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Application of nanoparticles in quartz crystal microbalance biosensors

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ABSTRACT

Nanoparticles are playing an increasingly important role in the development of biosensors. The sensitivity and performance of biosensors is being improved by using Nanoparticles for their construction. The use of these Nanoparticles has allowed the introduction of many new signal transduction technologies in biosensors. In this report, a comprehensive review of Application of nanoparticles in Quartz Crystal Microbalance biosensors is presented. The main advantages of QCM in sensing fields include high sensitivity, high stability, fast response and low cost. In addition, it provides label-free detection capability for bio-sensing applications.Firstly, basic QCM's design and characterization are described. Next, QCM biosensors based on modification of quartz substrate structure and their applications are digested. Nanoparticles and their utilizationin analysis are then illustrated. These include Nanoparticles in bio applications that cover Nanoparticles in Quartz Crystal Microbalance biosensors.

KEYWORDS

Nanoparticles;
Quartz crystal microbalance;
QCM;
Biosensors;
Review.

INTRODUCTION

Nanoparticle analysis is a rapidly growing research area with applications in a variety of fields including energy, nanocomposite materials, the environment and healthcare. Nanoparticles are being incorporated into materials such as solar panels, nanomachines, and a variety of thin films and coatings to generate more desirable properties. Between the different applications the use of NPs for biosensing is showing an increased interest for several areas such as clinical analysis[1,2], environmental monitoring[3-5]as well as safety and security.NPs involvements in DNA, protein and even cell sensing systems have recently been the hottest topics in nanobiotechnology. Although their use and benefits are unquestioned, there are still potential risks in their widespread use to health and the environment. Therefore, there is a need for new techniques for characterizing nanoparticles including their interactions with surfaces, each other and their stability in the environment. Quartz Crystal Microbalance (QCM) is an analytical technique that provides in-situ analysis of interactions taking place at surfaces. The main advantages of QCM in sensing fields include highsensitivity, high stability, fast response and low cost. In addition,it provides label-free detection capability for biosensingapplications. Being labeled free, it dispenses with the time and cost demanding labeling step, and also eliminates any possible interference of the "true" binding process due to the presence of the labels.Given the special importance of bio analysis, we intend in this review to overview some of the major advances and milestones in the field of Quartz Crystal Microbalance biosensors based on NPs underlying the different approaches reported so far. Since the nanoparticles andbiomolecules typically meet at the same nanometer length scale, thisinterdisciplinary approach will contribute to the establishment of anovel field, descriptively termed immunosensing nanotechnology ornanoimmunosensing. NP based immunosensing systems are proving to assist in the development of new and versatile protein detection methods.

In this report, firstly, basic QCM's design and characterizationare described.Next, QCM biosensors based on modification of quartz substratestructure and their applications are discussed.Nanoparticlesand their applications in analysisare then illustrated. These include nanoparticles in bio applications that cover nanoparticles in Quartz Crystal Microbalance biosensors.

QUARTZ CRYSTAL MICROBALANCE

Piezoelectricity is defined as electric polarization produced by mechanical strain incertain crystals, the polarization being proportional to the strain[6]. The Curies firstobserved piezoelectricity in 1880 as a potential difference generated across two surfacesof a quartz crystal under strain[7]. The converse piezoelectric effect, the deformation of apiezoelectric material by an applied electric field, was predicted by Lippman[8]. Thus,when a thin wafer from a piezoelectric crystal such as quartz is placed in an alternatingelectric field of the right frequency it will oscillate in a mechanically resonant mode ofthe wafer. The resonance frequency depends upon the angles with respect to the opticalaxis at which the wafer was cut from a single crystal and inversely on the crystalthickness. Quartz crystal microbalance (QCM) devices derive from piezoelectric quartz crystal resonators that resonate electromechanically in a thickness-shear mode (TSM) (Figures 1,2).Owing to piezoelectric effects, the resonant properties of crystal can be influenced by external physical loading, which may be classified into two main types, gravimetric and viscoelastic loading. In the first case, gravimetric force due to mass rigidly deposited on the crystal surface is balanced by a force originating from the shear gradient inside the crystal, leading to the resonance frequency shift (Δf_g), which is governed by Sauerbrey's equation[10,11]:

$$\Delta f_g = -\frac{2f_0^2}{\sqrt{\rho_q \mu_q}} \frac{\Delta M}{A}$$

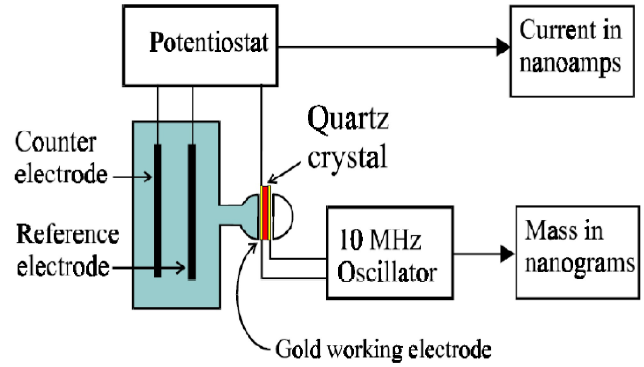

Figure 1 : Schematic diagram of Quartz Crystal Microbalanceinstrumentation. (Crystal is held between two o-rings) Reproduced with permission from ref[7], Copyright 2008 Elsevier

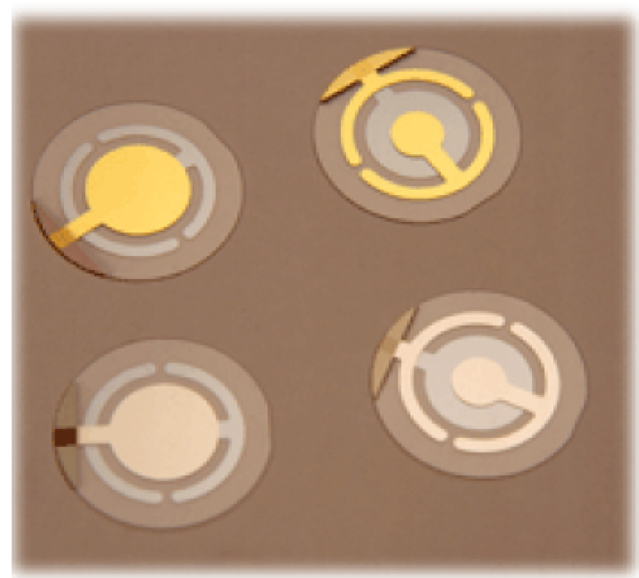

Figure 2 : Crystals used in a QCM.Reproduced with permission from ref[9], Copyright 2004 Elsevier

$$f_0 = \frac{v}{2t_q} = \frac{\sqrt{(\mu_q/\rho_q)}}{2t_q}$$

where f_0 is the fundamental resonance frequency, ΔM is the mass deposited on QCM surface, A is the active electrode area, v is the acoustic wave velocity, ρ_q is the density of crystal (2.648 g cm^{-3} for quartz), μ_q is the shear modulus of the cut face (2.947×10^{11} gcm^{-1} s2 for AT-cut quartz) and t_q is the crystal thickness. With this loading effect, QCM has been routinely used for thickness monitoring in physical vapor deposition processes. For the viscoelastic loading, resonance frequency is changed due to acoustic-fluid damping interaction when QCM operates in viscoelastic medium such as liquid[12]. Unlike gravimetric loading, there are large changes of dissipation factor and resonant resistance due to energy losses of shear wave that travels through non-rigidly adsorbed layers[13–16]. The viscoselastic resonance frequency shift (Δf_v), dissipation factor change (ΔD) and resonant resistance change (ΔR) are given by:

$$\Delta f_V = -f_0^{3/2}\sqrt{\frac{\eta_L \rho_L}{\pi \mu_q \rho_q}}$$

$$\Delta D = 2f_0^{1/2}\sqrt{\frac{\eta_L \rho_L}{\pi \mu_q \rho_q}}$$

$$\Delta R = \frac{(2\pi \eta_L \rho_L f_0)^{1/2} A}{k^2}$$

where ρ_L, γ_L and k are viscosity, density and electromechanical coupling factor of the liquid medium, respectively. Both dissipation factor and resonant resistance can be directly measured experimentally. The combined measurements of frequency shift and changes in the dissipation factor and resonant resistance have been termed, QCM-D and QCM-R, respectively[17]. With the combined mass and viscosity–density effects, QCM can be applied for bio/chemical sensing by coating with a chemically or biologically sensitive film to sense very small amount of adsorbed gases such as volatile organic compounds (VOCs)[18,19] and environmental pollutants[20–22] and liquid phase biochemicals.

A quartz crystal microbalance (QCM) measures a mass per unit area by measuring the change in frequency of a quartz crystal resonator. The resonance is disturbed by the addition or removal of a small mass due to oxide growth/decay or film deposition at the surface of the acoustic resonator. The QCM can be used under vacuum, in gas phase (“gas sensor”, first use described by King)[23] and more recently in liquid environments. It is useful for monitoring the rate of deposition in thin film deposition systems under vacuum. In liquid, it is highly effective at determining the affinity of molecules (proteins, in particular) to surfaces functionalized with 3to investigate interactions between biomolecules.

There has been a rapid increase in the number of publications related to QCM sensing applications during the last few decades (Figure 3). QCMs have been used for multifarious applications in various disciplines of science and technology for the detection of metals in vacuum, vapors, chemical analytes, environmental pollutants, biomolecules, disease biomarkers, cells, and

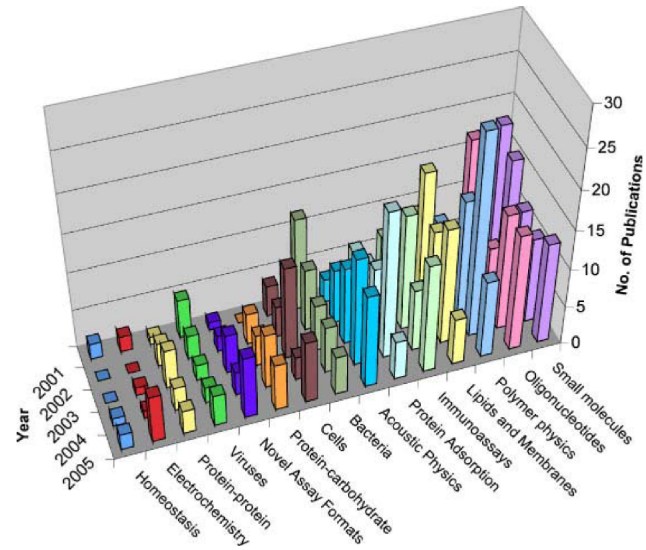

Figure 3 : A summary of the number of papers (for specific analytes by class) referencing‘QCM’ in the title, Keywords or Abstract[29]. Reproduced with permission from ref[29], Copyright 2007 John Wiley & Sons, Ltd

pathogens. They are also employed for deposition rate monitoring, electrochemical deposition, corrosion studies, detection in gas chromatography, and controlling thickness and composition of sputtered materials. Presently, there are several companies that are dealing with QCM-based systems.QCM provides oneof the most promising sensor technology based on its lowcost, rapid response, portability, nonhazardous label-freereal-time procedure, and high sensitivity, which is ideal forthe sensitive online detection of analytes and holds a greatpromise for the next-generation sensors.

QCM AS A BIOSENSOR

Nowadays the applications of sensors are a very promising research field since they ûnd application in many different areas[30-35]. Biosensors are an increasingly important technology in the detection of compounds ranging from pesticides to biological weapons. Typically, biosensors consist of a biological macromolecule that is immobilized on the surface of a signal transducer, as shown in Figure 4. As the macromolecule binds specifically to the ligand being detected, the signal transducer can measure a physical change due to the binding event. The transducer usually detects a change in resistance, pH, heat, light, or mass and then converts that data to an electrical signal to be collected and processed. One promising type of detector is the Quartz

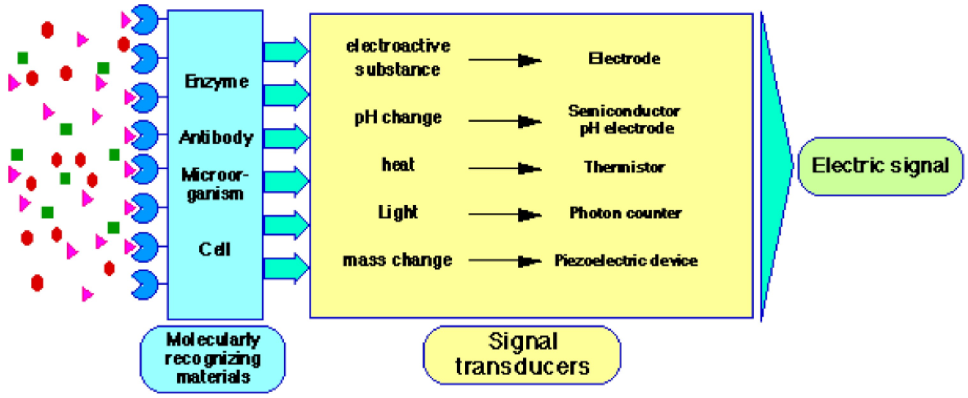

Figure 4 : Schematic for generic biosensor[36]

Crystal Microbalance (QCM). The QCM is a piezoelectric mass-sensing device. A QCM device works by sending an electrical signal through a gold-plated quartz crystal, which causes a vibration at some resonant frequency. The QCM then measures the frequency of oscillation in the crystal. When used as a biosensor, the QCM can detect changes in frequency of the crystal due to changes in mass on the surface of the crystal.

The sensitivity and specificity of QCM-based biosensorsis dependent on the immobilization of recognitionlayer. For example various strategies have been employed for theimmobilization of antibodies on the crystal surface. Passiveadsorption of antibodies has been the most widely usedmethod. However, it leads to random immobilization ofantibodies on the surface, which may not be in functionallyactive orientation. The crystallizable fragment (Fc)-bindingproteins such as protein A, protein G- and protein A/Gare employed for oriented immobilization of antibodieson the gold-coated QCM surface such that their antigenbindingsites (Fab region) are completely free for bindingantigens. Another strategy employs the initial generationof amino groups on the QCM surface by treatment with3-aminopropyltriethoxysilane followed by the activation ofamine-functionalized surface with glutaraldehyde to generatealdehyde groups, which bind to the antibody throughits amino groups. The interactions between thiols andgold are very strong and have also been exploited forantibody binding by employing self-assembled layers ofthiols and sulphides. The avidin-biotin interactions have alsobeen employed, where the avidin/streptavidin-coated QCMgold-surface is bound to the biotinylated antibody. Otherapproaches based on the use of polyelectrolytes, polymers,and Langmuir-Blodgett films have also been used.

