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1. Introduction

Phenol is an aromatic compound, with a wide variety of uses both medical and industrial. During its production, emissions of even low amounts have been demonstrated to be toxic at submicrogram/liter levels. Phenol is also a good model compound to assess environmental impact of its emission particularly in wastewater. However, the current approach, methodologies and application described herein can also be applied to other organic environmental / pharmaceutical pollutants. Phenol has been removed from wastewater through a number of different approaches and monitored using spectroscopy and chromatography. In the field the most common method is detection through electrochemical or colorimetric sensors, which are described in this study.

1.1 Source and properties of phenol

Phenol is an organic compound, which can be generated from petroleum by-products like tar (reviewed by Gerberding, 2002) or from the metabolism of benzene or organic matter containing appropriate motifs (Martus et al., 2003). Phenol also occurs in thyme oil, oil of wintergreen and methyl salicylate and has been generated as a by-product in various industrial processes, such as coke production, in the manufacturer of wood preservatives, fungicides and as a synthetic precursor in the synthesis of organic compounds used in pesticide, dye and pharmaceutical synthesis (Akai et al., 1998) and in disinfection (Chick, 1908). In the production of epoxy resins and nylon, phenol is required for synthesis of caprolactam and bisphenol A, which are carcinogenic intermediate molecules (Jones, 1981). The disinfectant properties of phenol have applications in over-the-counter medicines such as mouthwash, disinfectants, or fungicides, which have traces of phenol, and in throat lozenges. The approximate usage of phenol varies by industry, but is in the millions of kilograms per year range (Gilman et al., 1988). Phenols (or phenolic resins) if directly released into environment (air, soil or water) are toxic (reviewed by Gogate, 2008). The release is not common but can occur as a result of its widespread use, for example in the
automotive, construction, plywood, and appliance industries (Zhang et al., 2006) and in the manufacture of plastics as a plasticizer or antioxidant (reviewed by Xanthos, 1969). The type and degree of substitution would dictate the stability and reactivity of the phenol derivative (reviewed by Babich & Davis, 1981; Salkinoja-Salonen, 1981).

The ease of removing hydrogen ion at the hydroxy-position can give information on acidity/basicity, as a general rule more electronegative groups such as nitro led to stronger acids than the parent alone (reviewed by Kozak et al., 1979). Once phenol is synthesized, it can be converted to the end-product through the appropriate synthetic routes, for example if chlorophenol or trichlorophenol isomers are required, they can be synthesized through the Boehringer Process with iron salt as the catalyst, under low, similarly for the synthesis of pentachlorobenzene, or chlorobenzene, or hexachlorobenzene isomers, the appropriate precursor is hydrolyzed under alkaline conditions (reviewed by Buehler & Pearson, 1970; McKillop et al., 1974), noting that phenol can also be readily oxidized.

1.2 Toxicological effects of phenol

Phenol can induce skin cancer as documented in dermal studies of cutaneous application of phenol and can act as a tumor promoter or a weak skin carcinogen in mice (USDA, 1980a; Kreijl & Slooff, 1983). Teratogenic effects of phenol have also been reported in animal studies. With nasal and cutaneous exposure, the results are irritation of the skin, eyes and mucus membranes. Cutaneous application of phenol results in dermal inflammation and necrosis. Dermatological disorders including discoloration of the skin (Deichmann & Keplinger, 1962; reviewed by Bruce et al., 1987). Different derivatives have different toxicities (with the toxicity being related to acidity and persistence being related to degree of solubility in fats and lipids), with nitrophenol being the most toxic followed by chlorophenols which in turn are more toxic than phenol alone, however, chlorophenols are more difficult to biodegrade, therefore pose more of a problem than phenol in terms of toxicity and persistence (reviewed by Crosby, 1982; Folke, 1985).

1.3 Sampling and cleanup

Procedures have been developed to monitor the different species of phenol generated such that they are below toxic levels in a variety of matrices (Fichnolz et al., 1965; West et al., 1966; Chau & Coburn, 1974). Generally, the sample which is thought to contain phenol has its pH changed to non-neutral pH values to minimize microbial degradation and stored in brown glass vials to decrease the loss to adsorption and photodecomposition, respectively (Afghan et al., 1974). The extraction of phenol from the matrices as varied as water, fish, air, soil or plants has relied on organic solvents such as petroleum ether, benzene, or chloroform for polysubstituted phenols and butyl acetate or isomayl acetate for (monosubstituted) phenol (Taras et al., 1971; Afghan et al., 1974; Greminger et al., 1982). Liquid-liquid partitioning can be used to separate phenol from other organic compounds found in the matrices, or column chromatography, silica gel chromatography have been used to achieve separation (reviewed by Rao et al., 1978; EPA 1980; USDA 1980b; Renberg & Björseth, 1983; reviewed by Busca et al., 2008).

