REVIEW

Nanoparticles as biochemical sensors

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Abstract: There is little doubt that nanoparticles offer real and new opportunities in many fields, such as biomedicine and materials science. Such particles are small enough to enter almost all areas of the body, including cells and organelles, potentially leading to new approaches in nanomedicine. Sensors for small molecules of biochemical interest are of critical importance. This review is an attempt to trace the use of nanomaterials in biochemical sensor design. The possibility of using nanoparticles functionalized with antibodies as markers for proteins will be elucidated. Moreover, capabilities and applications for nanoparticles based on gold, silver, magnetic, and semiconductor materials (quantum dots), used in optical (absorbance, luminescence, surface enhanced Raman spectroscopy, surface plasmon resonance), electrochemical, and mass-sensitive sensors will be highlighted. The unique ability of nanosensors to improve the analysis of biochemical fluids is discussed either through considering the use of nanoparticles for in vitro molecular diagnosis, or in the biological/biochemical analysis for in vivo interaction with the human body.

Keywords: nanoparticles, quantum dots, nanomedicine, biochemical sensors, antibodies, in vivo, in vivo

Introduction

There are well-developed methods to synthesize nanomaterials (eg, nanorod,[1] nanocube,[2] nanoshell,[3] which have found real applications in practice. The light-scattering properties of new nanomaterials[4] in different composition, size, and shape have greatly attracted the attention of analysts, so the applications of nanomaterials for analytical purposes have grown dramatically. Because of their unique advantages, nanoparticles (NPs), with colorful light-scattering properties, are comparable to optical probes with various fluorescent dyes. For example, the light-scattering power of a single NP label is in order of magnitude greater than a single fluorescent label, and the light signals generated from these nanomaterials are not prone to photobleaching, even under illumination for a long time. What is more, in light-scattering detection, only a simple instrument is required, compared with a fluorescent system.[5]

Metal NPs with large diameter ($>$30 nm) exhibit strong light-scattering in the visible region, which could be used directly for light-scattering labels in biochemical assay. However, small NPs, which apparently do not feature light-scattering, can also be used to sense chemical interactions (eg, antigen–antibody, avidin–biotin, DNA hybridization, and electrostatic attraction), since enhanced light-scattering signals would be produced if these NPs were to aggregate during the interactions. Because

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the enhanced light-scattering signals from the aggregated species are sufficiently sensitive to monitor NP aggregation, in a simple procedure, biochemical assay based on such light-scattering signals has been widely used in the determination of DNA, proteins, and drugs.[5]

Immunoassay is important in basic research and clinical diagnostics. Sandwich-type immunoassay, using a primary antibody to capture the analyte, and a labeled, secondary antibody to detect antigen binding, is widely accepted procedure. The aggregation of nanomaterials, especially gold (Au), induced by the immunoreactions offers a new approach for immunoassay,[6] using light-scattering detection to obtain high sensitivity. The enhanced light-scattering signals from the AuNP aggregates, induced by the immunoreaction between the apolipoprotein and its AuNP-labeled antibody, have been successfully applied in clinical diagnostics.[7] There have been similar reports about light-scattering signals involving such different materials as silver nanoparticles (AgNP) and analytes (eg, fibrinogen and human immunoglobulin G [h-IgG]).[8] Interactions between proteins and nanomaterials have been applied in quantitative assays of proteins in biochemistry and clinical diagnostics. Electrostatic attraction between proteins and nanomaterials with opposite charge induces assembly of proteins or nanomaterials, resulting in enhanced light-scattering signals. Various nanomaterials (either metal or nonmetal), functionalized with the opposite charge to the protein, have been used to detect protein quantitatively with ng sensitivity.

This information stimulates our interest to review major research work related to the use of nanoparticles as biochemical sensors, and to highlight the advantages of using this strategy over the common analytical methods.

Nanoparticles in analytical biochemistry

One of the most important functions of nanoparticles is catalysis, especially with noble metal nanoparticles, which have high catalytic activity for many chemical reactions. Because nanomaterials also have good biocompatibility,

they are used to immobilize biomolecules for the fabrication of biosensors.

Glucose nanosensors

Glucose is a key metabolite for living organisms, especially in the case of patients suffering diabetes. Since the first enzyme electrode was reported in 1962 by Clark and Lyons.[9] There has been an increasing demand for the development of new methodologies for a rapid, simple, reliable, reproducible, and sensitive determination of glucose. The detection of glucose by electrochemical biosensors is based on the electrochemical oxidation of hydrogen peroxide (H_2O_2) generated by enzyme-catalyzed oxidation of glucose at anodic potentials (N +0.6 V vs Ag/AgCl).[10] The immobilization of glucose oxidase (GOx) on the electrode surface, which is one of the main factors that affects the performance of a glucose biosensor, has received considerable attention in recent years.[11] A new amperometric glucose biosensor was constructed, based on the immobilization of GOx with cross-linking in a matrix of chitosan (CHIT) on a glassy carbon electrode (GCE), which was modified by layer-by-layer-assembled carbon nanotube (CNT)/CHIT/gold nanoparticle (GNP) multilayer films. With the increasing of CNT/CHIT/GNP layers, the response current to H_2O_2 changed regularly, and the response current reached a maximum value when the number of CNT/CHIT/GNP layers was 8. The assembly process for the multilayer films was simple to operate. With GOx as an enzyme model, a new glucose biosensor was fabricated. The excellent electocatalytic activity and special structure of the enzyme electrode resulted in a detection limit of 3×10^{-6} M, estimated at a signal-to-noise ratio of 3, at a fast response time (less than 6 s). Moreover, it exhibited good reproducibility and stability. Human plasma samples were assayed in order to demonstrate the practical usage of the biosensor. Fresh plasma sample was first analyzed in the local hospital with the ASCA AGII Chemistry System (Landmark Scientific, Greensboro, NC). The samples were then re-assayed with the CNT/CHIT/GNP)8/GOx/GCE. A plasma sample (0.5 mL) was added into 2 mL of 0.067

