Biosensors Based on Nano-Particles

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Abstract

Nano-particles have various unique features , which include biocompatibility, rapid and simple chemical synthesis, excellent electro-activity, and efficient coating by biomolecules. So if biosensors are built from nano-particles, it is proved to be a benefactor. Taking this in account, the paper discusses important features of nano-particle biosensors and R&D bibliometric analysis. Since R&D bioliometric analysis show that gold nano-particles are the best in class, this paper evaluates them in detail.

Keywords: Gold Nano-Particles, Bibliometic analysis.

1. Introduction

Bio-sensors are devices that are routinely applied in applications such as monitoring glucose content in blood, quality analysis of fresh and waste water. This is because bio-sensors react to the presence of bacteria, viruses or bio-molecules such as proteins, enzymes and DNA. Research on nano-particles based biosensors is rapidly gaining attention in the research community. More specifically, there is tremendous interest in applying nano-scale materials to biological material for sensors. Nano-materials are ideal candidates for building sensor devices. This is because even a few molecules of nano-materials can alter the properties very drastically. Such changes can be easily detected by optical, electrical and chemical means. Start-of-the art nano-material based biosensors have high sensitivity. This makes it possible to use them in applications where we need to detect one particular molecule against a background of many others. Use of metal and semiconductor based nano-particles is also gaining increased popularity in bio-sensors. Changes in color, fluorescence intensity, emission colour and electrical current can be used as sensing mechanisms.

2. Nano-Particle Biosensors

With the recent advancement in the field of nano- particle biosensors, there are various biosensors which have come into picture. These nano-particle biosensors and their special features are listed below

Bio-sensors	Unique features
Gold nano-particle based	Show potential to facilitate molecular bonding to detect glucose in the micromolar concentration range.
Amperometric biosensors	Are aided by silver nano-particles. Show increased
	biocompatibility, which aids in pesticide detection.
Palladium nano-particles	Fabricate a sensitivity-enhanced electrochemical DNA biosensor
Functional nano-particles	Bound to biological molecules. Developed for use in biosensors to detect and amplify signals.

Bibliometric Analysis Bibliometric analysis is employed to ascertain R&D trends and research networks for nano-particle-based biosensors. Bibliometric analysis is a tool for extracting information from large databases looking for patterns and explaining reasons for apparently unstructured behavior. Figure 1 shows trend line of article counts, based on the cumulative number of publications by each of the three datasets i.e. SCI, FACTIVA, INSP. Apparently the overall trend of the publication counts keeps increasing, which shows that nano-particles have played a more and more important role in the this research and this trend is likely to increase further.

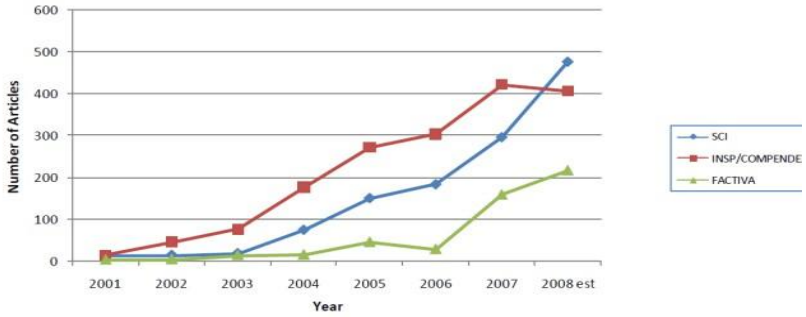

Figure 1: Trend of article counts for SCI, FACTIVA and ISP.

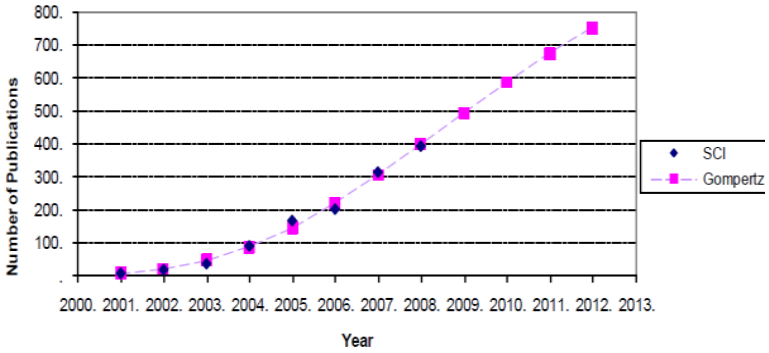

Figure 2: Trend of publication counts for SCI and Gompertz.