1.4 Sensor overview

With the advancement of science and technology sensor can change the data into a digital reading or some other form for easy perception of results (reviewed by Karube et al., 1995).
and Rogers, 1995). Actual sensor design and manufacturing for environmental (reviewed by Hart & Wring, 1997) monitoring or sensor architecture (reviewed by Lynch & Loh, 2006) for monitoring and reporting are beyond the scope of this chapter. The generic approaches and applications will be discussed with emphasis on environmental monitoring or chemical detection particularly for phenol. Since the development of a blood sugar monitoring sensor, the miniaturization of sensors has been advanced dramatically in detection of other molecules of interest (Kadish & Hall, 1965). In environmental and medical applications sensors have been used in the monitoring of phenol, the widespread use of sensors is due to their small size, operational suitability (e.g. good linear range, selectivity and sensitivity for the target molecule), robustness, ease of operation and the ability for micro-fabrication and auto-control (reviewed by Wang, 1997; Yu et al., 2003).

Sensors can be fabricated under ambient conditions with excellent pressure and temperature stability coupled to negligible expansion or swelling in protic / aprotic solutions (i.e. chemical inertness, Yu et al., 2003). These favourable operation parameters have led to their widespread adaptability for various applications. Most methods of analysis require site identification, collection, storage and shipment of the samples for further processing at the laboratory equipped to do the chemical / biological analysis. Poor handling during this sample acquisition process can lead to high statistical variability of the measured values (Chau & Coburn, 1974; Klein, 1988; Shammala, 1999). Due to their size and portability, sensors have been evaluated more in instant field testing of analytes as opposed to lengthy laboratory testing of analytes (reviewed by Rodriguez-Mozaz et al., 2005) where a quick determination is required. Sensors may be in the form of microchips, electrodes or thin films. The most common methods for sensor fabrication are sensors with amperometric detection (Hanrahan et al., 2004), although gas-chromatography and colorimetric sensors have also been used (Saby et al., 1997). The colorimetric method relies on the formation of a colored complex either as the final product or as stable intermediate (Martin, 1949). Common colorimetric tests include use of tritracynoethylene or tritracynoethylene ([(NC)2C≡C(NC)]2, Smith et al., 1963a), diamine ((C3H5)2NC=C=NC6H4NH2, Houghton & Pelly, 1937; Eksperianova et al, 1999) leading to the formation of indophenol (Ettinger & Ruchhoft, 1948; Smith et al., 1963b; Gupta, 2006), which is measured through titration extracted with carbon tetrachloride as summarised by Hill (Hill & Herndon, 1952; Benvenue & Beckman, 1967; Regnier & Watson, 1971; NRCC-18578, 1982). In addition, derivatization with 4-aminoantipyrine (C11H13N3O) followed by ultraviolet (UV) spectrophotometric / spectrofluorometric detection, usually at 254 (or 280) nm can be used with a limit of detection (LOD) in the sub microgram / liter range (Lykken et al., 1946; Dannis , 1951; Afgan et al., 1974; Norwitz et al, 1979; Realini, 1980; Farino et al, 1981). Other methods utilize gas chromatography (Renberg, 1981; Giger & Schaffner, 1983) coupled with flame ionization detection (FID) for derivatized phenol (e.g. acetylated, or heptafluorobutyl, or pentafluorobenzyl ether phenol) to increase volatility of phenol to enable GC-based analysis, (Corcia, 1973; Renberg, 1982, 1983) or liquid chromatography (LC) methods such as reverse phase (RP) with UV or electrochemical (EC) detection (ECD, Bhatia, 1973; Churatek & Houpek, 1975; Bidlingmeyer, 1980; Ogan & Katz, 1980). The RPLC method can give ultrahigh sensitivity in the parts-per-billion (ppb) range (Hoffsommer et al., 1980; Wegman & Wammes, 1983; Lee et al., 1984a, 1984b). Other chromatographic methods (Armentrout et al., 1979) include ion-pair chromatography (Tomlinson et al., 1978) followed by RP separation have been reported with a LOD in the (< 0.1 - 30) microgram / liter range (Goulden et al., 1973; Chau & Coburn, 1974; Kuehl & Dougherty, 1980; Mathew & Elzerman, 1981; Ribick et al., 1981; Lindinger et al., 1998).
Lastly, chemical / electron ionization and fast atom bombardment in positive-ion mode, have been applied in the analysis of phenol (reviewed by Lisk, 1970; and C. Staples et al., 1998; Santana et al., 2009). Over the last decade, there has been a shift from analysis by analytical biochemists to analysis by technicians. This has necessitated a re-design of sensors to be portable, rugged, with low manufacturing costs, the desired selectivity and sensitivity, and ease of interpretation of results. Colorimetric-based readouts (Folin & Denis, 1915; Rakestraw, 1923; Box, 1983) have inherent advantages over other EC sensors, in terms of cost, robustness and ease of operation. The sensor must consist of a: (i) trapping element in which the target molecule is selectively bound and held in place; and (ii) sensing or detecting element, which interacts with the target molecule leading to a quantified chemical reaction, as the output parameter for detection and quantification. In this regards, a colorimetric sensor based on Gibbs reagent as the sensing element and cyclodextrin and / or gold nanoparticles as the trapping elements were designed with the aim of detecting organic pollutants exemplified by phenol.