Table 1 Performances of some NADH sensors

NADH sensor method	Linear range	Detection limit	Reference
GC/AuNP/amperometry	1.25×10^{-6} M to 3.08×10^{-4} M	2.5×10^{-7} M	23
ITO/DA-TiO$_2$/pliotoelectrochemical	5.0×10^{-7} M to 1.2×10^{-4} M	1.4×10^{-7} M	19
CNF-CPE/amperometry	1.15×10^{-7} M	0.2×10^{-7} M	24
Pd-AuNP/spectroscopic	1.6×10^{-4} M	4.5×10^{-5} M	24

Abbreviations: NADH, dihydronicotinamide adenine dinucleotide; GC, glassy carbon electrode; AuNP, gold nanoparticles; ITO, indium-tin-oxide; DA, dopamine; TiO$_2$, titanium dioxide; CNF, carbon nanofibers; CPE, carboxypeptidase E; Pd, palladium; M, mole.

M phosphate buffer saline (pH 7.0) and the response was obtained at +0.6 V. The contents of glucose in blood could then be calculated from the calibration curve. The results are satisfactory and agree closely with those measured by the biochemical analyzer in the hospital.[11]

Recently, platinum nanoparticles (PtNPs) were deposited on mesoporous carbon material (CMK-3). GOx was immobilized in the resulting Pt nanoparticles/mesoporous carbon (Pt/CMK-3) matrix, and then the mixture was cast on a glassy carbon electrode using gelatin as a binder. The glucose biosensor exhibited excellent current response to glucose after cross-linking with glutaraldehyde. At 0.6 V (vs saturated calomel electrode) the response current was linear to glucose concentration in the range of 0.04–12.2 mM. The response time (time for achieving 95% of the maximum current) was 15 s and the detection limit (signal-to-noise ratio = 3) was 1 µM. The Michaelis–Menten constant (Km^{app}) and the maximum current density were 10.8 mM and 908 µAcm^{-2} respectively. The activation energy of the enzymatic reaction was estimated to be 22.54 kJmol^{-1}.[12] The biosensor showed good stability. It achieved the maximum response current at about 52°C and retained 95.1% of its initial response current after being stored for 30 days. In addition, some fabrication and operational parameters for the biosensor were optimized in this work. The biosensor was used to monitor the glucose levels of serum samples after being covered with an extra Nafion film to improve its anti-interferent ability, and satisfactory results were obtained. The interference of some electroactive compounds to the glucose response was examined by Yu et al.[12] When the glucose concentration was 5.6 mM, the deviation of response was calculated according to (Ii-Ig)/Ig, where Ii and Ig were the steady-state currents recorded for solutions containing glucose and interferent, or glucose alone. At Pt/CMK-3-GOx-gelatin/GCE, the deviations caused by ascorbic acid (AA) (0.1 mM), p-acetaminophenol (0.05 mM) and uric acid (UA) (0.5 mM) were 0.44%, 4.69%, and 13.2% respectively. This indicates that the biosensor suffersed some influence from coexistents in serum samples. Therefore, 1 µM percentage by weight Nafion was cast on the surface of Pt/CMK-3-GOx-gelatin/GCE to prevent the interference. And then, at Nafion/Pt/CMK-3-GOx-gelatin/GCE, the deviations were in the acceptable range (±10%).[12]

A silver hexacyanoferrate nanoparticle (AgHCFNP)/CNT modified GCE was fabricated and then successfully used for the simultaneous determination of AA, UA, and dopamine (DA) by cyclic voltammetry (CV). Meissam et al[13] reported for the first time a simultaneous determination of AA, DA, and UA using a glassy carbon electrode modified with a mixture of AgHCFNPs and multiwalled carbon nanotubes. Compared with the existing reports about simultaneous determination of AA, DA, and UA, the proposed method is much more convenient, as to preparation of the GCE/CNT-AgHCFNP-modified electrode.

