specific examples of nano-materials/nano-sensors focusing primarily for water industry are described. Specific examples illustrating the strengths of micro-and nano-technology for microbial detection are highlighted. A comparison is made between whole cell and nucleic acid based detection schemes for waterborne pathogens. A brief summary about sample processing and a novel DNA concentration scheme that could be used to eliminate substrates inhibitory to PCR is discussed. Selected studies related to market trends are also summarized.

13.2 Characteristics of Sensors for the Water Industry

A number of parameters must be considered during the development of any sensor for the water industry. These include specificity, sensitivity, speed, cost, sample throughput, and target multiplexing (Figure 13.1a). Most micro and nano-scale sensors attempt to optimize the assay for speed and cost followed by sensitivity and specificity. Increasing target and sample throughput are often not the goals for these sensors, although exceptions do exist. The ability to discriminate between closely related organisms/nucleic acid sequences is critical in screening assays, because of their widely differing characteristics. Sensitivity or limit of detection is the smallest amount of a species of interest that can produce a measurable output signal. The cell based detection systems represents the limit of detection in the units of colony forming units for bacteria and plaque forming units for viruses.

A lower limit of detection for a given agent helps minimize the risk from its exposure. The time required to complete an assay is also important because detection should be rapid enough to allow time to warn the consumer or take preventive action before the water reaches the consumer. Approaches that eliminate the need for sample concentration and/or processing and target labeling e.g., nanocantilevers (Carrascosa et al., 2006), surface plasmon resonance (Chinowsky et al., 2007), and nanoparticles/ nanowires (Jianrong et al., 2004) when combined with real time concentration techniques may be able to play a key role in continuous monitoring of water for selected pathogens. In order the make the sensors cost effective, integration of complex fabrication steps, batch fabrication, and use of cheaper materials are necessary. Micro and nano-scale sensors are employed to detect pathogenic microorganisms in both clinical and environmental (water industry) samples. However, with respect to sample processing, background causing cross hybridization, and sample volume, are two different problems (Figure 13.1b). Environmental samples usually have high volume, low amount of targets, and high amount of background while clinical samples have just the opposite. Processing of environmental samples is complex because of the need to concentrate small number of targets (1–10,000 cells) from a large volume of water (1–100 liters) and the presence of high background. This complexity in dealing with environmental samples limits the widespread use of sensors in the water industry.

13.3 Working Mechanisms and Types of Sensors

13.3.1 Working Mechanism of Sensors

Micro- and nano-sensors can be classified according to the type of bioreceptors, transducers, and applications (Vo-Dinh and Cullum, 2000). Bioreceptors are biological molecules (antibody, enzyme, protein, nucleic acid, cell etc.) that exploit the biochemical interaction for analyte (antigen, nucleic acid) recognition. Transducers are electrical/electronic components that convert the biological detection event into a measurable signal. The conversion of biological event signal to a specific physical signal differs for the type of transducer, e.g., i) an electrochemical transducer converts the biochemical event to an electrical event by measuring the potential difference between electrodes, ii) an optical transducer converts the biochemical event to a photochemical event by measuring the difference in light intensity, and iii) a mechanical transducer converts the biochemical event to an electrical event by measuring the difference in mass or surface stress.

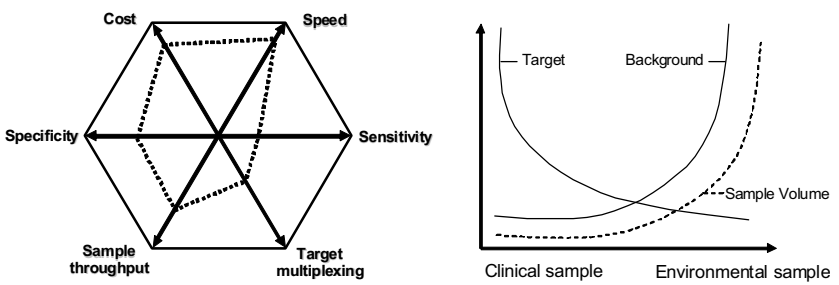

Figure 13.1 (a) Representation of parameters of interest during methods development for micro and nano-scale sensors. Envelope shown by dark and dotted lines represents the ideal and real scenario of biosensor development studies, (b) comparison of the level of complexity for environmental and clinical samples applicable to sensors. High volume, low amount of targets, and high amount of background in environmental samples are bottlenecks in the widespread use of sensors in the water industry.