1.4.1 Gibbs-based sensor

Dihalogen-substituted quinonechloroimides (such as 2,6-dichloroquinone-4-chloroimide and 2,6-dibromoquinone-4-chloroimide, also known as Gibbs reagent) give the most stable indophenols. The test employing the 2,6-dichloroquinone-4-chloroimide, has a sensitivity of at least 1 part of phenol in 20x10^6. The indophenol formation can be measured quantitatively by means of the spectrophotometer. The maximum absorption for 2,6-dichloroquinoneimine was 280.5 nm with a change at 275 nm (at pH 8.5, Svobodová et al, 1977a) where indophenol formation was measured at 610 nm in the spectrophotometer. Subsequently, it was proposed to use 2,6-dichloroquinone-4-chloroimide as the standard Gibbs’ reagent for the detection and determination of uric acid (Raybin, 1945). The quinonechloroimides do not react with all phenols and the primary requisite is that the position para to the hydroxyl must be unsubstituted (Gibbs, 1927a, 1927b). Different indophenol formations require different pH, but all are formed in the alkaline region (Gibbs, 1927a). Phenols substituted in the 2- and 6-positions (2,6-di-tert-butyl- and 2-tert-butyl-6-methyl-4-methoxyphenol) tended to give a typical magenta color (absorption region 565-575 nm, Dacrel, 1971). For photometric purposes, acetone, dioxan, methanol and ethanol are required for preparation of stock solutions. Sensitivity is hampered by reagent instability, which can be minimised through careful preparation against changes in temperature, light and moisture (Svobodová & Gasparič, 1971; Svobodová et al., 1977a, 1977b).