Figure 3 shows that the demand of metal nano- particle is increasing gradually. It further shows that the demand for gold nano-particles far exceeds the demand for platinum and silver nano-particles.

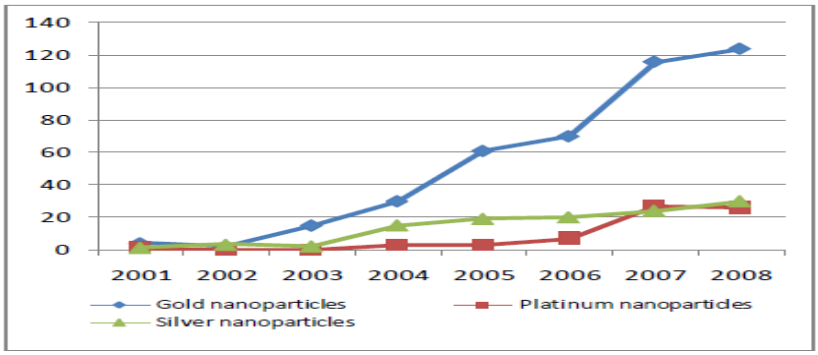

Figure 3(a): Increasing demand for gold nano-paticles Figure 4 shows that metal nano-particles are on the top if we compare all types of nano-particle biosensors.

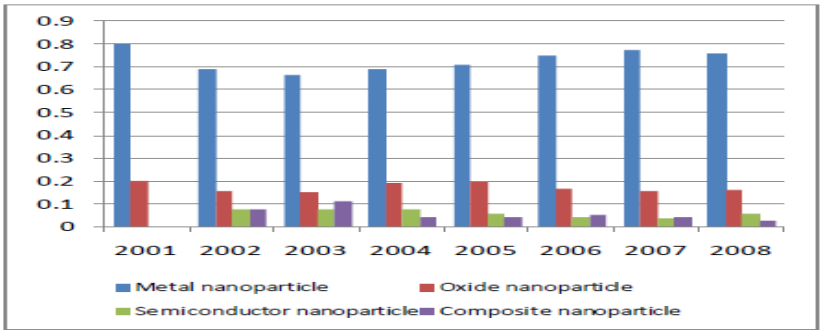

Figure 3(b): Increasing demand for metal based nano- particles

Gold Nano-particle Biosensors Gold nano-particles typically have dimensions ranging from 1-100nm. In addition, gold nano- particles display many interesting electrical and optical properties. Metals (like the gold in the nanoparticles) are good conductors, which is why they are used in electronics and wiring. Metals are good conductors for two reasons. First, electrons are not bound to individual atoms. Instead, they form a cloud around the atomic cores. This cloud of electrons is mobile allowing metal to transport charge (electrons) easily. Second, light is reflected off the surface of metals back to the eye. This is due to the electron cloud that surrounds the metal. Photons (individual units) of light cannot be absorbed by the atomic cores because they are blocked by the electron cloud. Consequently, photons are reflected back to the eye producing the sheen associated with metals.However, we also know from quantum mechanics that electrons can behave as either a wave or a particle. If we imagine electrons in the electron cloud as a wave with a certain energy value, we can envision a situation where it is possible for light of the same wavelength to be absorbed by the electron cloud, producing resonance. This is similar to what happens on stringed instruments, when a vibration occurs that matches the natural length of the string or one of its harmonics.

3. Principle

Metals are typically characterized by the presence of "free" electrons. In nanometer sized metal particles, there will be a strong absorption of light by the collective excitation of these unbound electrons. This absorption is referred to as a plasmon resonance and in the case of gold nano-particles they will have a spectral position and width that depends on its size, shape and to some extent the size distribution of the ensemble. When a metal absorbs light of a resonant wavelength, it causes the electron cloud to vibrate, dissipating energy. This process usually occurs at the surface of a material (as metals are not usually transparent to light) and is therefore called surface plasmon resonance. Figure 4 shows the plasmon resonance phenomena.