In general, a biosensor platform has three important components: i) bioreceptor, ii) transducer, and iii) output system. The target species (antigens, nucleic acids), when interacting with these bioreceptors, produces a biological signal, which is sensed by a transducer and transformed into a measurable response (current, potential, or light intensity etc.). The signal obtained at such small scale is quite low and noisy, which can be further processed, amplified, and stored for analysis by an amplifier and

microcontroller (output systems). The conceptual diagram of biosensing principle is shown in Figure 13.2. The most common forms of biosensing by these biological recognition elements/bioreceptors are: i) antibody/antigen interaction, ii) nucleic acid/nucleic acid interaction, and iii) cellular interaction. The commonly employed transducers are based on: i) optical measurements, ii) electrical/electrochemical measurements, and iii) mechanical measurements.

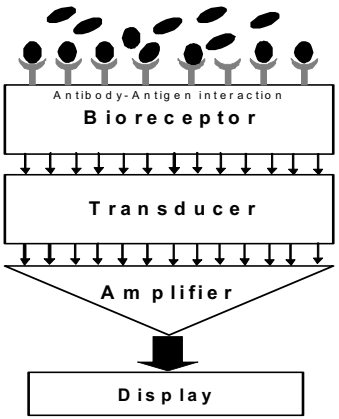

Figure 13.2 Schematic illustrating the general principle used in most micro- and nano-sensors.

13.3.2 Biomolecular Interactions Exploited in Sensors

There are two main classes of biomolecule interactions that have been exploited more than others to detect waterborne pathogens: i) antibody/antigen, ii) nucleic acid/nucleic acid. Specificity i.e., the ability to discriminate between two different closely related targets is governed by this interaction. Nucleic acid/nucleic acid based interactions have better specificity than the antibody/antigen (immunological) based interactions. The antibody/antigen interaction is analogous to lock and key mechanism (Figure 13.3), where the antigen-specific antibody interacts with the antigen in a highly specific manner. This interaction is exploited in various immunosensors, where the antibodies are generally immobilized on the surface of a substrate, which interacts with the specific antigens to produce biological signals. The antibody/antigen binding can be detected by a change in fluorescent intensity, refractive index, or reflectivity. Antibody/antigen interaction is mainly dependent on the immobilization strategy. Biomolecule immobilization is considered to be an important step in the development of sensors. Immobilizations that result in disoriented or overcrowded antibodies are known to induce non-specific antibody/antigen interactions. The most popular method to

control the orientation of antibodies is by immobilizing the antibodies on a self-assembled monolayer of alkanethiols. The interaction of thiol groups (-SH) on a gold surface forms robust SAMs with different terminal group functionalities (amine, carboxyl, methyl etc.) depending upon the chemical structure of alkanethiol molecule. The orientation in self assembled monolayer molecules induces the similar orientation on the immobilized antibodies (Oh et al., 2004). Immobilization of antibodies on Langmuir-Blodgett (LB) monomolecular films or protein films are also widely applied (Oh et al., 2003).

For nucleic acid/nucleic acid interaction, a short sequence of nucleic acid, called "probe" is immobilized on a surface and the complementary sequence of nucleic acid, called "target" is hybridized to the probe. Probes are signature sequences that are specific to the target organism. Usually they are part of a virulence or marker gene that is highly specific to the organism. Databases related to such virulence and marker genes of pathogenic organisms (e.g., VFDB located at: http://www.mgc.ac.cn/VFs/ and Virulence Database of Lawrence Livermore National Laboratory located at: http://mvirdb.llnl.gov/) are slowly becoming available to help in the design of such signature sequences. The target molecule is usually labeled with an optically detectable compound (fluorescent dye, quantum dot, gold particle), which interacts with the complementary probe and gives a specific signal. If assay has a low detection limit, target amplification using polymerase chain reaction (PCR) may be necessary prior to the labeling and hybridization step. Nucleic acid based sensors provide superior specificity (Lian et al., 2004) as well as genotyping (Lindroos et al., 2002), and multi-target detection capabilities.