The voltametric behaviors of a mixture of AA, DA, and UA on bare GCE, GCE/CNT, and GCE/CNT-AgHCFNP elctrodes were studied, and a comparison of the CV results is shown in Figure 1. DA showed broad oxidation peak and could not be discriminated from AA and UA when a bare GC electrode was used (Figure 1A). For DA, the anodic and cathodic peaks appear at 0.482 V and 0.552 V, respectively, and the separation of peak potential is about 0.070 V. Moreover, the cathodic peak current is much smaller than the anodic peak current. It is clear that the electrochemical reaction of DA at the bare GCE is irreversible, indicating the sluggish electron transfer kinetic of DA at the bare GCE, which may be related to electrode fouling caused by the deposition of this compound and its oxidation product on the electrode surface.[14] When GCE/CNT or GCE/CNT-AgHCFNPs was used as the working electrode, the detection sensitivity was improved significantly, and effective separation of the anodic peaks of AA, DA, and UA was obtained.[13]

It could be suggested that the modification of the GCE surface by CNT-AgHCFNPs not only improves the electrochemical catalytic activities towards the oxidation of AA, DA, and UA, but also resolves the overlapped oxidation peaks of AA, DA, and UA into three well-defined peaks at potentials 0.193 V, 0.444 V, and 0.620 V in CV, respectively. Comparison between GCE/CNT and GCE/CNT-AgHCFNPs demonstrates that GCE/CNT-AgHCFNPs better facilitates the simultaneous determination of AA, DA, and UA with good stability, sensitivity, and selectivity. In comparison with other electroanalytical methods that report for the simultaneous determination of ascorbic acid, dopamine, and uric acid, this method has better figures of merit. The proposed method can be applied to the simultaneous determination of AA, DA, and UA concentrations in real samples with satisfactory results.[13]

Choline nanosensors

Choline is distributed in the central and peripheral nervous systems of mammals.[15] It is also an important component of phospholipids (lecithin and sphingomyelin), which are required for the synthesis of the neurotransmitter precursor acetylcholine.[16] Classified as vitamin-like, choline is generally uptaken by the human body from both dietary and endogenous sources. Determination of choline is carried out
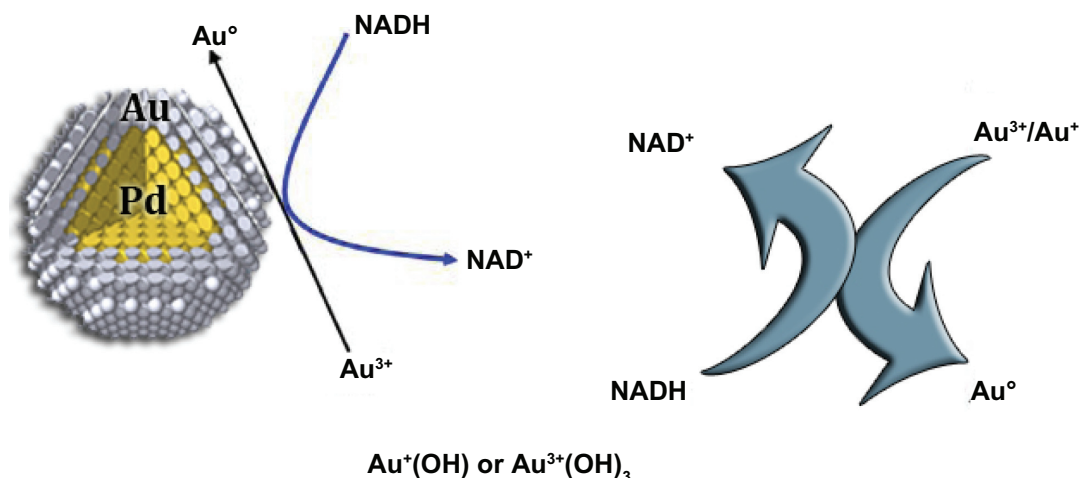
Figure 1 Proposed mechanism for Pd (core)-Au (shell) NPs catalyzed oxidation of NADH to NAD.[24]
Abbreviations: NADH, dihydronicotinamide adenine dinucleotide; NAD+, nicotinamide adenine dinucleotide; NPs, nanoparticles; Au, gold; Pd, palladium.

by a number of techniques nowadays. As the most promising alternative, enzyme-based biosensors have emerged in the past few years for direct monitoring of choline. A number of enzyme electrodes, with immobilized ChOx, were reported for choline determination based on the detection of liberated H_2O_2.[17,18] The manganese dioxide (MnO_2) nanoparticle-modified electrodes show a bidirectional electrocatalytic ability toward the reduction/oxidation of H_2O_2. Based on this property, a choline biosensor was fabricated via direct and facile electrochemical deposition of a biocomposite that was made of chitosan hydrogel, ChOx, and MnO_2 nanoparticles onto a glassy carbon electrode. The biocomposite is homogeneous and easily prepared, and provides a shelter for the enzyme to retain its bioactivity. The results of square wave voltammetry showed that the electrocatalytic reduction currents increased linearly with the increase of choline chloride concentration in the range 1.0×10^{-5} M–2.1×10^{-3} M, and no obvious interference from AA and UA was observed. Good reproducibility and stability were obtained.