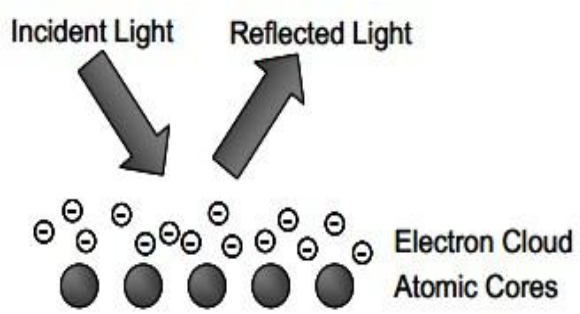

Figure 4: Plasmon resonance phenamenon

3.1 Equipments used

There are various equipments used to make measurements on nano-particles. This paper discusses three equipments listed below.

3.2 Scanning electron microscope (SEM) creates images of invisibly tiny things by bombarding them with a stream of electrons. This allows us to look at features on a scale as small as 10 nanometers (billionths of a meter). An SEM shoots a beam of electrons at the examination spot, transferring energy to the spot that it hits. The electrons in the beam (called *primary electrons*) break off electrons in the specimen. These dislodged electrons (called *secondary electrons*) are then pulled onto a positively charged grid, where they are translated into a signal. Moving the beam around the sample generates a whole bunch of signals, after which the SEM can build an image of the surface of the sample for display on a computer monitor [3].

3.3 Atomic force Microscope (AFM) scans the movement of a really tiny tip made of a ceramic or semiconductor material as it travels over the surface of a material. The tip positioned at the end of a *cantilever* (a solid beam) is either attracted to, or pushed away from the sample's surface. This deflects the cantilever beam and a laser measures the deflection. AFM then produces a visible profile of the little hills and valleys that make up the sample's surface.

3.4 Transmission electron microscope (TEM): Bouncing electrons off a sample is only one technique; you can also shoot electrons through the sample and watch what happens. That's the principle behind a transmission electron microscope (TEM). In effect, it's a kind of nano-scale slide projector. Instead of

shining a light through a photographic image (which allows certain parts of the light through), the TEM sends a beam of electrons through a sample. The electrons that get through then strike a phosphor screen, producing a projected image. Darker areas indicate that fewer electrons got through it, hence indicating that portion of the sample was denser. Lighter areas are where more electrons got through it, hence indicating that portion of the sample was less dense)[4].

4. Fabrication

There are various methods to fabricate gold nano- particle biosensors. This paper discusses two methods listed below.

4.1 Method: A glass slide is thoroughly cleaned in ethanol and deionized water for 15 min under ultrasonic agitation. Then, it is pretreated in a 30:70(v/v) mixture of H2O2 (30%) and H2SO4 (conc.) at 60-80˚C for 45 min, washed with deionized water for 15 min and dried in an oven at 110˚C for 45 min[7]. Surface of the substrate is modified by putting the glass slide in a solution of modifying agent, i.e. MPTMS, APTMS and PEI, in methanol for predetermined time (4, 12 and 20 hours). Then, the glass slide is washed with methanol and deionized water respectively, in an ultrasonic bath. Subsequently, the glass slide is immersed in gold colloidal solution prepared earlier. The deposition time for AuNPs is varied from 4, 12 and 20 hours, after which the glass slide is extensively rinsed with deionized water. Surface of the coated sample was characterized by X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM).