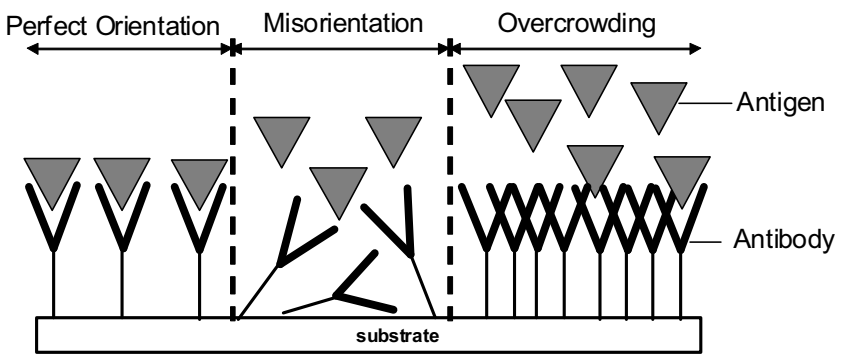

Figure 13.3 Schematic of antibody (hollow V-shaped) and antigen (filled triangle) interaction mechanism. As illustrated, disorientation and overcrowding of immobilized antibody induces non-specific interactions.

13.3.3 Types of Transducers

Transducers are electrical/electronic components that convert the signal associated with a biological detection event into a measurable signal. There are three types of transducers: i) electrochemical, ii) mechanical, and iii) optical. Sensors are often classified according to the type of transducer used as described below.

Electrochemical Transducers. Electrochemical sensor measures the change in current or potential during the biorecognition event (Wang, 2006). It can be classified according to the observed parameter as amperometric (current), potentiometric (potential), and impedimetric (impedance). One of the main advantages of electrochemical sensors is their ability to detect the analytes without labeling. For example, ultrasensitive detection of cancer genes was demonstrated based on the catalytic oxidation of guanine base by redox-threading intercalator, without amplifying the genes of interest (Tansil et al., 2005). Similarly, an electrochemical sandwich immunoassay was used to detect small quantities of *E. coli* O157:H7 cells in less than 6 minutes (Muhammad-Tahir and Alocilja, 2003). Electrochemical techniques can be applied to nucleic acid as well. However the lower specificity of electrochemical systems is an issue that needs to be addressed (Odenthal and Gooding, 2007).

Mechanical Transducers. Mechanical sensors can sense change of mass/stress by the bending (static mode) or resonant frequency (dynamic mode) (Carrascosa et al., 2006). Adsorption of analytes on the sensor surface produces stress that bends the surface and changes its resonance frequency. The bending and change in resonance frequency can be monitored by optical, piezoresistive, piezoelectric, capacitance, or interferometric technique. There are many advantages for using a mechanical sensor including extreme simplicity as electrodes can be used for sensing changes in frequency or bending instead of optical lasers and targets do not need to be labeled, thus higher sensitivity, low assay time, and portability are achieved. Cantilevers based systems have the potential to detect single target molecule of DNA (Ilic et al., 2004). This was demonstrated in a recent study where antibody functionalized piezoelectric-excited cantilevers were used to measure *E. coli* O157:H7 cells in a 1-liter water sample at 1 cell/ml concentration (Campbell and Mutharasan, 2007). Such techniques have the greatest potential for online continuous monitoring of selected pathogens and indicators – a key need for the water industry.

Optical Transducers. Optical sensors can transduce a number of phenomena like fluorescence, phosphorescence, refraction, dispersion etc. which can be quantified (Erickson et al., 2008). Optical transduction is the most widely used phenomena for sensors. Biomolecules to be measured are tagged with an optically detectable compound (fluorescent dye, quantum dot, gold nanoparticle) and exposed to a light with sufficient energy. Electrons absorb the light and jump from the valence band to the excited state.