Carbon nanotube-modified electrodes have shown excellent electrocatalytic activity towards some electromotive substances, such as H_2O_2, due to the fast electron transfer ability of CNTs.[19]

Nicotinamide adenine dinucleotide nanosensors

Nicotinamide adenine dinucleotide (NAD+) and dihydronicotinamide adenine dinucleotide (NADH) are the key central charge carriers in living cells. NAD+ is a ubiquitous cofactor utilized by more than 300 dehydrogenase enzymes. The nicotinamide region is the site of reversible redox processes in living cells. NAD+ and phosphorylated NAD+ (NADP+)

are the two forms of coenzyme. NADH and NADPH are reduced forms of NAD+, NADP+ respectively. While NAD+ and NADP+ accept electrons from other molecules, NADH and NADPH donate electrons. NADH plays a key role in the multi-enzyme redox system in the microchrondrial transport chain.[20] A large amount of byproduct is often produced during the direct electrode process. Also, the adsorbed NAD+ could cause electrode fouling. Further, the high potentials used for the oxidation of NADH could also cause simultaneous oxidation of many interfering substrates, such as AA.[21]

The sensitivity of detection of NADH was determined from the plot of absorbance (at 340 nm) vs C_{NADH}. Typical calibration plots were deduced from ultraviolet visible spectroscopy (UV-vis) recorded at 10 min and 90 min. The sensitivity of NADH was determined to be 0.45 mM per unit of absorbance. The detection limit of NADH was estimated to be 0.45 µM. The spectrophotometer can measure absorbance with an accuracy of 0.001. Because the sensitivity of detection for NADH is 0.45 mM for unit absorbance change, the detection limit for NADH could be 0.45 µM. Nanoparticle-NADH sensors performances are different depending on the composition of the sensor, showing different detection limits and linearity.[22,23]

A plausible mechanism for Palladium (Pd) (core)-Au (shell) NP-catalyzed conversion of NADH is proposed by Gopalan et al (2009).[24] It is clear that the position of surface plasmon resonance (SPR) bands of AuNPs showed significant shift during the catalytic transformation of NADH to NAD+. It is therefore envisioned that the surface of the Au layer catalyzes the reaction. They presume that oxygen molecules adsorbed on the surface of the Au layer may induce oxidation of NADH to NAD+. It is known that reactions such as low

temperature oxidation of carbon monoxide or propylene that involve oxygen as oxidant are catalyzed by AuNPs.[25] In the Gopalan model,[24] Au^+ or Au^{3+} species are expected to present in the shell layer (Au) of the Pd (core)-Au (shell) catalyst. NADH oxidation was facilitated by the conversion of Au^{3+}/Au^+ to Au^0 (Figure 1).

Lactate nanosensors

L-lactate is constantly produced from pyruvate by lactate dehydrogenase (LDH) in a process of fermentation during normal metabolism and exercise. L-lactate concentration plays an important role in clinical diagnostics, medicine validation, and food analysis. For example, the concentration of L-lactate in blood is a fundamental parameter for the prevention and diagnosis of a number of clinical disorders such as hypoxia[26,27] some acute heart diseases, and drug toxicity tests. Over the past few years, various approaches based on LDH or lactate oxidase have been developed for the detection of lactate. They employ different methods to attach enzymes on the sensing layer, including adsorption,[28] cross-linking,[29] covalent attachment,[30,31] conducting polymer entrapment,[32,33] and confinement in sol-gel matrix.[34] Flow injection analysis[35,36] and mediator- based lactate biosensors[37] have also been developed. In the LDH-based amperometric biosensor, LDH catalyzes the oxidation of lactate to pyruvate in the presence of the oxidized form of NAD^+. However, the LDH-based biosensors have some common drawbacks, including the fact that they are unstable and that the electrochemical oxidation of the reduced form of NAD commonly occurs at high potentials, which can allow interference from other electroactive compounds usually present in real samples. Moreover, the intermediates produced during the oxidation reaction can lead to electrode fouling. Therefore, there is a demand for the development of a biosensor that is stable, reproducible, and sensitive for lactate detection in real samples. Recently, Rahman et al[38] developed a lactate biosensor based on a conducting polymer, poly-5, 20-50,200-terthiophene-30-carboxylic acid (pTTCA), and multiwall carbon nanotube (MWNT) composite on a gold electrode. LDH and the oxidized NAD^+ were subsequently immobilized onto the pTTCA/MWNT composite film. Analytical performances and dynamic ranges of the sensor were determined, and the results showed that the sensitivity, stability, and reproducibility of the sensor improved significantly using the pTTCA/MWNT composite film. The calibration plot was linear (coefficient of determination = 0.9995) over the range of 5 to 90 μM. The sensitivity was approximately 0.0106 μA/μM, with a detection limit of 1 μM, based on a signal-to-noise ratio of 3. The applicability of the sensor for the analysis of L-lactate concentration in commercial milk and human serum samples was demonstrated successfully.

Triglyceride nanosensors

Estimation of triglycerides (triacylglycerols) is extremely important, since its high concentration (normal range: 40–160 mg/dL in men, and 35–135 mg/dL in women) can cause hyperlipidemia.[39–41] Apart from coronary diseases, hyperlipidemia is associated with several disorders, including diabetes mellitus, nephrosis, liver obstruction, and endocrine impairment. Moreover, estimation of triglyceride content in food has become important due to increased health awareness and stringent regulatory laws.[42] The conventional methods for triglycerides detection, such as colorimetric or fluorometric techniques, are based on enzymatic hydrolysis of triglycerides to glycerol and free fatty acids.[43–45] Most triglyceride biosensors reported to date are based on multi-enzymes, wherein a biochemical reaction depends upon the enzyme kinetics of another enzymatic reaction. McGowan et al[46] have described an enzymatic method for serum triglyceride detection that involves simultaneous catalysis of four bio-enzymes (lipase, glycerol kinase, L-a-glycerophosphate oxidase, and peroxidase).[46] These methods are, however, time-consuming, complicated, and expensive. Recently, lipase has been immobilized onto a sol-gel-derived nanostuctured cerium oxide (Nano-CeO$_2$, 35 nm) film deposited onto indium-tin-oxide-(ITO) coated glass plate for tributyrin detection.[47] The Nano-CeO$_2$/ITO electrode and lipase/nano-CeO$_2$/ITO bioelectrode have been characterized using scanning electron microscopy (SEM) and CV. The electrochemical response of the lipase/nano-CeO$_2$/ITO bioelectrode towards tributyrin, investigated using CV studies, exhibits linearity, detection limit, and shelf life as 50–500 mg/dl, 32.8 mg/dl, and 12 weeks respectively. The value of the apparent Michaelis–Menten constant obtained as 22.27 mg/dl (0.736 mM) for the lipase/nano-CeO$_2$/ITO bioelectrode, indicating a high affinity of lipase with tributyrin samples (Figure 2).[47]