1.4.2 Gold modified Gibbs sensor

Nanoparticles (NPs) as the sensing element can be synthesized in solution and operated at ambient temperature. Gold NPs (Au-NPs) provide facile route toward synthesis, precise control of formulation and low cost of synthesis and application (Kim et al., 2005). Noble metals have desirable properties for use in sensors such as chemical resistance to oxidation, chemical inertness in the bulk form, which is lost in the nanoform, leading to enhanced catalytically. These properties are due to the nanosurfaces intrinsic properties, and ultrahigh surface area, leading to excellent electrical response of the sensor at the nanoscale (Kim et al.; 2005). An additional advantage in consideration of Au is its optical properties, for example, surface plasmon resonance (SPR) of Au-NPs has widely been used in sensing bio-molecules (Nath &
Chilkoti, 2002; reviewed by Aslan et al., 2004). Au-NPs display wide variation in optical properties including variation in the dielectric in part due to the nanoscale size and in part due to the microenvironment (e.g. solvent) via surface plasmon resonance (SPR, Liz-Marzan, 2004; Hornyak et al., 1997; reviewed by Link & El-Sayed, 2003) effects. This property can enable the fabrication of tuneable colorimetric sensor for detection of different environment pollutants, such as phenol which was used in our study. These fabricated Au-NPs /Au-NRs can therefore have wavelengths which vary at least by an order of magnitude from infra-red to visible spectrum of light. Likewise, Au nanoclusters can be fabricated with precise size and absorption / emission properties (‘tunability’) through formulation of surfaces of defined size (Aslan et al., 2004). This tunability can be achieved in the fabrication process through judicious use of solvents, dispersing agents and synthesis parameters to finely control surface size, layering and dimensionality. One application of Au-NP-based sensors is detection of alterations in SPR, due to changes in the local environment (via changes in the dielectric constant). The measured changes may be due to Au-NP-analyte (e.g. phenol) interactions due to surface adsorption, or nanocluster formation or aggregation due to analyte effects (J. Liu & Lu, 2004). The most common approach to tap into SPR changes is through functionalization of the Au-NPs, for example, through incorporation of gum Arabic (GA) or other long chain macromolecules, which have detergent-like properties aiding in Au-NP size selection (Bashir & Liu, 2009) in aqueous-based devices..Au-NPs increase the specificity of sensor by binding of the sensing element to the sol-gel and increasing electron tunneling to Gibbs reagent (as detecting agent) for detection of phenol.

1.4.3 Cyclodextrin as trapping agent

Cyclodextrins (CDs) are oligomers of D-glucose, which are linked through the 1→4 position. The oligomers can have from six (α-CD) to eight (γ-CD) residues linked end-to-end in cyclic fashion. In this manner, CDs can form 3D like ‘bucket’ structures, which can facilitate CD (host) and analyte (guest) interactions, including formation of non-covalent complexes, particularly with alkali metal cations (Kutner & Doblhofer, 1992; Bashir et al, 2003). These host-guest interactions can be exploited towards construction of trapping elements with in-built selectivity (due to ring size) for certain sensor applications, with the most common CD being β-CD. CDs exhibit two distinct ‘faces’ including an ‘inner’ and ‘outer’ face. The primarily face has a narrower entrance than the secondary face with the narrower/wider sides being associated with primary/secondary hydroxyl groups, respectively. This size difference can thus be exploited in group modification or decoration. Decoration and end-capping of CDs, particularly β-CDs has led to their application as selective agents in chromatography, as tagging agents in pharmacetics and also as host-guest probes (reviewed by Gattuso et al., 1998, and Khan et al., 1998, and Engeldinger et al., 2003) in biomedicine. The degree of binding or interaction between the host-guest is determined by hydrophobicity and van der Waals forces. CDs bind to the Gibbs reagent by intermolecular forces, such as dipole-dipole forces. Other factors include expulsion of water molecules from the core, hydrophobic interactions, van der Waals forces and hydrogen bonding (Salústio et al., 2009). As such CDs have been used as separation surfaces or chromatography media for the separation of drugs, or as encapsulation matrices for enzymes or drugs (Sahoo et al., 2008) for drug interaction, catalytic reactions to occur under normal or photoactive conditions (reviewed by Mallick et al., 2007) in addition to enhancement of transport of molecules such as phenol in animal models.

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1.5 Evaluation of sensor performance
Sensors should be a part of the environmental monitoring system and can provide early-warning about build-up or release of pollutants or molecules of interest. Colorimetric sensors meet the pre-requisites (of reliability and dynamic response), in addition to appropriate operational parameters and as such have been utilised in studies for evaluation of xenobiotic / environmental pollutants or chemicals of interest (Smyth et al., 2008) in which a change in color upon interaction with the target substrate allow for substrate determination, either quantitative or qualitative. In our study, indophenol, (the colored complex), is formed from phenol reacting with 2,6-dichloro-p-quinone-4-chloroimide or 2,6-dibromo-p-quinone-4-chloroimide. The sensor is evaluated for its performance, the detection of phenol, reproducibility and stability at different known concentrations of this analyte.

1.6 Nanotechnology: definition, fabrication and characterization techniques
The design of an optimal sensor necessitates material characterization at the nanoscale level, using nanotechnological tools and resources. Nanotechnology revolves around fabrication of materials of dimensions of less than 100 nm which are used in designing, constructing and utilizing functional structures. Nanomaterials are synthesized or fabricated by top-down and bottom-up processes. Common top-down processes of operation are to grind materials into extremely fine powder, or via ball milling and lithographic processes. Bottom-up methods of fabricating nanomaterials commonly employed include epitaxial deposition of thin films and sol-gel fabrication. In this study, a bottom-up approach was used to prepare the sensing element.