QCM biosensors have been employed in the label-free detection of an incredibly broad range of analytes; from interfacial chemistries and lipid membranes to small molecules and whole cells[37]. They provide a unique method for observing in situ events involving ‘soft matter’, in which changes in contact mechanics, interfacial dynamics, surface roughness, viscoelasticity, density and mass can be monitored in real time[38-44]. This level of information content goes beyond that produced by many more widely adopted optical techniques that are used to analyse molecular changes occurring in biochemical processes. Acoustic sensor technology is thus highly interdisciplinary and has encompassed advances and improvements from electrical engineers to cell biologists. Braunhut et al adapted the quartz crystal microbalance (QCM) biosensor technique to study responses of human mammary epithelial

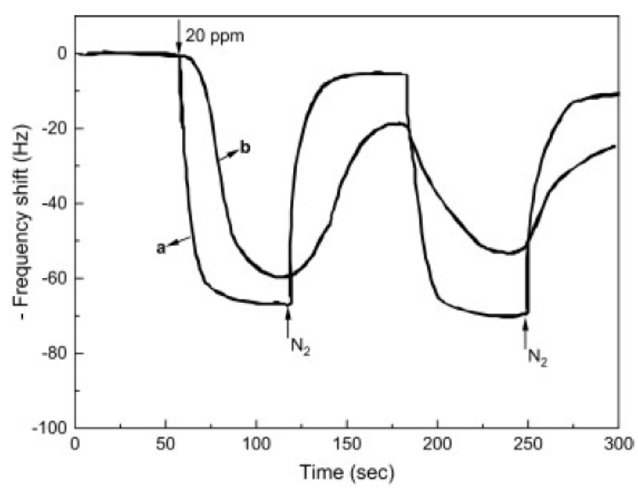

Figure 5 : Typical response of PEDOT particles to NH_3 gas (20 ppm) (a) nanoparticles and (b) conventional particles Reproduced with permission from ref[95], Copyright 2007 Elsevier

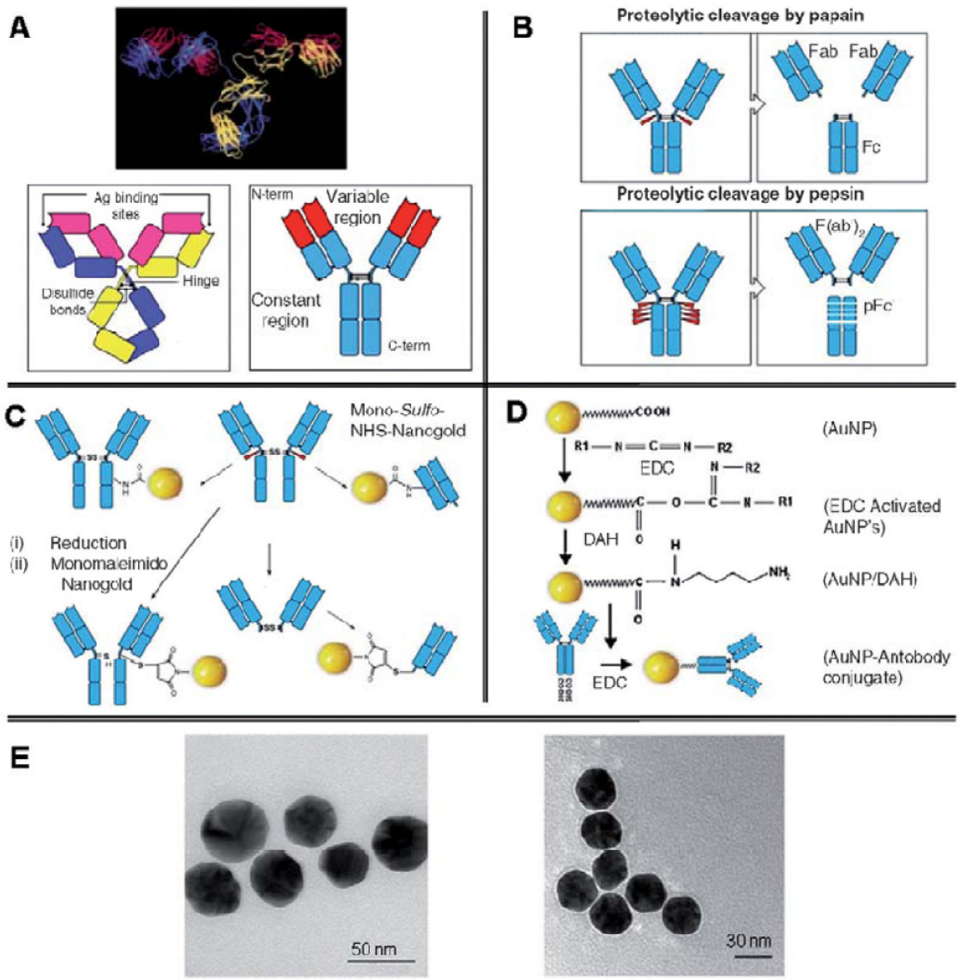

Figure 6 : (a) X-ray crystallographic structure of an IgG antibody (upper part); A schematic representation of IgG and a simplified scheme of the antibody's structure(lower part). (b) The papain (upper part) and the the pepsin (lower part) cleavages. (c) Functionalization of a whole IgG or a Fab by a Mono-Sulfo-NHS-AuNP viafree amino groups (upper part); monomaleimidoAuNP binds the S atom after the reduction of the disulfide bond starting from the whole IgG (lower part, left) orfrom F(ab') 2 (lower part, right)[53]. (d) An activated by EDC AuNP coated with gluthatione, as spacer arm, reacts with an IgG, also activated by EDC[54]. (e) TEM images ofAuNPs (left part) and AuNPs conjugated with IgG (right part). Image shows a thin white layer, called "halo" effect, surrounding the surface of the AuNPs indicatingcoating with protein.Reproduced with permission from ref[55], Copyright 2010 Elsevier

tumor cells to taxanes[45]. Taxanes are used for the treatment of many human cancers, as first- and second-line chemotherapeutics.

The ultimate goal in any biosensor development project is its use for actual sample detection. Recently, there has been an interest in biosensors with aptamers as bio-recognition elements, but reported examples all deal with standards, not human serum.In comparison to antibodies, aptamer receptors have a number of advantages. The main advantage is the avoidance of the use of animals for their production. Moreover, the aptamer select ion process can be manipulated to obtain aptamers that bind to a specific region of the target and with specific binding properties under different binding conditions[46-50]. In order to verify the differences of aptamer-based biosensor and antibody-based biosensor in clinical detection, Yao et al carried out a comparison of the performance of aptamer-based and antibody-based quartz crystal microbalance (QCM) biosensors for the detection of immunoglobulin E (IgE) in human serum[51]. Wu et al developed a DNA piezoelectric biosensing method for real-time detection of Escherichia coli O157:H7 in a circulating-flow system[52]. The QCM system was also applied in the detection of PCR-amplified DNA from real samples of E.coli O157:H7. The resultant H% of the PCR-amplified double-strand DNA was comparable to that of the synthetic target T-104AS, a single-strand DNA. The piezoelectric biosensing system has potential for further applications. This approach lays the groundwork for incorporating the method into an integrated system for rapid PCR-based DNA analysis.

NANOPARTICLES

One of the most important functions of nanoparticles iscatalysis, especially with noble metal nanoparticles, whichhave high catalytic activity for many chemical reactions.Because nanomaterials also have good biocompatibility, they are used to immobilize biomolecules for the fabricationof biosensors. Sensitivity and other attributes of biosensors can beimproved by using nanomaterials in their construction. Nanomaterials, or matrices with at least one of their dimensions ranging in scale from 1 to 100 nm, display unique physicaland chemical features because of effects such as the quantum size effect, mini size effect,surface effect and macro-quantum tunnel effect.