Ochratoxin A detection

Among the various metal oxides, nanostructured zinc oxide (ZnO) has been used for immunosensor applications.[48–51] Its unique properties, such as high isoelectric point (IEP ≈ 9.5) and biocompatibility, facilitate immobilization of an enzyme and protein having low IEP via electrostatic interactions. Besides this, the positively charged ZnO nanoparticles not only provide a friendly microenvironment for immobilizing negatively charged rabbit antibodies (r-IgGs; IEP ≈ 5.5)
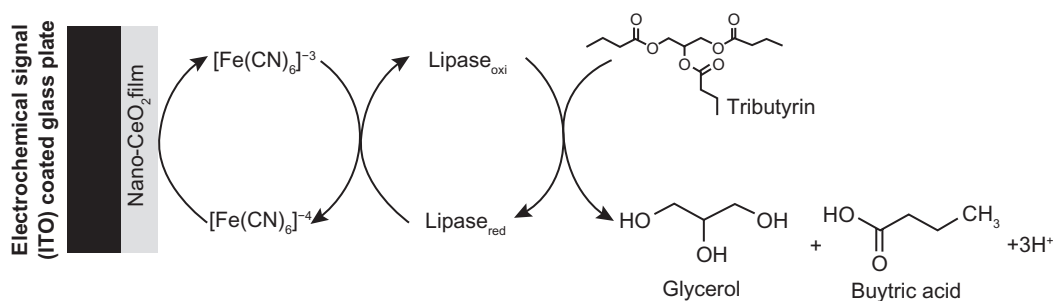
Figure 2 Electrochemical reaction at lipase/nano-CeO$_2$/ITO bioelectrode.[47]
Abbreviations: CeO$_2$, cerium oxide; ITO, indium-tin-oxide.

and retain bioactivity, but also accelerate electron transfer communication between the protein and the electrode to a large extent.[48,49] Moreover, nontoxicity, high chemical stability, and high electron transfer capability make Nano-ZnO a promising material for immobilization of desired biomolecules for fabrication of an immunosensor. The sol-gel derived Nano-ZnO has recently emerged as an attractive material due to its ease of preparation under ambient conditions, tunable porosity, high thermal stability, chemical inertness, and negligible swelling in aqueous and nonaqueous solutions for immobilization of desired biomolecules (enzymes, proteins, and antibodies)[52] Liu et al[51] have prepared stable, nanosized, flower-like ZnO film by a hydrothermal method for a H$_2$O$_2$ sensor. ZnO nanocomb has been fabricated in bulk quantity, by the vapor phase transport method, for glucose detection.[48] Wei et al[48] have grown ZnO nanorods on Au electrodes for the development of an enzymatic glucose biosensor.

Ochratoxin-A (7-[L-β-phenylalanylcarbonyl]-carboxyl-5-chloro-8-hydroxy-3,4-dihydro-3R-methylisocumarin, OTA) is one of the most abundant food-contaminating mycotoxins.[53–57] OTA is found in tissues and organs of animals, including human blood and breast milk, and is known to produce nephrotoxic, tetratogenic, carcinogenic, and immune toxic activity in several animal species. OTA contamination has been reported in cereals, coffee, wines, dried fruits, and animal feeds, as well as in tissues and blood of animals and human beings.[55,56,58] It affects humans mainly through consumption of improperly stored food products, and causes carcinogenicity. The International Agency of Research on Cancer has classified OTA as a possible carcinogenic compound (Group 2B, possibly by induction of oxidative DNA damage) for humans, since it causes immunosuppression and immunotoxicity.[55,56]

Nano-ZnO film has been deposited onto ITO glass plate for co-immobilization of r-IgGs and bovine serum albumin (BSA) for OTA detection. The results of x-ray diffraction studies reveal the formation of Nano-ZnO with average particle size ≈ 5.0 nm. Fourier transform infrared spectroscopy, SEM, and electrochemical impedance spectroscopy techniques have been used to characterize the Nano-ZnO/ITO electrode and the BSA/r-IgGs/Nano-ZnO/ITO immunoelectrode. The electrochemical impedimetric response of the BSA/r-IgGs/Nano-ZnO/ITO immunoelectrode, obtained as a function of OTA concentration, exhibits linearity as 0.006–0.01 nM/dm^3, detection limit of 0.006 nM/dm^3, response time of 25 s, and sensitivity of 189 Ω/nM/dm^{3}cm^{-2}, with a regression coefficient of 0.997.[55]