Most commonly used instruments for characterization of nanomaterials are ‘nano’ microscopy related techniques, such as scanning electron microscopy (SEM), transmission electron microscopy (TEM), atomic force microscopy (AFM) or spectroscopy based techniques, such as X-ray powered diffraction (XRD), single-crystal X-ray diffraction (SCD), or energy dispersive spectroscopy (EDX). With the advancement of technology, electron microscopy has become primary tool for characterization of nanomaterials. In this study, all of the above except SCD were used for nanostructural analyses of the phenol sensor.

Another important feature to evaluate the sensing element stability is measurement of the electrokinetic potential (also known as zetapotential, ζ). ζ is the difference in potential between the fluid in the stationary and continuous phase respectively attached to the dispersion phase. ζ arises due to dissociation of atoms capable of producing ions, such as an electrolyte on the surface of the nanoparticle and solvent ions at the surface. The net change in charge on the nanoparticle will influence the distribution of ions in the local microenvironment, for examples by either increasing the concentration of anions (if the surface is positive) near the nanoparticle surface. As a consequence of this counter charge (between the ions) an electrical double layer is created near the nanoparticle surface micro-environment or interface. ζ exists for colloidal systems and indicates the stability of the colloidal suspension. Lower ζ values, indicate that the attractive cohesive forces dominate, leading to aggregation, whereas higher (±) values indicate the contrary, leading to dispersion and nanoparticle stabilization. Colloid suspensions with ζ values from ±30 to ±40 (mV) are moderately stable with values of ±40 to ±60 (mV) exhibiting good stability. Electrophoretic mobility is often used as a starting pointing in theoretical calculations to compare with ζ values, which in turn are based upon electroacoustics (Lyklema, 1995) measurements. Au-NPs (of diameter of 30-40 nm) are suspended as a dispersion phase and the measurement of ζ is a valuable method of determining the stability of sol for the preparation of sensor.
2. Experimental approach

In this chapter, we are designing a nanoscale sensor to overcome the shortcomings of phenol sensor designs, limited detection resolution, and slow response time. The overall experiments are carried out for the construction of phenol film sensor derived by sol method. The structural characterization of the phenol sensor is by optical and nanomicroscopy and colorimetric evaluation of sensor performance. Importantly, as previously indicated in the introduction, this approach can also be applied to the detection of other organic environmental / pharmaceutical pollutants, along the same principles as described in the following sections. All chemicals used are of analytical grade and were obtained from various vendors as listed. 2,6-dichloroquinomine-4-chlorimid (Fluka, St Louis, MO), gold (III) chloride trihydrate (Sigma–Aldrich, St Louis, MO), α-cyclodextrin hydrate (Alfa Aesar, Ward Hill, MA), L-ascorbic acid (Fisher, Thermo scientific, Pittsburgh, PA), gum Arabica (M.P. Biomedicals, Morgan Irvine, CA) were used without modification. α-CD interacts with the Gibbs reagents as the trapping element through a color change. This color change is due to transfer of electrons from the Gibbs molecule to the phenol molecules. In this research, the Gibbs reagent (also known as the 'detecting/sensing agent') and CD (as the 'trapping agent') are used to detect for phenol. The detecting agent coupled to the trapping agent, which is embedded in a sol-type matrix. Other biological sensing elements (such as gold with trapping element such as: cyclodextrins (Janshoff et al., 2000), laccase, (Y. Liu et al., 2006), dendrimers, (Manna et al., 2001) gold-nanotubes-chitosan, (Y. Liu et al., 2006) or C_60–sugar composites (reviewed by Pumera et al., 2007) with gold nanoparticles for building a biosensor can also be utilized. In our case, we utilized cyclodextrin-Gibbs as the trapping/detecting elements with Au-NPs. Separately, Au-NPs and Gibbs (with Arabica Gum as the dispersing agent to minimise aggregation of the Au-NPs) towards enhanced detection of phenol was also used in our specific case, although other general approaches (e.g. Au-laccase-Gibbs) summarized above could have also been used.