4.2 Method 2: 1.3 μm polystyrene latex (PSL) is used for template microsphere of colloidal crystal and 30 - 40 nm colloidal gold NPs is used to prepare assembled structure for SERS substrate. This process selected larger size gold NPs than 15 nm gold NPs which Kuncicky had used. This is because the apparent intensity of local plasmon resonance of 40 nm gold NPs is relatively larger than that of 15 nm and therefore more active substrate is expected as a result of experiments of colloidal aggregates. PSL and colloidal gold NPs were mixed and the volume fraction of PSL was adjusted from 2.5×10^{-3} to 5×10^{-3}. A glass plate is rinsed by the mixture solution of ethanol and water, and irradiated by ultraviolet light to make hydrophilic surface. Rinsed O-ring silicone rubber (5 mm diameter, 0.5 mm thickness) is put on the glass plate and the suspension of particle mixture is dropped into the ring for the fabrication of colloidal crystal with assembled structure of gold NPs. This O-ring is available to conserve the amount of gold NPs in the circumference of dried spots. After the drying process of the suspension, the PSL as template particles is removed by submersing the glass plate in methylene chloride for 15 min. A scanning electron microscope (S-4300, Hitachi, Tokyo, Japan) is used to observe the nanostructure of the substrates. SERS spectra is measured by a Raman spectroscope with a 785 nm incidental laser. A 10 μL of 30, 100 or300 nM 4,4'-bipyridine (4bpy) aqueous solution is dropped on the SERS substrate and then the measurement is immediately performed. Raman spectrum of pure water is also measured as the background. The collection time of each SERS measurement is 10 seconds. Time course of SERS spectra were measured at the time of 1, 3, 5, 7 and 15 min after addition of 4bpy.

Figure 5 shows the schematic of the above prescribed method.

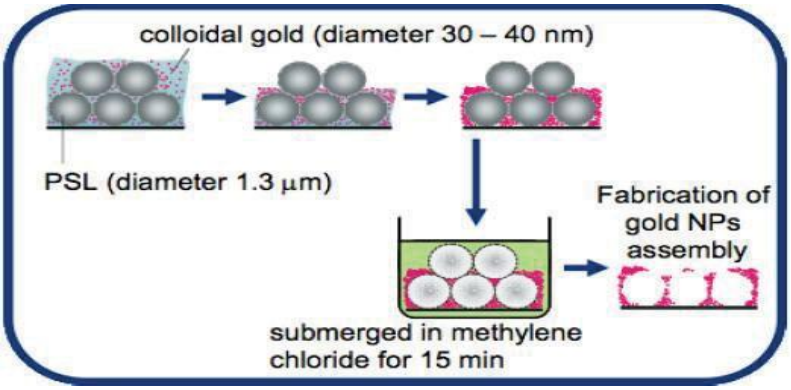

Figure 5: Schematic of Method 2

5. Characterization

Model gold nano-particles have diameter of 200 nm and length 2.5 – 4.0 μm. Surface modifications of gold nano-particles is done by the chemical compound 3-Mercapto-1-Hexanol ($C_6H_{14}OS$).

This paper focuses on surface characterization which includes size measurement (Inverted microscope Olympus IX70), electro-kinetic properties (ZetaPALS), and hydrophobicity (VCA Optima Goniometer). Electro-kinetic property is a particle's ability to move in the electromagnetic field. ZetaPALS measures the particles' mobility, and then calculates to give zeta potentials or the surface charge values. Figure 6 shows that the optimum concentration occurs at (OD_{546nm}): **0.15 - 0.30**

Effect of concentration

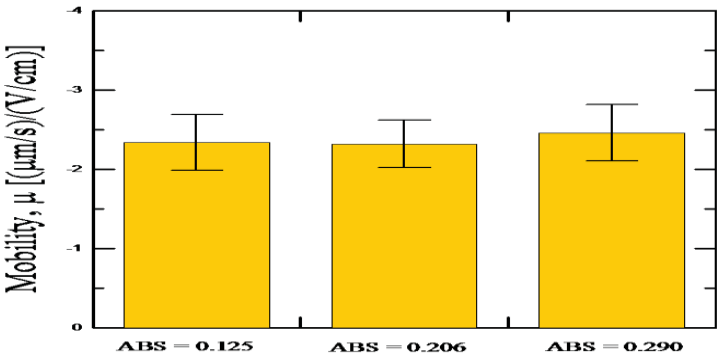

Figure 6: Mobility versus concentration

Effect of Size

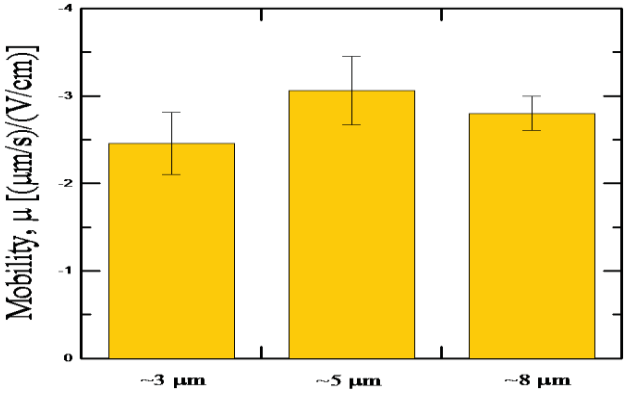

Figure 7: Mobility versus size, shows that mobility is not a function of size.

