reconstitution of the apo-enzyme: tailoring of novel glucose biosensors. Anal Chim Acta 385: 45–58.
76. Savitri D, Mitra CK. 1998. Electrochemistry of reconstituted glucose oxidase on carbon paste electrodes. Bioelectrochem Bioenerg 47:67–73.
77. Glaxier SA, McCurley MF. 1995. Biosensor applications for bioprocess monitoring and drug analysis. Biopharmacology 8:38–48.
78. Karube I, Ikebukuro K, Mukakami Y, Yokoyama K. 1995. Micormachining technology and biosensors. Ann NY Acad Sci 26:101–108.
79. Atanasov P, Yang S, Salehi C, Ghindilis AL, Wilkins E, Schade D. 1997. Implantation of a refillable glucose monitoring-telemetry device. Biosens Bioelectron 12:669–680.
80. Fang Q, Shi XT, Sun YQ, Fang ZL. 1997. A flow injection microdialysis sampling chemiluminescence system for *in vivo* on-line monitoring of glucose in intravenous and subcutaneous tissue fluid microdialysates. Anal Chem 69:3570–3577.
81. Shen Z, Cho W.1995. Highly efficient immobilization of phospholipase A2 and its biomedical applications. J Lipid Res 36:1147–1151.
82. Bernstein H, Yang VC, Lund D, Randhawa M,

Harmon W, Langer R. 1987. Heparinase immobilization. Characterization and optimization. Kidney Intern 321:452–463.

83. Shpigel E, Goldlust A, Efroni G, Avraham A, Eshel A, Dekel M, Shoseyov O. 1999. Immobilization of recombinant heparinase I fused to cellulose-binding domain. Biotechnol Bioeng 65:17–23.
84. Lizano C, Sanz S, Luque J, Pinilla M. 1998. *In vitro* study of alcohol dehydrogenase and acetaldehyde dehydrogenase encapsulated into human erythrocytes by an electroporation procedure. Biochem Biophys Acta 1425:328–336.
85. Jones MN, Hill KJ, Kaszuba M, Creeth JE. Antibacterial reactive liposomes encapsulating coupled enzyme systems. Int J Pharm 126:107–117.
86. Hill KJ, Kaszuba M, Creeth JE, Jones MN. 1997. Reactive liposomes encapsulating a glucose oxidase-peroxidase system with antibacterial activity. Biochem Biophys Acta 1326:37–46.
87. Magnani M, Fazi A, Mangani F, Rossi L, Mancini U. 1993. Methanol detoxification by enzyme-loaded erythrocytes. Biotechnol Appl Biochem 18: 217–226.
88. Niesman MR, Johnson KA, Penn JS. 1997. Therapeutic effect of liposomal superoxide dismutase in an animal model of retinopathy of prematurity. Neurochem Res 22:597–605.
89. Cruz MEM, Gaspar MM, Lopes F, Jorge JS, Ferezsoler R. 1993. Liposomal L-asparagines-*in vitro* evalution. Int J Pharm 96:67–77.
90. Gaspar MM, PerezSoler R, Cruz MEM. 1996. Biological characterization of L-asparaginase liposomal formulations. Cancer Chemother Pharm 38:373–377.
91. Walfe EA, Chang TMS. 1987. Orally ingested microencapsulated urease and adsorbent, zirconium-phosphate, to remove urea in kidney failure. Int J Artif Organs 10:269–274.
92. Garin M, Rossi L, Luque J, Magnani M. 1995. Lactate catabolism by enzyme-loaded red blood cells. Biotechnol Appl Biochem 22:295–303.
93. Karle P, Muller P, Renz R, Jesnowski R, Saller R, von Rombs K, Nizze H, Liebe S, Gunzburg WH, Salmons B, Lohr M. 1998. Intratumoral injection of encapsulated cells producing an oxazaphosphorine activating cytochrome P450 for targeted chemotherapy. Adv Exp Med Biol 451:97–106.
94. Lohr M, Muller P, Karle P, Stange J, Mitzner S, Jesnowski R, Nizze H, Liebe S, Salmons B, Gunzburg WH.1998. Targeted chemotherapy by intratumour injection of encapsulated cells engineered to produce CYP2B1, an ifosfamide activating cytochrome P450. Gene Ther 5:1070–1078.
95. Gunzburg WH, Karle P, Renz R, Salmons B, Renner M. 1999. Characterization of a human cell clone expressing cytochrome P450 for safe use in human somatic cell therapy. Ann NY Acad Sci 880:326–336.
96. Petrikovics I, Hong K, Omburo G, Hu QZ, Pei L, McGuinn WD, Sylvester D, Tamulinas C, Papahadjopoulos D, Jaszbereny JC, Way JL. 1999. Antagonism of paraoxon intoxication by recombinant phosphotriesterase encapsulated within sterically stabilized liposomes. Toxicol Appl Pharm 156:56–63.
97. Pei L, Petrikovics I, Way JL. 1995. Antagonism of the lethal effects of paraoxon by carrier erythrocytes containing phosphotriesterase. Fundam Appl Toxicol 28:209–214.
98. Axley MJ, Dad LK, Harabin AL. 1996. Hydrogenase encapsulation into red blood cells and regeneration of electron acceptor. Biotechnol Appl Biochem 24:95–100.
99. Regnault C, Roch-Arveiller M, Tissot M, Sarfati G, Giroud JP, Postaire E, Hazebroucq G. 1995. Effect of encapsulation on the anti-inflammatory properties of superoxide dismutase after oral administration. Clin Chim Acta 240:117–127.
100. Yarosh DB, O'Connor A, Alas L, Potten C, Wolf P. 1999. Photoprotection by topical DNA repair enzymes: Molecular correlates of clinical studies. Photochem Photobiol 69:136–140.
101. Yarosh DB, Klein J, Kibitel J, Alas L, O'Connor A, Cummings B, Grob D, Gerstein D, Gilchrest BA, Ichihashi M, Ogoshi, M, Ueda M, Fernandez V, Chadwick C, Potten CS, Proby CM, Young AR, Hawk JLM. 1996. Enzyme therapy of xeroderma pigmentosum: Safety and efficacy testing of T4N5 liposome lotion containing a prokaryotic DNA repair enzyme. Photodermatol Photogr 12:122–130.
102. Jorgensen K, Kiebler T, Hylander I, Vermehren C. 1999. Interaction of a lipid-membrane destabilizing enzyme with PEG-liposomes. Int J Pharm 183:21–24.
103. Alvarez FJ, Herraez A, Murciano JC, Jordan JA, Diez JC, Tejedor MC. 1996. *In vivo* survival and organ uptake of loaded carrier rat erythrocytes. J Biochem 120:286–291.
104. Johnson KM, Tao JZ, Kennan RP, Gore JC. 1998. Gadolinium-bearing red cells as blood pool MRI contrast agents. Magn Reson Med 40:133–142.
105. Kravtzoff R, Desbois I, Lamagnere JP, Muh JP, Valat C, Chassaigne M, Colombat P, Ropars C. 1996. Improved pharmacodynamics of L-asparaginaseloaded in human red blood cells. Eur J Clin Pharmacol 49:465–470.
106. DeLoach JR, Droleskey RE. 1993. Endocytosis during the preparation of mouse and human carrier erythrocytes. Biotechnol ApplBiochem18: 83–92.
107. DeLoach JR, Droleskey RE, Andrews K. 1991. Encapsulation by hypotonic dialysis in human erythrocytes: A diffusion or endocytosis process. Biotechnol Appl Biochem 13:72–82.