2.1 Experimental procedure

Colorimetric studies have been implemented toward study of agents (Lante et al., 2000), as potential detecting or sensing elements related to alterations in color upon interaction with the target molecule. In our study, indophenol complex was formed from phenol reacting with Gibbs reagent. The sensor was involved in a number of fabrication parameters (described in § 2.2), which were extensively characterized using microscopy to determine the nanostructure of the catalytic surface (described in § 2.3) and dynamic light scattering to determine electrokinetic behavior of the colloidal suspensions (described in § 2.4). Once the sensor was optimized in terms of limit of detection (LOD) of phenol through a trial-and-error approach, its performance was evaluated through colorimetric measurement of relative optical densities (described in § 2.5). The workflow of the nanofabrication and performance evaluation of phenol sensor is summarized in Figure 1A.

2.2 Fabrication of sensor

The experimental workflow for construction of phenol sensor is shown in Figure 1B. Briefly, the phenol sensor was created by dissolving different concentrations of Gibbs reagent in the mixture of water and methanol (1:1 volume ratio, solution A). Solution A was mixed continuously with a magnetic stirrer for 30 min. Gum Arabica (GA, as a surfactant) was added (2 % by mass) to solution A to improve the uniformity of the resulting film and to
control the particle size of the sensing elements. To solution A, an aqueous solution of gold (III) chloride trihydrate (HAuCl₄·3H₂O) and separately an aqueous solution of ascorbic acid (C₆H₈O₆) at different concentrations were simultaneously injected. The molar ratio of Au³⁺ to ascorbic acid in solution A was controlled at 1:2 to produce 16 formulations with Gibbs concentration ranging from 0.025-0.25 µM, phenol concentration from 6-50 Vol %, Au-NPs concentration from 0.01-0.04 M to form the gold modified Gibbs hybrid (GGH) composite to extend the detection range of the sensor (cf. Table 1, § 3.6). The precursor sols were heated between 60 and 80 °C for 2 hrs to increase the sol viscosity, through evaporation of methanol.

Fig. 1. The workflow employed in the fabrication and characterization of the sensor (A) and schematic of the synthesis procedure and likely nanostructure (B).

2.3 Structural characterization
Advanced instrumentation techniques provide higher magnification for analyzing elemental composition and particle size distribution of the sensing elements and structural changes with addition of phenol. A phase contrast optical microscope (M 021, Olympus, Olympus America Incorporation, Irving, TX) was used to determine the texture of the GGH sensor and textural changes on interactions of the phenol substrate on to the surface of the sensor. A field emission SEM (JSM-6701F, JEOL Ltd, Peabody, MA) was used to determine the thickness and surface morphology of the sensor surface sol-derived phenol sensor. An accelerating voltage of 10 kV, current of 5 µA and a high vacuum of 10⁻⁵ Pa for specimen chamber were employed to obtain the optimal resolution. An air-dried GGH phenol sensor films were mechanically fractured using a diamond blade before mounting to the aluminum (Al) stubs. A thin layer of Au was sputtered using Denton Vacuum LLC Desk IV Sputter, (Mooresetown, NJ) operating at a vacuum of 50 mTorr. Au coating was applied to improve surface conductivity and prevent charging by the electron beam with high energy. A TEM (Tecnai G² F20, FEI Company, Hillsboro, OH) was used to determine the fine structure of the sol-derived GGH phenol sensor. Tungsten (W) crystal field emission gun with extraction voltage of 4 kV was the source of electrons and electrons were accelerated in the vacuum chamber to 200 kV and a high vacuum of 0.39 mbar was employed to obtain optimal resolution. Samples were diluted and sonicated for 10 minutes for uniform distribution of the particles and deposited onto carbon coated copper (Cu) grids. The crystal
structure was studied with electron diffraction resulting from either single crystal units or polycrystals of the sensing elements. As a complementary approach of crystalline phase identification, an Ultima III XRD with Cu diffractometer and visual XRD Jade 7 software (Rigaku Americas Incorporation, Houston, TX) was used to determine the phase structure. The operating voltage and current were controlled at 40 kV and 44 mA, respectively. An AFM (Nanoscope III, Veeco Cooperation, Santa Barbara, CA) was used to determine the topography, surface uniformity and three-dimension (3D) surface image of the GGH sensor. Samples for analysis were greatly diluted and loaded on to the surface of the mica film. Point probe cantilevers (monolithic silicon AFM probe) were used for analysis in tapping mode of operation. A drive frequency of 362.99 kHz and drive amplitude of 144.1 mV was applied for topographic analysis of the sensor surface.