Nanoparticles in bio applications

Nanoparticles have numerous possible applications in biosensors. For example, functionalnanoparticles (electronic, optical and magnetic) bound to biological molecules (e.g.peptides, proteins, nucleic acids) have been developed for use in biosensors to detect andamplify various signals. Some of the nanoparticle-based sensors include the acoustic wavebiosensors, optical biosensors, magnetic and electrochemical biosensors.The structure of IgG, the most used antibody in immunosensig assays,has been determined by X-ray crystallography (Figure 6a, upper part)which shows a Y-shape form consisting of three equal-sized portions,loosely connected by a flexible tether[53]. The antibodies are constitutedby two heavy and two light polypeptide chains linked between themas shown in Fig. 6a (lower part). The C regions determine the isotype of the antibody whereas the variable regions of one heavy and onelight chain constitute an antigen binding site (ABS). The digestion ofantibody with papain (Figure 6b upper part) and pepsin (Figure 6b lowerpart) proteases produces several smaller fragments that may be usedinstead of the original antibody for immunosensing applications.Immunoassays are based on the interaction between the antibodyand the antigen, in particular between the ABS and the epitope.In fact this interaction gives high specificity and sensitivity to theimmunoassay. In order to functionalize the Ig, the connection oflabels through three main groups: $-NH_2$, -COOH and –SH have beenperformed. Figure 6c (upper part) shows two examples of conjugation ofmono-sulfo-NHS-AuNPs with an antibody through the amino group.A conjugation of monomaleimidoAuNPs (Figure 6c, lower part) with anantibody, through the –SH group, is also shown. The use of the thiolgroup for the functionalization is a good way to control the directionof the bond between the label and the antibody, and prevent theinvolvement of the ABS. Figure 6d is an example of conjugation through the carboxyl group reported by Ahirwal et al[54]. They connected thecarboxi-

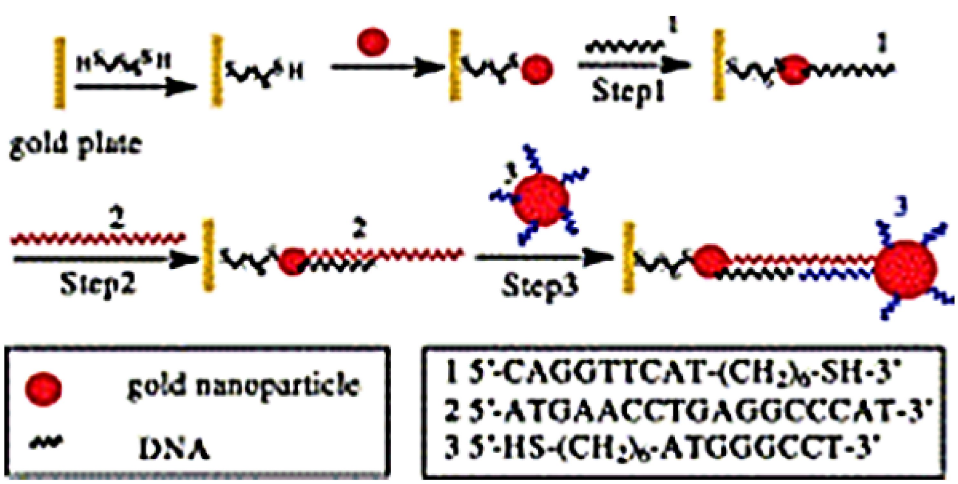

Figure 7 : Schematic illustration of the sensing process of the amplifying system based on Au nanoparticle-covered QCM surface. Reproduced with permission from ref[82], Copyright 2004 Elsevier

term of the antibody with anAuNP by glutathione used as aspacer. The C-term region is a good point to attach the label, since itis far from the ABS and should allow the molecule interaction with theantigen. The AuNP modification with protein can even be observed byTEM (Figure 6e)[55].

Nanoparticles in Quartz Crystal Microbalance biosensors

Quartz crystal microbalances have emerged inthe last few years as versatile biosensors demonstrating remarkable achievements such as high sensitivity and label free detection[56-58]. In the quartz crystal microbalance, thebioreaction generates a change in the mass, reordering the charges inthe surface of the piezoelectric material and giving rise to a changein the resonant frequency of the microbalance. The use of NP labelsin sandwich assays can increase both the surface stress and the massof the immunocomplex, allowing the increase in sensitivity of theseelectromechanical assays.This was the case in work reported for the detectionof human IgG[59] and aflatoxin B1[60] at clinical relevant levels using AuNPs as amplification agents in adynamic mode quartz crystal microbalance biosensor.

Quartz crystal microbalance biosensors were developed to greatly improve the sensitivity and limitsof detection[61]. In the mass-amplified quartz crystal microbalanceassay variant of this technology, antibody modified sol particles indirectly bind to anelectrode surface by complexing to an analyte that has been itself captured by an antibodyimmobilized on the electrode surface. The large mass of the bound sol particles greatlyaffects the vibrational frequency of the quartz crystal and this is used as the basis fordetection. The assay can be carried out in the competitive mode. The preferred diameter ofsol particles is in the range of 5–100 nm. Other high-density particles (e.g. Au, Pt, CdS,TiO2, polymers) may be also suitable[62,63].

Recently chu et al developed a label-free gliadinimmunosensor by applying 25 nm gold nanoparticles (AuNPs) to the surface of a bare QCM electrode[64]. Also a rapid and sensitive quartz crystal microbalance (QCM) biosensor combined with gold nanoparticle amplification for diagnosis Mycobacterium tuberculosis was developed byKaewphinitet al[65]. The label free QCM was successfully developed with high sensitivity, specificity, low cost and convenience, this technique may prove to be a powerful tool for an early diagnosis of M. tuberculosis.

DNA-conjugated nanoparticles can be used to enhance the signal produced upon hybridization to a surface-bound single-stranded template[66-74]. They can also be used to increase the surface area available to immobilize the DNA template on a sensor[75-77]. Detection sensitivity is generally in the region of 10^{-15}–10^{-16} M, with one report of zeptomolar (10^{-21} M) quantities of specific nucleic acid[78]. Ma et al.[79] used a mixture of $HAuCl_4$ and NH_2OH to enlarge the gold particles. However, it has been shown that 20 nm is the optimal particle size for maximally increasing the sensitivity as the larger particles suffer from weaker binding to the surface and are therefore lost on exposure to flow[80]. A quartz crystal microbalance (QCM) DNA sensor, based on the nanoparticle amplification method, was developed for detection of Escherichia coli O157:H7[81]. A thiolated single-stranded DNA (ssDNA) probe specific to E. coli O157:H7 was immobilized onto the QCM sensor surface through self-assembly (Figure 8). The