Figure 8 shows the effect of **valence** and **ionic strength.** As ionic strength increased in the presence of salt solutions, mobility became less negative (charge on particle approached neutral). Valance had an important role on mobility, in the presence of divalent cations, mobility was less negative than that in the presence of monovalent cations.

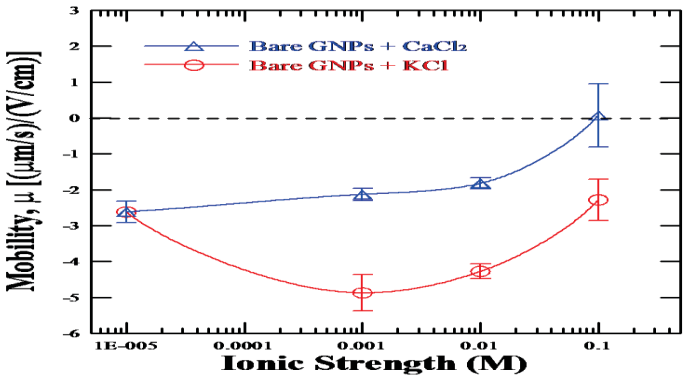

Figure 8: Mobility versus ionic strength

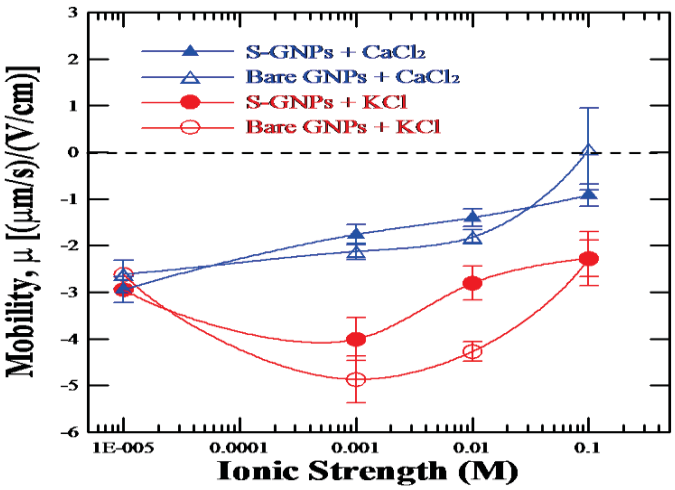

Figure 9: Mobility versus ionic strength for bare GNP versus S-GNP

Figure 9 shows the electro-kinetic properties of Bare GNPs vs. S-GNPs. The mobility of S-GNPs was less negative than that of bare GNPs in the presence of KCl. However, the difference was not significant in the presence of $CaCl_2$. **Valence** played an important role on GNPs' mobility regardless of the presence of 3-mercapto-1-hexanol groups.Moving on the other side, hydrophobicity refers to a surface's property of being water-repellent. Contact Angle method is used to know at what degree is GNPs hydrophobic. It is said to be hydrophobic if the contact angle is greater than 90˚ and hydrophilic otherwise. Solution concentration used is OD_{546nm} : 1.684 (2.5x dilution). The optimum angle is observed at the concentration of 100μL. Contact angle of S- GNPs: 135.8 ± 3.2 ^{O}. This shows that the surface of S-GNPs is hydrophobic. Functional groups 3- mercapto-1-hexanol did not affect the hydrophobicity significantly.