2.4 Electrokinetic behavior of GGH colloidal suspension
ZetaPALS™ (Brookhaven Instrument Corporation, (BIC), New York City, NY) was utilized for particle size analysis and colloidal stability of sol prepared. Diluted samples of the GGH were analyzed for particle distribution and zetapotential measurement. The instrument was operated at room temperature and the samples were analyzed for 8 runs and each run for 15 sec to give the best results. The angle of incidence light was 90° and wavelength of operation was 659.0 nm. Results were analyzed with BIC particle sizing software and BIC zetapotential analyzer.

2.5 Evaluation of sensor performance
In order to evaluate the sensor performance, a colorimetric study was undertaken to determine the limit of detection (LOD), response time, and sensitivity of the sensor. Formation of the indophenols with significant color changes was the key principle for the colorimetric studies. Studies were conducted in a ceramic watch plate with 12 wells, each well with a surface area of approximately 3.14 cm². Various volumes and concentration of Gibbs reagent and phenol buffer were added to observe the color changes (Table 1, also cf. § 3.6). At intervals of 15 minutes, a photograph of the sample was collected with computer assisted camera, and the whole experiment was conducted over 24 hr. The collected samples were re-imaged after two weeks and the initial and two-week intensities were compared. No difference was found, indicating that the samples are at least stable up to two weeks. The significance of the background and maximum values were to enable normalization of the different colors in grey-scale mode with background subtraction. In this manner, different intensities due to differences in background color could be compensated for and different formulations directly compared. The grey-scale calibration tablet provided ‘internal’ calibration allowing changes due to actual phenol to be determined.

3. Results and discussion
The optical properties of the GGH sensor will be discussed first (described in § 3.1), followed by electron and atomic microscopy morphological analysis (described in § 3.2-3.4). The electrokinetic properties will be discussed next (described in § 3.5) followed by actual sensor performance with literature comparison (described in § 2.4) and a conclusion on sensor testing to close the chapter (described in § 4.0).
### Table 1. The measurement of relative optical density as function of fabrication variable.

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<th>Sl. No</th>
<th>Gibbs conc. (µM)</th>
<th>Parameter 2: Au NPs conc. (M)</th>
<th>Parameter 3: Phenol Conc. (Vol %)</th>
<th>R.O.D = 1/O.D</th>
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</tbody>
</table>

Average back ground-174.02  Maximum value-255

3.1 Optical microscopic study on GGH phenol sensor

Optical microscopic images showing the morphological structures of the Gibbs reagent were captured with and without the addition of the phenol. Figure 2A (100×) micrographs show cylindrical and linear nature of the Gibbs reagent (without AgNPs) which were sparsely distributed. The cylindrical properties of these compounds increase the surface area for exposure to phenol for better sensitivity. Figure 2B (400×) indicates that the Gibbs reagent is impregnated with Au-NP clusters. Au-NPs show uniformity in size and shape increase the sensitivity of the sensor due to SPR and the rapid reaction rate between the two (Gibbs) and target molecule (phenol) elements. Figure 2C (400×) also shows the morphological changes to the trapping element upon addition of buffered phenol, for the enhanced GGH sensor. These morphological changes were recognized as loss of linear cylindrical structure of Gibbs reagent polymer and of the indophenol complex, whereas Au-NPs remain intact, which provides conspicuous color changes and consequently improves the LOD of phenol. It can be surmized that Au-NPs act as heterogeneous catalysts to increase the reaction rate between phenol and Gibbs via lowering the activation energy and creating a new pathway. The indopenol complex with various color schemes serves as a delicate indicator to display the sensor performance.
Fig. 2. Optical microscopic photographs of gold-modified Gibbs hybrid sensing element and sensing mechanism, A: the sensor image at 100 \( \times \); B: the sensor image at 400 \( \times \); C: the sensor image at 400 \( \times \) after addition of buffered phenol analyte.