While returning back to the ground state, they emit light with a different wavelength than the exposed light. Fluorescein isothiocyanate, tris (2'2-bipyridyl) dichlororuthenium (II) (Rubpy), 6-carboxyl-X-rhodamine are some of the commonly used markers in bioassays. In order to improve the sensitivity and photostability of organic fluorophores, dye doped silica nanoparticles have also been used (Yan et al., 2007). Using the cage structure of a nanoparticle, a high number of organic fluorophores (hundreds to thousands) can be incorporated inside a single silica particle. Labeling of biomolecules, however, may be a time-consuming process. Alternatives like surface plasmon resonance (SPR) permit the detection of label free molecular interactions and shows improved specificity, sensitivity, and reproducibility and allows portability (Chinowsky et al., 2007).

13.4 Fabrication and Synthesis of Micro- and Nano-Scale Materials

Fabrication of micro- and nano-sensors is accomplished using techniques already developed in the integrated circuit manufacturing technology. It consists of four steps: i) thin film growth/deposition, ii) photolithographic patterning, iii) etching, and iv) surface and bulk micromachining. Earlier, photolithography had some limitations because the then available 157-nm wavelength could produce features as small as 75 nm (Service, 2001). However, recent advances using deep ultraviolet light can generate 40 nm sized features (Totzeck et al., 2007). The fabrication of more complex structures (~nm sized) is also possible by the advancements in e-beam and X-ray lithography techniques. The integrated circuit manufacturing technology, originally limited to silicon based materials (e.g., silicon, silicon dioxide, and silicon nitride) is also now routinely used for the fabrication of complex devices made out of glass, plastic, and quartz, and other novel materials. These materials have specific electrical, mechanical, and thermal properties enabling cost effective and tailored characteristics by combining two or more materials

Microcantilever-based sensors show high potential to detect biological molecules with extraordinary accuracy. A number of commercial entities like Concentris (www.concentris.ch), Cantion (www.cantion.com), Veeco (www.veeco.com) now produce micro-cantilevers with typical lengths of 10 to 500 μm and thickness of a few nm. These cantilevers are batch fabricated by thin film processing technology from silicon-based materials. High throughput platforms using arrays of cantilevers can be used as an alternative to microarrays. For example, the VeriScan 3000 system from Protiveris (www.protiveris.com) uses 64 cantilevers to detect biomolecular interaction events in real time. Recently a company originating out of the work done at the University of California, Berkley named Kalinex Inc. (http://otl.berkeley.edu) demonstrated the high throughput detection of 100 to 200 compounds by employing 1000 micro-cantilevers on a chip (Carrascosa et al., 2006). In recent years, considerable effort has been devoted to the nano-patterning of biomolecules on surfaces. These are

termed as nanoarrays - a miniaturized version of microarrays. They have become invaluable tools for genomics and proteomics research. Common methods for the fabrication of nanoarrays are dip-pen nanolithography (Piner et al., 1999), nano-grafting (Liu et al., 2002), and finely focused ion beam lithography (Bergman et al., 1998). Nanoarrays provide highly parallel, multiplexed, and ultrasensitive format for diagnostic applications of biomolecules (Ekins, 1998).

In addition to the above-mentioned two- and three-dimensional nano-structures, nanoparticles (NPs) are also becoming a critical component in sensors. Nanoparticles are often presented as a more effective alternative to organic flourophores. NPs are attractive candidates in sensor applications because of their: i) smaller size (1-100 nm) and correspondingly high surface to volume ratio, ii) chemically tunable physical properties related to size and composition, iii) excellent photostability and high luminescent signal, and iv) possibility of surface modification for biocompatibility. Gold nanoparticles, quantum dots (QDs), and organic dye doped silica nanoparticles are the more common examples of NPs used in bioimaging and biosensor applications. NPs possess very high surface area for the immobilization of analytes. In addition, most of the nanoparticles are easily synthesized using routine chemical procedures. Gold NPs, for example, are commonly synthesized by reduction of $HAuCl_4$ by sodium citrate solution (Turkevich et al., 1951), two phase reduction method (Brust et al., 1994), and seeding technique (Murphy and Orendorff, 2005). Extensive information is available on the synthesis and characterization of QDs (Boatman et al., 2005).