108. Chiarantini L, Antonelli A, Rossi L, Fraternale A, Magnani M. 1994. Red blood cell phagocytosis following hexokinase inactivation. Cell Biochem Funct 12:217–220.
109. Sanz S, Pinilla M, Garin M, Tipton KF, Luque J. 1995. The influence of enzyme concentration on the encapsulation of glutamate dehydrogenase and alcohol dehydrogenase in red blood cells. Biotechnol Appl Biochem 22:223–231.
110. Alvarez FJ, Herraez A, Tejedor MC, Diez JC. 1996. Behaviour of isolated rat and human red blood cells upon hypotonic-dialysis encapsulation of carbonic anhydrase and dextran. Biotechnol Appl Biochem 23:173–179.
111. Jordan JA, Alvarez FJ, Tejedor MC, Diez JC. 1999. Band-3 crosslinking-induced targeting of mouse carrier erythrocytes. Biotechnol Appl Biochem 29:59–65.
112. Jordan JA, Alvarez FJ, Lotero LA, Tejedor MC, Diez JC. 1998. *In vivo* behaviour of rat band 3 cross-linked carrier erythrocytes. Biochimie 80: 325–332.
113. Dusserre N, Lessard C, Paquette N, Perron S, Poulin L, Tremblay M, Beauchamp D, Desormeaux A, Bergeron MG. 1995. Encapsulation of foscarnet in liposomes modifies drug intracellular accumulation, *in vitro* anti-HIV-1 activity, tissue distribution and pharmacokinetics. AIDS 9: 833–841.
114. Harvie P, Desormeaux A, Bergeron MC, Tremblay M, Beauchamp D, Poulin L, Bergeron MG. 1996. Comparative pharmacokinetics, distributions in tissue, and interactions with blood proteins of conventional and sterically stabilized liposomes containing 2′,3′-dideoxyinosine. Antimicrob Agents Chemother 40:225–229.
115. Petrikovics I, Hong K, Omburo G, Hu QZ, Pei L, McGuinn WD, Sylvester D, Tamulinas C, Papahadjopoulos D, Jaszberenyi JC, Way JL. 1999. Antagonism of paraoxon intoxication by recombinant phosphotriesterase encapsulated within sterically stabilized liposomes. Toxicol Appl Pharmacol 156:56–63.
116. Allen TM. 1994. Long-circulating (sterically stabilized) liposomes for targeted drug delivery. Trends Pharmacol Sci 15:215–220.
117. Allen TM. 1998. Liposomal drug formulations. Rationale for development and what we can expect for the future. Drugs 56:747–756.
118. Bunn B, Miller JM, Dave BC, Valentine JS, Zink JI. 1998. Strategies for encapsulating biomolecules in sol-gel matrices. Acta Mater 46:734–741.
119. Avnir D, Braun S, Lev O, Ottolenghi M. 1994. Enzymes and proteins entrapped in sol-gel materials. Chem Mater 6:1605–1614.
120. Gill I, Ballesteros A. 1998. Encapsulation of biologicals within silicate, siloxane, and hybrid sol-gel polymers: An efficient and generic approach. J Am Chem Soc 120:8587–8598.