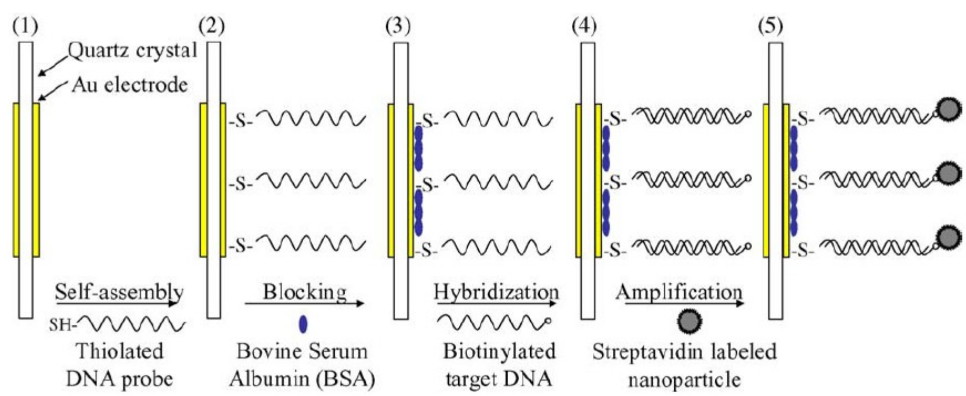

Figure 8 : llustration of the sensor fabrication and detection procedure Reproduced with permission from ref[81], Copyright 2006 Elsevier

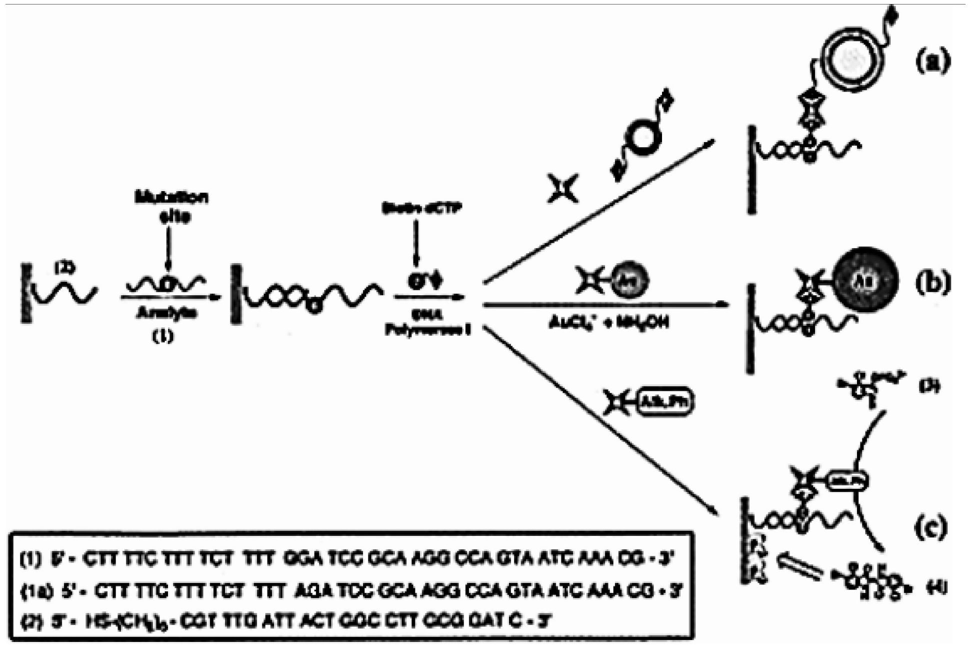

Figure 9 : Amplified detection paths of a single-base mismatch in nucleic acids: (a) using avidin and biotin-labeled liposomes. (b) Using an avidin–Au-nanoparticle conjugate and the catalyzed deposition of gold. (c) Using an avidin–alkaline phosphatase bioconjugate and the biocatalyzed precipitation of the insoluble product 4 Reproduced with permission from ref[84], Copyright 2002 Elsevier

hybridization was induced by exposing the ssDNA probe to the complementary target DNA, and resulted in the mass change and therefore frequency change of the QCM.

The enhancement of a single strain DNA probe linked to the sensor surface is of crucial importance in DNA molecule recognition. By means of nanogold modification of the sensor surface in addition to the nanogold amplifier, DNA detection sensitivity higher than 10^{-16}mol/L was obtained in a Quartz Crystal microbalance (QCM) system, much higher than the ordinary QCM sensor without surface modification by nanogold (Figure 7)[82].

Recently a DNA probe functionalized quartz crystal microbalance (QCM) biosensor was developed to detect B. anthracis based on the recognition of its specific DNA sequences, i.e., the 168 bp fragment of the Ba813 gene in chromosomes and the 340 bp fragment of the pag gene in plasmid pXO1[83]. Willner et al described three different methods for the amplified detection of a single-base mismatch in DNA using microgravimetric quartz-crystal-microbalance as transduction means (Figure 9)[84]. All methods involve the primary incorporation of a biotinylated base complementary to the mutation site in the analyzed double-stranded primer/DNA assembly. The double-stranded assembly is formed between 25 complementary bases of the probe DNA assembled on the Au-quartz crystal and the target DNA. One method of amplification includes the association of avidin- and biotin-labeled liposomes to the sensing interface. The second method of amplified detection of the base mismatch includes the association of an Au-nanoparticle-avidin conjugate to the sensing interface, and the secondary Au-nanoparticle-catalyzed deposition of gold on the particles. The third amplification route includes the binding of the avidin–alkaline phosphatase biocatalytic conjugate to the double-stranded surface followed by the oxidative hydrolysis of 5-bromo-4-chloro-3-indolyl phosphate to the insoluble product indigo derivative that precipitates on the transducer. Comparison of the three amplification routes reveals that the catalytic deposition of gold on the Au-nanoparticle/avidin conjugate is the most sensitive method, and the single-base mismatch in the analyzed DNA is detected with a sensitivity that corresponds to 3×10^{-16} M.

A novel amplification route for DNA detection based on the deposition of gold on a 10 nm Au-colloid/avidin conjugate label acting as a 'seeding' catalyst is described by Weizmann et al (Figure 10)[85].

Microgravimetric quartz-crystal-microbalance measurements are employed to transduce the catalyzed deposition of gold on the piezoelectric crystals. Three different DNA detection schemes are described: (i) analysis of a 27-base nucleic acid fragment; (ii) analysis of the entire M13f DNA (7229 bases); and (iii) detection of a single-base mismatch in a DNA. Ultrasensitive detection of DNA is accomplished by the catalyzed deposi-