Urea nanosensors

Urea is widely distributed in nature, and its analysis is of considerable interest in clinical and agricultural chemistry.[60,61] It is known to be an important marker for evaluating uremic toxin levels. The normal level of urea in serum is from 15 to 40 mg/dL (2.5–7.5 mM/l). In patients suffering from renal insufficiency, urea concentrations in serum vary from 180 to 480 mg/dL and, at elevated levels above 180 mg/dL, hemodialysis is required. Apart from clinical applications, there is a growing demand for robust, reliable instrumentation toward the estimation of urea in other fields (eg, food science and environmental monitoring). As the principal component of nonprotein nitrogen in cow milk, urea is utilized as an indicator of protein-feeding efficiency.[62] Nanopatterned porous alumina, prepared by electrical anodization in acid solution, has been used as an enzyme electrode and pH sensor for urea detection. The biocompatibility of nano-size porous alumina as an immobilization matrix for biocolloidal systems has stimulated interest for improved sensor sensitivity. The sensor was tailored to enlarge the active surface area, which in turn increased the sensitivity of the sensor. Some other supports used for urea biosensor fabrication include bilayer lipid membranes,[63] carbon paste electrodes,[64] composite hydrogel membranes,[65] gelatin membranes,[66] inorganic matrices (eg, clays, laponite, and

zinc-aluminum-layered double hydroxide matrices),[67] silicon dioxide (SiO_2) films, glass beads, diatomite, silica, porous glass, hornblende, and molecular sieves.[68]

Srivastava et al[66] have described a urea biosensor that utilizes urease immobilized on gelatin beads. The system was successful in achieving a long storage stability, with a half-life of 240 days. The characteristics of this sensor include a detection limit from 0.8 to 23 mM and a response time of 6 min. Another sensing system has been proposed for urease immobilization on porous glass beads.[69] The sensor setup proposed by Seki's group[70] used urease immobilized on SiO_2 films as the pH-sensitive layer in light-addressable potentiometric sensors, with a detection range from 5–15 mM. However, more studies must be done in this field to fabricate a cost-effective, commercial sensor using these novel materials. The use of enzyme mixtures can provide improved performance in urea sensors. Seo et al[71] determined ammonia via amperometric methods, using the immobilization of L-glutamate dehydrogenase on the immobilon-AV affinity membrane. Incorporation of the enzyme into a carbon paste was performed by Yang et al[64] for the construction of an amperometric enzyme electrode. The enzyme-modified carbon paste electrode resulted in improved specificity and sensitivity. This system made use of bi-enzyme systems involving glutamate dehydrogenase and urease for urea estimation (Figure 3).

Nanoparticles in bioassays
Human immunoglobulin G

Bioassays are widely used in medical applications for sensitive and specific detection of biomolecules such as antibodies and antigens. The enzyme-linked immunosorbent assay (ELISA) is a well-known and commonly used tool for this purpose.[72] However, in typical ELISA systems, several washing steps are performed that lead to an increased risk of, for example, residual contamination or loss in reagent, thereby reducing the apparent sensitivity, accuracy, and/or reliability.
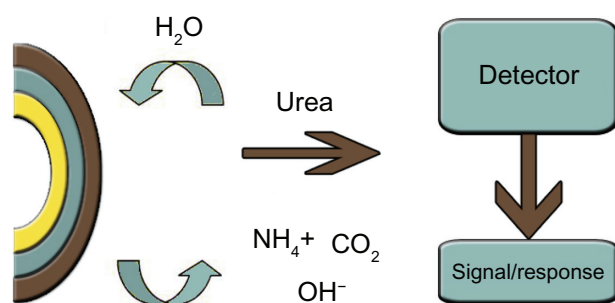

Figure 3 Schematic representation of a urea biosensor.[66]

Moreover, ELISA measurements give a static picture of the sample in terms of concentration of analyte, but provide no information about the binding kinetics or the dynamics of the reactions. A new approach for bioassays based on frequency- and time-domain measurements of magnetic nanoparticles (MNP) was recently introduced by Oisjoen et al.[73] They demonstrate a one-step wash-free bioassay measurement system capable of tracking biochemical binding events. They combine the high resolution of frequency- and high speed of time-domain measurement in a single device in combination with a fast one-step bioassay. The one-step nature of their magnetic nanoparticle-based assay reduces the time between sample extraction and quantitative results, while mitigating the risks of contamination related to washing steps. The bio-functionalized MNPs they use for their experiment are multi-core particles containing single domains of cobalt ferrous oxide. According to the manufacturer, the MNP system has a median hydrodynamic radius of 50 nm. The size distribution is a critical factor in determining the sensitivity of this system. Larger MNPs have smaller relative change in hydro-dynamic radii associated with molecules binding to their surfaces. The particles are functionalized with streptavidin, a biomolecule that has high affinity for biotin. According to specification from the provider, the MNPs can bind 80 pmol of biotinylated protein per mg of MNPs, which corresponds roughly to 30 poly(sebacic anhydride) (PSA) molecules per MNP. This system has an advantage in that it does not require a separation step, which is usually employed for PSA detection through Brownian relaxation measurements of functionalized MNPs.[73] There is no potential loss of signal associated with competitive binding of free analyte. This system has a sensitivity to 2 µL samples, which means that less sample volume from a patient is needed, which provides the capability to perform additional parallel screening-type measurement on a single sample.