Figure 13.4 b depicts silica nanoparticle doped with dye molecules to enhance the resulting signal for detection of *E. coli* O157:H7. Several novel organic dye-doped silica nanoparticles were designed for high dispersibility in water, excellent photostability, easy surface modification capacity, size uniformity and tunability and produced high-quality luminescent signals (Santra et al., 2001). A large number of dye molecules (hundreds to thousands) can be incorporated inside a single silica particle. Moreover the already developed silica chemistry allows for surface modifications of dye-incorporated silica NPs. Two methods that are commonly applied for the synthesis of dye doped silica NPs are the method of Stäber et al. (Shibata et al., 1997) and the reverse microemulsion method (Yamauchi et al., 1989).

13.5 Detection Limit as Key Parameter for Pathogens in Water

There are many challenges to achieve rapid and accurate detection of small amounts of target microorganisms present in complex matrices. Thus, concentration of target and its enrichment or amplification is always required. Genetic targets can be amplified by polymerase chain reaction, ligase chain reaction, Klenow fragment amplification, strand displacement amplification or tandem amplification process. As a

reference, PCR sensitivity level can be assumed as 100 copies of target gene/mL (derived from 5 copies per 50 μL volume in a PCR vial). PCR and real time PCR, of course, can be optimized to detect 1 copy in a reaction volume but it is seldom achieved in routine analysis, especially when reaction volumes are also small. Methods without some type of amplification or concentration have much poorer detection limit. For example, conventional glass slide based DNA array using Cyanine-3 labeling and confocal laser scanning microscopy can only detect 5 pM which is approximately 3.00×10^9 copies of target gene/mL (Taton et al., 2000). As illustrated in Table 13.2, the issue of detection limit persists in most microarray-based approaches that do not use PCR as a pre-amplification step. Certain nanoparticle-based techniques using indirect observation of the target do report much better sensitivity, in the zeptomolar range. The bio bar-code amplification technique reported by (Nam et al., 2004) is one such example.

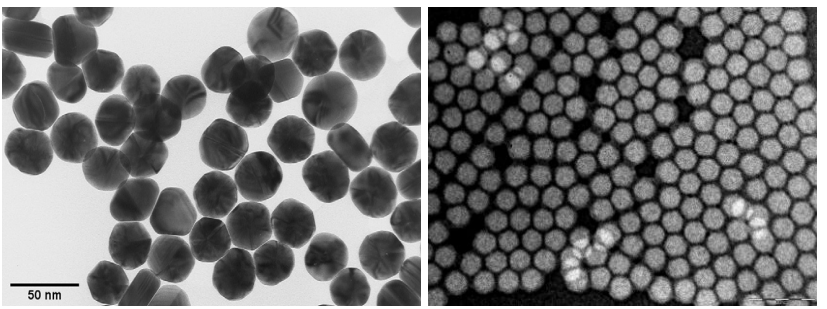

Figure 13.4 (a) Transmission electron microscopy (TEM) image of octanethiol functionalized gold NPs (Canu et al., 2008), and (b) TEM image of Rubpy dye-doped silica NPs (Zhou et al., 2004). Copyright Elsevier 2004 and 2008, reproduced with permission.

13.6 Labeling Approaches

Use of nanoparticle-based approaches is emerging as a competing approach to the use of dyes (e.g., used in microarrays). The large surface area of nanoparticles serves as a compatible and versatile substrate for biomolecule immobilization (Niemeyer, 2001). This facilitates the conjugation with signaling reporter groups and thus renders each nanoparticle a high-density signaling probe. For example, many fluorophore molecules can bind to one nanoparticle probe, and each binding site on the target can thus conjugate more fluorophore molecules. In addition to the contribution to high assay sensitivity, nanoparticle labels have not only extraordinary target recognition

Table 13.2 Sensitivity of fluorophore and nanoparticle labeling-based microbial detection assays.