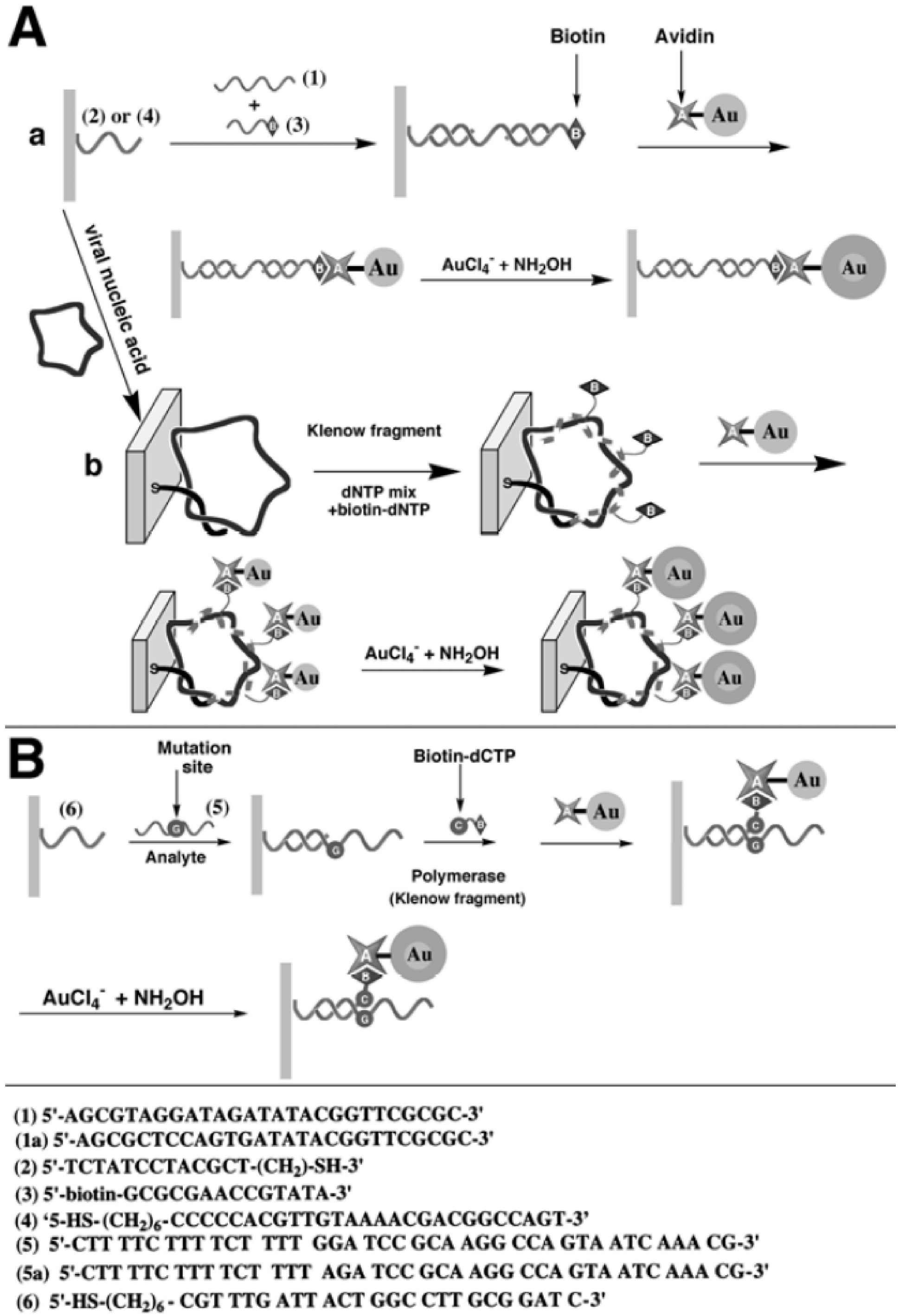

Figure 10 : (A) Amplified detection of DNA by: (a) association of an Au–avidin conjugate and catalytic deposition of Au; (b) polymerase-induced replication of DNA followed by the electroless deposition of gold on an Au-avidin conjugate linked to the double-stranded assembly. (B) Amplified detection of a singlebase mismatch in DNA by the catalytic deposition of Au on an Au–avidin conjugate linked to the labeled double-stranded assembly.Reproduced with permission from ref[85], Copyright 2001

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tion of gold, detection limit 4 1×10^{-15} M.

A rapid and sensitive detection method for the early diagnosis of infectious dengue virus (DENV) urgently needs to be developed by chen et al[86]. In this study, a circulating-flow quartz crystal microbalance (QCM) biosensing method combining oligonucleotide-functionalized gold nanoparticles (i.e. AuNP probes) used to detect DENV has been established. The sensitivity and specificity of the present DNA–QCM method with nanoparticle technology showed it to be comparable to the fluorescent real-time PCR methods. Moreover, the method described herein was shown to not require expensive equipment, was label-free and highly sensitive.Scodeller et al reported a fully integrated core”shell nanoparticle system responsive to glucose[87]. The system is comprised of self-assembled glucose oxidase and an osmium molecular wire on core”shell Au nanoparticles.

In an interesting work Krozer et al, investigated a new and generic approach for integration of molecularly imprinted nanoparticles into sensing systems[88]. Nanoparticles can be easily immobilized on flat transducer surfaces through a supporting polymer film using spin-coating, and the thickness of the nanoparticle coating can be easily controlled by changing the nanoparticle/polymer ratio and the solid content of the nanoparticle suspension.

Recently a simple and sensitive sensor method for cancer biomarkers [prostate specific antigen (PSA) and PSA-alpha 1 antichymotrypsin (ACT) complex] analysis was developed, to be applied directly with human serum (75%) by using antibody modified quartz crystal microbalance sensor and nanoparticles amplification system[89]. A QCM sensor chip consisting of two sensing array enabling the measurement of an active and control binding events simultaneously on the sensor surface was used in this work. With the achieved detection limit in serum samples, the developed QCM assay show a promising technology for cancer biomarker analysis in patient samples.

Ma et al reported a new amplification route for human lung carcinoma cell detection based on the detection of gold deposition on an Au-antibody conjugate label acting as a “seeding” catalyst[90]. Quartz crystal microbalance (QCM) was employed to measure the catalyzed deposition of gold on the piezoelectric crystals. The gold-coated quartz crystal was modified by polystyrene films, which provided a substrate for antibody immobilization by physical adsorption (Figure 11). The polystyrene films significantly improve the surface smoothness of the electrode. Compared to the conventional EnzymelinkedImmunosorbentAssay (ELISA), the method described herein is a less time consuming procedure and gives comparable detection sensitivity of human lung carcinoma cells, *ca.* 100 cells mL^{-1}. This will be of great significance in the diagnosis of this disease.

A highly sensitive quartz crystal microbalance with dissipation monitoring (QCM-D) biosensor for protein was developed using aptamer-functionalized gold nanoparticles (Apt-GNPs) for amplification[91]. Human α-thrombin, an important physiological protease found in blood, was chosen as the target protein. Captured by immobilized aptamers, thrombin was determined on-

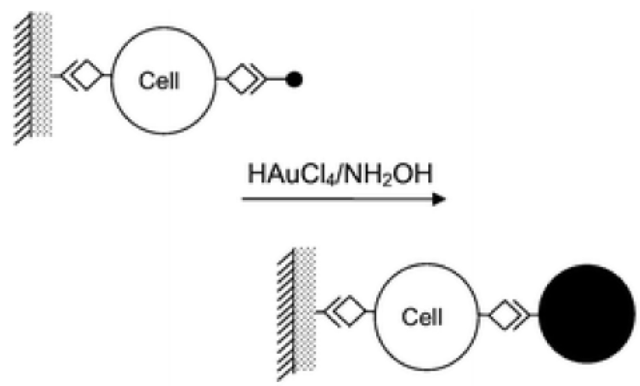

Figure 11 : The gold-coated quartz crystal was modified by polystyrene filmsReproduced with permission from ref[90], Copyright 2002 Royal Society of Chemistry