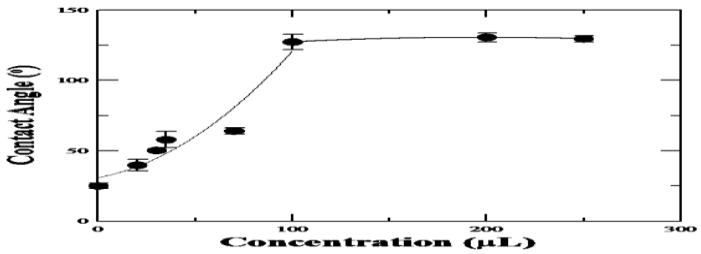

Figure 10: Contact angle as a function of concentration

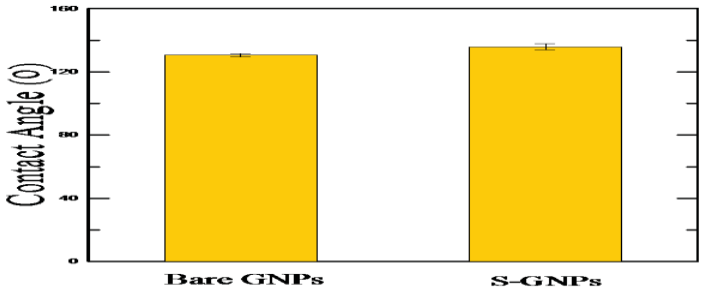

Figure 11: Comparison of contact angles for bare GNPs and S-GNPs

6. Applications

The most familiar application of nano-particles in sensing is the home pregnancy test. Nano-particles (< 50 nm) are bound to antibodies, complementary to a hormone produced by pregnant women. When the stick is submerged in urine flow, if the hormone is present it will bind to the microspheres (~ 500 μm) and nano-particles causing aggregates to form. The solution then passes through a paper filter. If the pregnancy hormone is present, the aggregates will be trapped by the filter producing a colored product. If the pregnancy hormone is not detected, the nano- particles will pass through the filter because of their

6 . 1 small size.

Urine passes from the flow stick to a central reservoir containing gold nano-particles and latex micro- particles. If pregnancy hormone is present, particles aggregate and are prevented from passing through a downstream filter. This produces a red signal in the viewing window. Nano- and micro- particles are modified with antibodies (blue) that bind to pregnancy hormone. If pregnancy hormone (yellow) is present in urine, particles will aggregate and are unable to pass through the downstream filter[8].

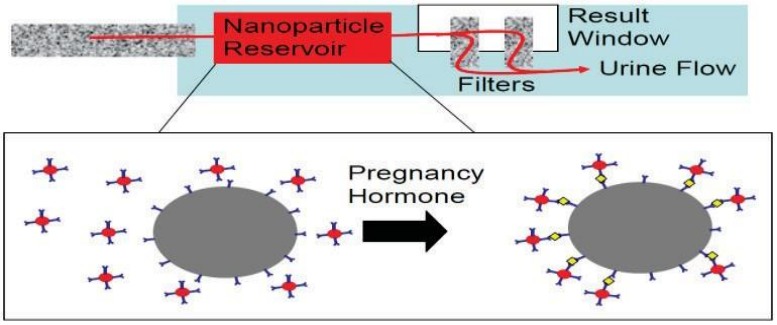

Figure 12: Application of nano-particles for home- pregnancy test

Functions	Biosensor	Sensor Advantage	Typical Examples
Bio-molecule Immobili- zation	Ampero-metric Biosensor	Improved Stability	For the determination in inulin of food
Catalysis of reactions	Glucose Biosensor	Improved sensitivity And	Glucose in gold nanoparticles
Labelling Bio- molecules	Optical Biosensor	Improved sensitivity and indirect detection	Self assembled nanoparticles probes for recognition and detection of biomolecules
Enhance-ment of electron transfer	Electroche mical Biosensor	Improved sensitivity and direct electroche mistry of protiens	Colloidal gold enhanced DNA immobilization for electrochemica l detection of sequence specific DNA.

Other applications of gold nano-particles in different biosensor systems are listed in the table above.

7. Conclusion And Future Prospects:

Various aspects of gold nano-particles applications are presented in this paper. The properties of gold nano-particles can be modified by the adsorption of both polymers and biopolymers. The typical preparation strategies and applications of these gold nano-particle–polymer hybrids are summarized. The unique optical properties, special catalytic properties of gold nano-particles allow the use of these particles as labels for colorimetric detection of biomolecules, for developing sensitive biosensors. There is immense potential of gold nano-particles for cancer diagnosis and therapy. One of the most promising areas of gold nano-particles application is photothermal therapy, also antibacterial activity of drugs conjugated with gold nanoparticles.

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