Many immunoassay techniques have been developed. Some substances such as radioisotopes, enzymes, chemiluminescent, and fluorescent compounds have been used as labels in immunoassay. However, water-soluble cadmium tellurium (CdTe) nanocrystals have attracted considerable attention as novel biological luminescent labels, compared to the traditional labels, in recent years. They have some unique physical and chemical properties, such as excellent brightness, narrow and precise, tunable emission, negligible photobleaching, fairly high quantum yields, and good chemical stability. Lately, some luminescent nanoparticles are also becoming very attractive for immunoassays. Crystalline europium oxide

nanoparticles,[74] water-soluble poly (acrylic acid) grafted luminescent silicon nanoparticles[75] and cadmium/selenium-zinc/sulphur core-shell quantum dots[76] have been successfully applied as fluorescence labels in fluoroimmunoassays. The experimental procedure for determination of h-IgG using CdTe is very simple, involving the mixing of ten microliters of an analyte h-IgG with 0.8 ml of iron oxide (Fe_3O_4) and primary antibody solution. The mixture was incubated for 30 min at 37°C. The Fe_3O_4/primary antibody/h-IgG conjugate was formed, and then 2.0 mL of CdTe-secondary antibody conjugate was added into the tube to form the sandwich-type complex. Li et al[77] were able to use this technique and show that the sandwich-type complex in an immunoassay can be sensitively detected by colloidal CdTe nanoparticles as labels. The CdTe label has better sensitivity and reproducibility than the traditional fluorescent labels. In addition, the CdTe nanoparticle labeling procedure is very simple, and the biochemical activity of the labeled compound is almost unaffected. Dextran–Fe_3O_4 magnetic nanoparticles were used in the immunoassays, so the separation procedure is simple and rapid. This new technique may be applied in many types of antibody–antigen system.

Surface-enhanced Raman scattering (SERS)-based immunoassay, a new technique with high sensitivity, showing the ability to detect picomole to femtomole amounts, is known as a potent detection means for protein determination. Raman reporter-labeled immuno-gold nanoparticles are utilized, and the biologic proteins are finally assayed qualitatively or quantitatively by the characteristics SERS peaks of the reporter.[78,79] It is well known that the response ability of the SERS-based immunoassay is critically related to the size of the gold nanoparticles, as well as the aggregation degree of the immune aggregates.[80,81] Beermann et al[82] have investigated the surface enhancement ability of an isolated gold nanoparticle and found that a single nanoparticle with an average dimension of 25 nm was insufficient to enhance the Raman signals of the reporter. Similarly, it was discovered that no signal could be detected using small gold nanoparticles as a substrate.[77] Conversely, bigger gold nanoparticles will result in greater SERS signals. But their stability may decrease significantly with the increasing sizes when they are modified with Raman reporters.[82] Thus, taking into account the higher stability, many researchers have to carry out the SERS-based immunoassay with small gold nanoparticles. Meanwhile, an additional treatment (eg, silver stain) for SERS activation must be executed to ensure immunoassay with higher sensitivity.[83–85]

A highly sensitive immunoassay based on SERS has been developed with a novel immune marker, a Raman reporter-labeled immuno-gold aggregate on a SERS-active immune substrate. The features of those immune aggregates were characterized by UV-vis extinction spectra, transmission electron microscope images, SEM pictures, and SERS spectra. It is found that stable gold aggregates in appropriate morphologies can be induced by mixing proper amounts of reporter molecules with gold nanoparticles. Based on those reporter-labeled Au aggregates, immune aggregates with high stability can be prepared successfully by immobilizing antibody to the surface of the aggregates. Using this proposed immunoassay structure, the concentration detection of h-IgG was performed, and a calibration curve was obtained in the range from 100 ngmL^{-1} to 100 fgmL^{-1}. This opens a new avenue for sensitive immunoassay and other biochemical analysis based on SERS.

Steroids

Steroids are lipid compounds. With the exception of cholesterol, steroids are natural hormones or hormone precursors. The determination of the levels of steroid hormones is an important issue for the inspection of endocrinological disorders related to adrenal or gonadal function. Among analytical methods used to determine the concentrations of steroid hormones or their precursors are immunoassays,[86–88] fluorescence resonance energy transfer,[89] SPR,[90] gas chromatography-mass spectrometry (GC/MS),[91] and liquid chromatography-mass spectrometry (LC/MS).[92–94] A sensitive assay for hormone detection that typically involves a mass spectrometer coupled with either a gas chromatograph or a liquid chromatograph has been developed by Fenlon et al.[95] For these hormones, like anabolic steroids, according to the differences in fragmentation caused by collisions of medium energy related to the structure, it is difficult to find a common product ion or neutral loss. Furthermore, not only do most ELISA-like assays lack the sensitivity required to determine >90% of hormone derivatives,[94] but also these analytical procedures might introduce artefacts. The detection limits of the above methods range from ng/mL to pg/mL.[96] One-dimensional nanostructures such as carbon nanotubes and silicon nanowires (SiNW) have been demonstrated to be sensitive chemical and biological sensors.[78] That detection results from the disturbance of charge on the surface of the functionalized nanostructure on which the target molecules are specifically recognized. For instance, the real-time detection of various antigens,[97] oligonucleotides,[98,99] proteins,[100,101]and charged small molecules has been shown to be feasible on devices