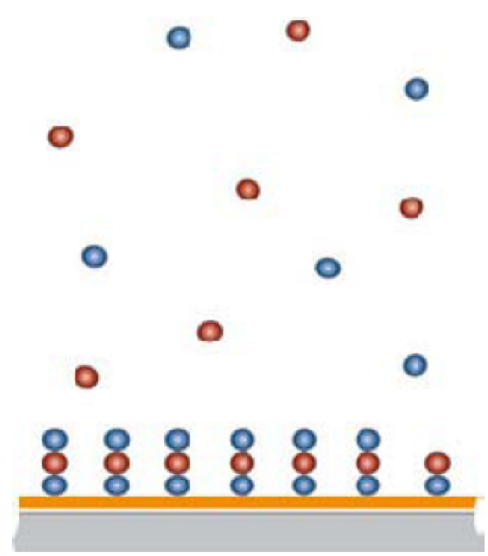

Figure 12 : Self assembly processes[96]

line using Apt-GNPs to enhance both frequency and dissipation signals. The fabricated sandwich of aptamer/thrombin/Apt-GNPs on chip surface was confirmed by atomic force microscopy (AFM). Compared to direct assay, the detection limit for thrombin was down to 0.1 nM, yielding about 2 orders of magnitude improvement in sensitivity. For the first time, the dual-signal enhancement of Apt-GNPs on QCM-D sensing was demonstrated, and such design could provide a promising detection strategy for proteins with two binding sites.In an interesting work Kaufman et al, described the adsorption characteristics of three proteins [bovine serum albumin (BSA), myoglobin (Mb), and cytochrome *c* (CytC)] onto self-assembled monolayers of mercaptoundecanoic acid (MUA) on both gold nanoparticles (AuNP) and gold surfaces (Au)[92]. The combination of quartz crystal microbalance measurements with dissipation (QCM-D) and pH titrations of the *ú*-potential provide information on layer structure, surface coverage, and potential. A new quartz crystal microbalance immunoassay method based on a novel transparent immunoaffinity reactor was developed for clinical immunoassay byTang et al[93]. To construct such an affinity reactor, resonators with a frequency of 10 MHz were fabricated by affinity binding of functionalized gold nanoparticles (nanogold) to quartz crystal with immobilized specific ligand for the label-free analysis of the affinity reaction between a ligand and its receptor. [Recombinant human tumor markers, carcinoembryonic antigen (CEA) was chosen as a model ligand.]. Compared with the conventional enzyme-linked immunosorbent assay, the proposed immunoassay system was simple and rapid without multiple labeling and separation steps. Importantly, the proposed immunoassay system could be further developed for the immobilization of other antigens or biocompounds.

Su et al fabricated Novel low-humidity sensors by the in situphotopolymerization of polypyrrole/Ag/TiO_2 nanoparticles (PPy/Ag/TiO_2 NPs) composite thin films on a quartz-crystal microbalance (QCM)[94]. The sensitivity increased with the doping amount of TiO_2 NPs. Additionally, based on the dynamic analysis of adsorption, the association constants of water vapor molecules with PPy/Ag and PPy/Ag/50 wt% TiO_2 NPs composite thin films were estimated to be 81.6 and 227.9M^{-1}, respectively, explaining the effect of adding 50 wt% TiO_2 NPs to PPy/Ag; the sensitivity to low humidity increased as the association constant increased. Also a sensitive method for ammonia gas analysis was developed with Conducting polymer poly (3,4-ethylenedioxythiophene) (PEDOT) nanoparticles by reverse micelle method, which could be well dispersed in water or alcoholic solvent with ultrasonic treatment[95]. Compare to electrical conductivity of conventional PEDOT particles (ca. 0.5 S/cm), the as-prepared palletized nanoparticles have higher conductivity (ca. 10.2 S/cm) and the conductivity varies with the doped and de-doped states of PEDOT. As for sensing property, it has been found that QCM device coated with nanoparticles shows faster response and recovery to 20 ppm ammonia gas (ca. 50 s) than that of conventional PEDOT particles (Figure 5). The PEDOT nanoparticle covered device exhibits almost linear relation to lower NH_3 gas concentration and shows a saturate tendency of gas sensitivity with the increase of gas concentration over 500 ppm.

One of the applications of QCM technique is study nanoparticle self-assembly processes, nanocomposite materials and track nanoparticle interactions in the environment.Self-assembly is a process where similar particles spontaneously bind to one another to form new materials, coatings or films with a variety of desirable properties (Figure 12). These type of mass sensitive technique is highly useful to study monolayer formation, enzyme immobilization, selective sensing of small organic or large biomolecules. For example, recently

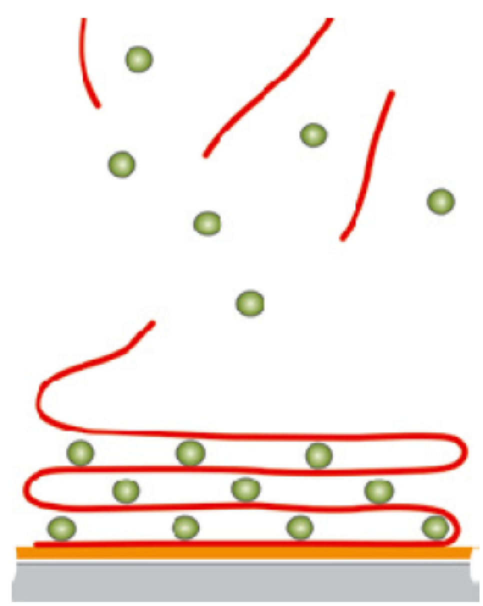

Figure 13 : Nanocomposite materials analysis[96]

Crooks and coworkers have shown that hyperbranch polymer monolayers and poly(amidoamine)dendrimer monolayers can interact with small organic molecules[97-98]. Similarly Kepley and coworkers have used copper(II) ions bound on SAM to selectively and reversibly detected organophosphonate[99]. Host–guest interactions can be monitored by highly ordered cavitated monolayers as shown using calix[4] resorcinarene[100]. These cavited monolayers have a high affinity towards perchloroethylene[101]. The QCM technology enables one to measure the kinetics of nanoparticle self-assembly processes, measure the resulting thickness, mass and structural properties of the self-assembled film and com-pare the self-assembly process under different conditions such as temperature, solvent type or concentration, flow or static environment and particle type.

Modifying polymer films with nanoparticles to form nanocomposite materials has become a useful method to modulate the structural properties and other characteristics of thin films to have more desirable properties and better stability (Figure 13). The QCM-D technology can be used to study changes in film thickness and

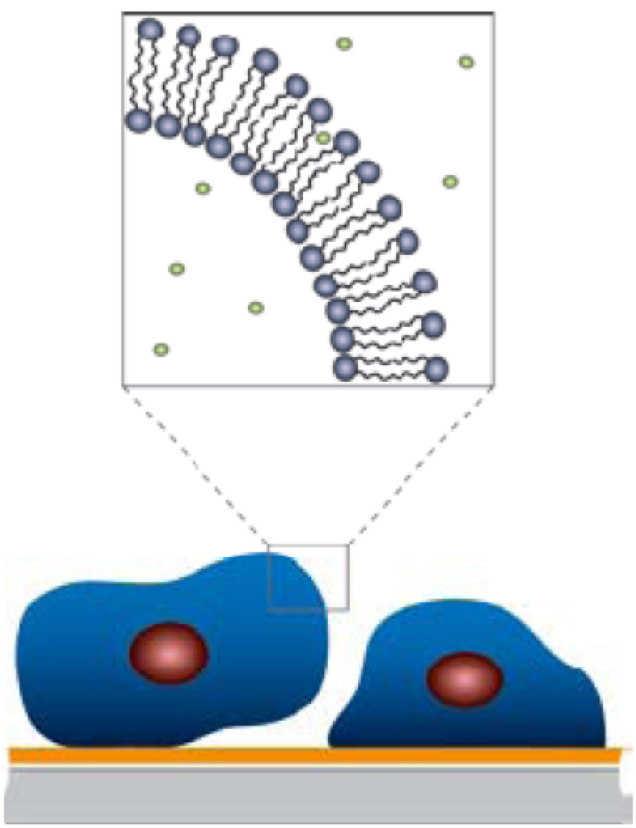

Figure 14 : Nanoparticle interactions in the environment[96]