Scheme description	Detection limit	Reference
DNA hybridization on glass slide; Cyanine3 labeling and fluorescent signaling; imaged by confocal microscopy.	$\geq 3.01 \times 10^9$ copies of target gene /mL	(Taton et al., 2000)
Sandwich-structure DNA hybridization for target recognition; Gold nanoparticle labeling for target identification; Silver enhancement; gray level signaling of silver coating.	3.01×10^7 copies of synthetic target gene /mL	(Taton et al., 2000)
Sandwich-structure, DNA/RNA hybridization for target recognition; Gold nanoparticle labeling for target identification; Ag enhancement; Surface-enhanced Raman scattering signaling.	1.20×10^7 copies of synthetic target gene /mL	(Cao et al., 2002)
Bar-code amplification (BCA); sandwich-structure hybridization for target protein (prostate-specific antigen) recognition; Au nanoparticle labeling for target identification; sandwich-structure hybridization for bar-code DNA detection; Ag enhancement; gray level signaling of silver coating.	1.81×10^4 copies of PSA /mL	(Nam et al., 2003)
BCA; sandwich-structure hybridization of target DNA recognition; Gold nanoparticle labeling for target DNA identification; sandwich-structure hybridization for bar-code DNA recognition; Silver enhancement; gray level signaling of silver coating.	3.01×10^2 copies of functional gene /mL	(Nam et al., 2004)
Tetramethylrhodamine-doped silica nanoparticle labeling of synthetic target DNA sequence; sandwich DNA hybridization for target recognition; TMR fluorescent signaling.	4.82×10^7 copies of synthetic DNA sequences/mL	(Zhao et al., 2003)
RuBpy-doped silica NP labeling of target genomic DNA; sandwich DNA microarray for target recognition; RuBpy fluorescent signaling.	$\sim 2.35 \times 10^5$ copies of gDNA/mL	(Lian et al., 2004)
RuBpy-doped silica nanoparticle labeling; immunological chemistry (mAbs labeling); Rubpy fluorescent signaling.	2.5 cells/mL	(Zhao et al., 2004)

abilities but may also have built-in coding systems for rapid and multiplex target identification. Nanoparticle labeling is expected to be more sensitive, more flexible in target selection (e.g., adding new genes or single-nucleotide mutations), more diversified in bar coding, faster in binding kinetics due to the pseudo-homogeneous reaction, less expensive to produce and less physically interferential in the biological recognition events due to the nanoscale-size particles (Han et al., 2001).

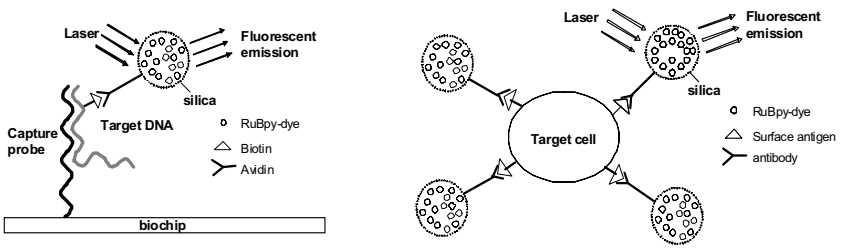

Figure 13.5 (a) RuBpy-doped silica nanoparticle based DNA labeling scheme using in (Lian et al., 2004), (b) RuBpy-doped silica nanoparticle labeling based immunological scheme used in (Zhao et al., 2004).

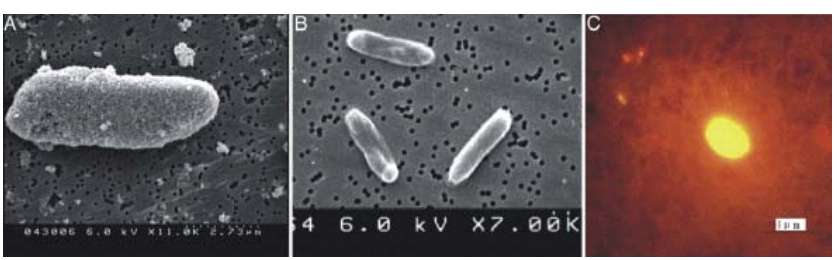

Figure 13.6 (a) Scanning electron microscopy (SEM) image of *E. coli* O157:H7 cell incubated with dye doped NPs (b) SEM image of *E. coli* DH5 cells incubated with dye doped NPs (negative control) (c) fluorescence image of a single *E. coli* O157:H7 cell. Copyright PNAS 2004. Reproduced with permission.

It should be noted, however, that the gain in signal enhancement (hence the improvement in detection limit) by using nanoparticle-based labeling approaches may vary by several orders of magnitude at the cell and the DNA level. This is illustrated in Figure 13.5 and Table 13.3, which compares the enhancement in detection limit from two studies using similar dye-doped nanoparticle labeling; one uses the label at the cell