using nanowire or carbon-nanotube transistors as active transducers. The sensing mechanism in an electrically-based biosensor relies on an altered conductance or threshold voltage induced by the attachment of the charged analytes. Chang et al[102] attempted to integrate protein engineering with the sensitive nature of a SiNW-field-effect transistor (FET) in charge disturbance to overcome the intrinsic weakness of SiNW-FET in detecting uncharged analytes. They chose an uncharged steroid, 19-norandrostenedione, as the target analyte. Delta 5-3-ketosteroid isomerase has served as a receptor for steroid recognition because of its well understood enzyme function. The primary concept of the sensing mechanism is based on intramolecular binding of a charged ligand, functioning as a reporter, to mimic the binding of an analyte to a protein. The major driving force favoring this association is generally thought to be the hydrophobic effect that prompts the hydrophobic ligand to bind with the protein. The thermodynamics of protein-ligand binding can be altered by a favorable control of enthalpy and, particularly in this model, the characteristic "entropy-driven" thermodynamic signature of the steroid. The analyte might replace the pre-situated ligand, which becomes thus exposed to the surface of the SiNW and perturbs the charge density and conductance of the nanostructure. In the presence of a steroid, the negatively charged 5-(2-aminoethylamino)-1-naphthalenesulfonic acid (1,5-EDANS) moiety, which presumably occupies the steroid-binding site, is expelled and exposed to the nanowire surface (Figure 4). The electrical response produced from the 1,5-EDANS moiety is measured and the concentration is calculated accordingly. The sensitivity of this novel nano-bio-device can attain a femtomolar level.[102]

C-reactive protein

Recently a label-free quartz crystal microbalance (QCM) immunosensor for an important biomarker, C-reactive protein (CRP),[103–105] related with coronary heart disease, hypertension, and inflammation, has been developed with picomolar sensitivity.[106] However, a possible matrix effect caused by serum viscosity might be present, like coloring substances in the case of ELISA.[107] One way to avoid it is to dilute samples, including serum and plasma, to the degree at which sample viscosity approaches that of the reaction buffer. Recently, Kim et al[107] conspicuously increased the sensor signal, and thus sensitivity of a label-free QCM immunosensor for CRP, by introducing a streptavidin-coated gold nanoparticle during antibody-antigen complexation, based on an indirect competitive assay format. When 200 μL of the modified antibody, having the concentration of 0.250 mg/mL, was added with GNp to the immunosensor system, the frequency shift obtained was 139.8 ± 0.3 Hz. Compared to the frequency shift of 91.1 ± 1.3 Hz found with the addition of the unmodified antibody only, the signal augmentation after GNp binding amounted to 53.4%, which resulted in sensitivity improvement of the immunosensor.

Conclusion

Clinicians, food technologists, and environmentalists all have an interest in generally increased sensitivity and limits of detection for a range of analytes. While the precise demands to meet today's requirements may be modest in these respects, few would contest the longer term benefits of reliable detection of trace amounts of various diagnostic indicators, additives, or contaminants.

As chemical analysis becomes simpler and more widely available, we can expect more accurate diagnosis and prognosis for different diseases. Nanosensors, capable of providing data through unique technology, could find wide application in monitoring our personal health, the food we eat, and our environmental health. The performance of nano-biochemical sensors is excellent in terms of sensitivity, selectivity, linearity, stability, response time, and reproducibility compared to
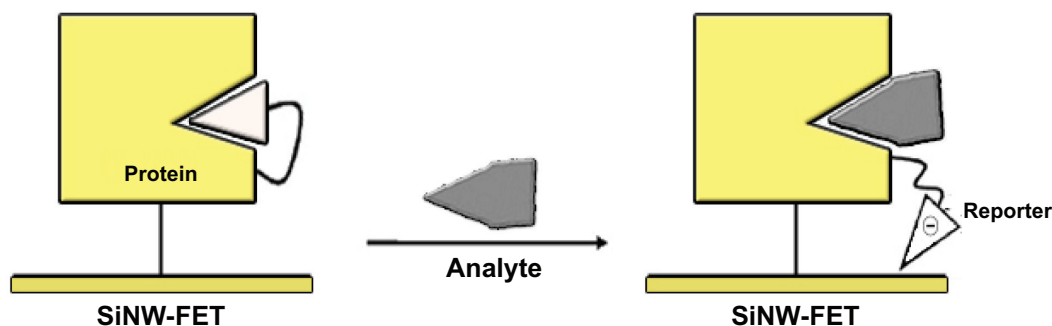

Figure 4 SiNW-FET platform for the detection of uncharged steroid-related analyte.[105]
Abbreviation: SiNW-FET, silicon nanowire field-effect transistor.

the traditional biosensors. Nanoparticle labeling procedure is very simple, and the biochemical activity of the labeled compound is almost unaffected. This new approach is critically useful in preventing interference between chemically related analytes.

The use of core-shell NPs for the biocatalytic transformation of an enzyme could form the basis for the fabrication of an optical sensor for redox compounds. Moreover, the low Km^{app} value recorded when enzymes were immobilized on nano-biosensors indicates high affinity of sensors to analytes, which is interesting and promising for biosensor electrode construction.

Disclosure

The authors report no conflicts of interest in this work.

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