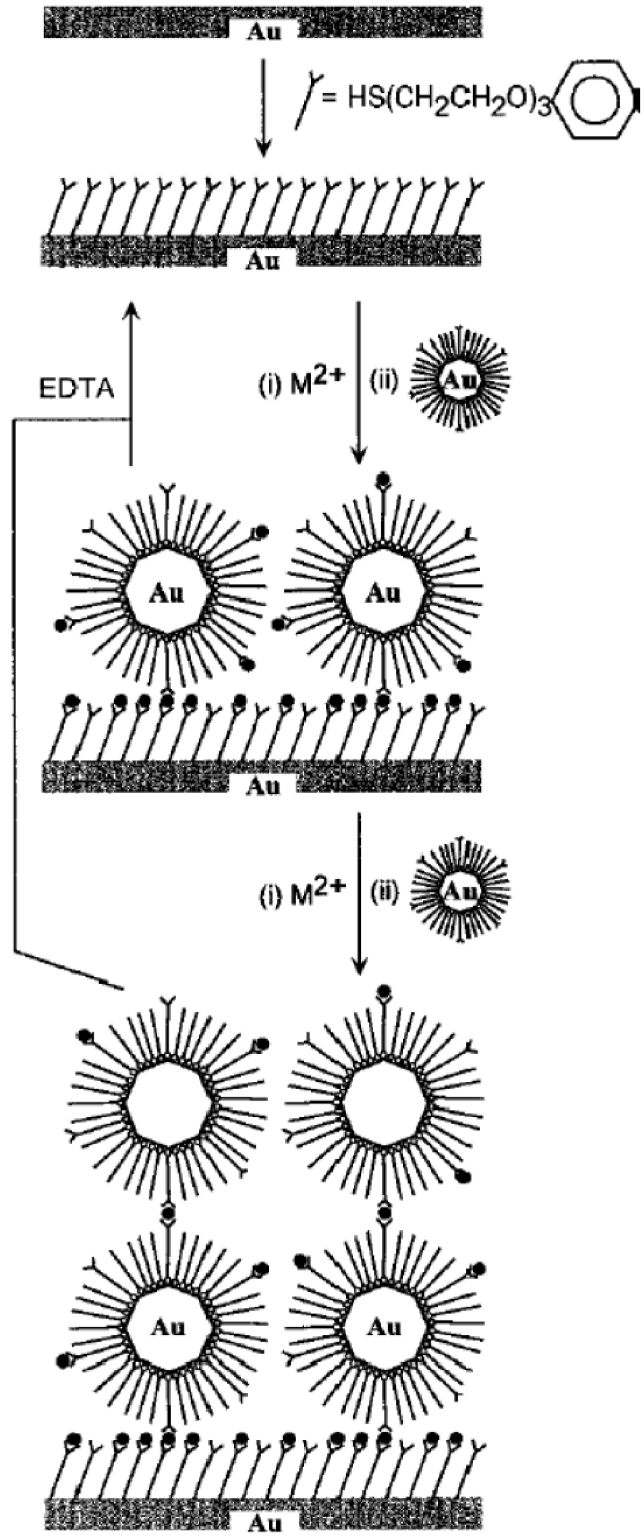

Figure 15 : Procedure for Nanoparticle Assembling bythe Chelating Interactions between Divalent (Transition)Metal Ions and Pyridine Moieties[102] Reproduced with permission from ref[102], Copyright 2002 American Chemical Society

rigidity due to the additional of nanoparticles. Properties such as thickness, viscosity, storage and loss modu-

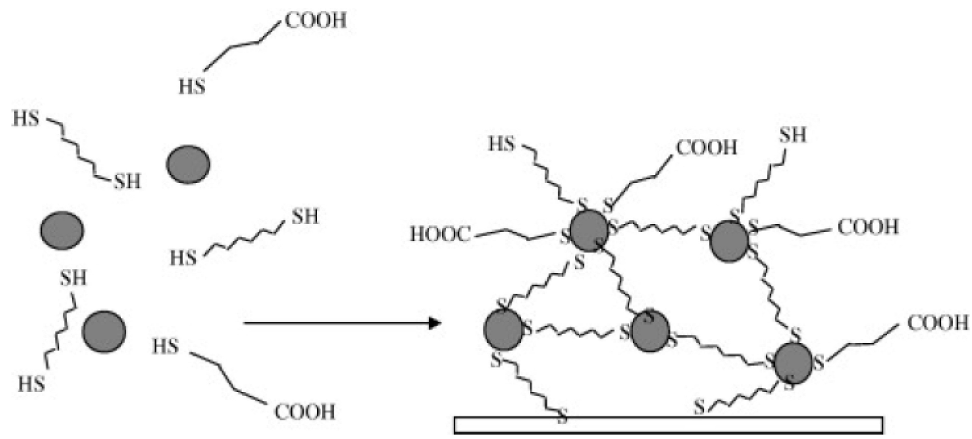

Figure16 : Schematic of the mixed–assembly cross-linking route for the preparation of Au nanoparticle network–type thin film. Reproduced with permission from ref[104], Copyright 2007 Elsevier

lus of nanocomposite materials can be measured, in real time, by QCM. There is concern that nanoparticles stable in the environment could cause significant health issues. The QCM technology can also be used to track nanoparticle stability and interactions under environmental conditions with a variety of real-life surfaces such as glass, metals and natural organic matter (Figure 14). The technique can track the kinetics, extent and stability of nano-particle deposition onto different surfaces and in different environmental conditions.

Chen et al described the fabrication of gold nanoparticle surface thin films by taking advantage of the strong complexation interactions between transition metal ions and pyridine functional groups (Figure 15)[102]. The thickness (layers) of the particle thin films was readily controlled by the repetition of the alternate dipping cycles, as monitored by quartz crystal microbalance (QCM).

Liu and hu showed interaction between myoglobin and hyaluronic acid in their layer-by-layer assembly by quartz crystal microbalance and cyclic voltammetry studies[103]. Quartz crystal microbalance (QCM) and CV were used to confirm the film growth and characterize the films. Shen et al investigated a means of producing Au nanoparticle network-type thin films that are derived by a one-step mixed-assembly cross-linking route, can avoid nonspecific interactions, and provide maximum binding to a specific target (Figure16)[104].

CONCLUSION

Quartz crystal microbalances have emerged in the last few years as versatile biosensors demonstrating remarkable achievements such as high sensitivity and label free detection.In the quartz crystal microbalance, the bioreaction generates a change in the mass, reordering the charges in the surface of the piezoelectric material and giving rise to a change in the resonant frequency of the microbalance. Its detection range and sensitivity are not quite as goodas some other label-free sensor technologies (e.g. surface plasmon resonance). The use of NP labels in sandwich assays can increase both the surface stress and the mass of the immune complex, allowing the increase in sensitivity of these electromechanical assays. This progress will require an increased understanding of both theoretical and experimental aspects of the relation among changes in frequency, energy dissipation, and NPs. These advancements will have a tremendous impact on the versatility and effectiveness of the QCM in future applications.In this review, application of nanoparticles in Quartz Crystal Microbalance biosensors has been extensively reviewed. The theoretical/experimental analysis, designand applications of quartz crystal microbalance biosensors combined with nanoparticles amplificationhave been discussed. These biosensors present as a detection option that can beeffectively employed to specific pathogen related requirements.The prospects are low detection limit, less time consuming,economical, biocompatible, precise and accurate like features.New nanoparticles need to be explored for use in these biosensors. Therefore, QCM is a relatively new and promisingresearch area worthy for further exploration.

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