3.2 Scanning electron microscopic study on GGH phenol sensor
SEM micrographs also exhibit the linear cylindrical nature of the Gibbs reagent (Figure 3A) coated with the spherical Au-NPs (before addition of phenol) and GHH sensing element forms mesh-like network, upon addition. Au-NPs display near-spherical shape and their particle sizes vary from approximately 30-40 nm in diameter. Although Au-NPs aggregate and form several large-size clusters, it was determined that evenness of the GGH sensor increased with electrical activity and resulted in improved sensitivity of Gibbs reagent. Figure 3B is an SEM image of GGH sensing element which interacts with the phenol compound when the buffered phenol analytes with various concentrations were introduced. With the addition of phenol to the surface of the GGH sensor, morphological changes of dissolution of porous nature of Gibbs was seen with the formation of the indophenol (colored complex) and loss of mesh work was characterized. Therefore, the formation of the indophenol becomes a delicate indicator to display the regional differentials in color and morphology of the GGH sensor. In turn, this will enhance the sensor sensitivity and its LOD, which is classified by colorimetric values.

Fig. 3. The SEM top view morphological analyses of sensor, A: the GGH morphology before addition of phenol and B: the GGH morphology after addition of phenol.

3.3 Transmission electron microscopic study on GGH phenol sensor
TEM images also depict the inner structural morphology with high spatial resolution of the film as indicated by SEM. TEM images (Figure 4A-D) of GGH depict linear and cylindrical...
nature of Gibbs reagent with uniform Au-NP coating. Linear nature of the polymer synthesized in sol method possess better sensitivity for the detection of phenol because large area of contact for electron exchange and consequently for the indophenol complex formation (Figure 4A). Loss of network structure of GGH sensing element was observed upon substrate inclusion via a chemical reaction of the substrate phenol and detecting element, the Gibbs reagent (Figure 4B). These changes were in consistent with the optical and SEM characterization techniques. The significant morphological changes enhanced the limit of detection of the GGH sensor, which allows us to develop sensor with high performance. Figure 4B also depicts the size and shape of Au-NPs of 10-15 nm in diameter. Au-NPs were agglomerating slightly and possess surface plasma resonance. The lattice fringe (Figure 4C) indicates ultrafine Au-NPs were obtained. The ring pattern of Ag-NPs (Figure 4D) results from polycrystals, suggesting high degree of crystallinity was acheived. The indexing of ring pattern suggests that the face-center cubic Au (PDF 00-004-0784, a_0 = 0.408 nm, \( \alpha = 90^\circ \)) was obtained. This observation is confirmed by XRD analysis (Figure 4E-insert). However, the Gibbs reagent was vaporized under high energy beam of electrons, which is the reason why no ring pattern or electron diffraction was observed. The XRD pattern indicated that the Gibbs reagent (Figure 4E) is well-aligned with the standard 2,6-dichloro-p-benzoquinone-4-chlorimine (C_6H_2Cl_3NO, PDF 25-1917) using lower energy.

### 3.4 Atomic force microscopic study on GGH phenol sensor

AFM images depicted the surface morphology and measured the size of the Au-NPs. Figure 5A showed the sparse distribution of the Au-NPs and figure 5B represents the topography of sensor film active surface at nano-range. Size of coated Au-NP cluster in this study was measured as approximately 30-40 nm in diameter. The Au-NPs was coated on the surface by Gibbs reagent. Since AFM studies the top surface layer of sensor film, it depicts the actual layer which is in contact with phenol but it cannot reveal the inner composition and build up of sensor. Au-NPs were measured to have an effective diameter of 12 nm for Au by itself. With the addition of gum Arabic (GA) to the Au-NPs, increase in the size of NPs was observed with an average cluster dimension of 169.6 nm and polydiversity of 0.267 (dimensionless) for the cluster of coated particles. Analysis shows the mean particle size of GGH was 485 nm and the distribution of (up to 20) particles (polydiversity of 0.05), which was consistent with the SEM findings.

### 3.5 Electrokinetic behavior of GGH colloidal suspension

The measured zetapotentials (\( \zeta \), with ZetaPALS\textsuperscript{TM}) of GGH colloidal suspension were averaged at -23.0 mV. The negative sign indicates repulsive forces between Au-NPs and GGH preventing flocculation and aggregation of particles and the numerical value indicates samples had colloidal stability (Figure 6). \( \zeta \) results indicate that the GGH colloid is stable and agglomeration is successfully prevented using GA as the surfactant. The electrokinetic study also confirms that nano-dispersion of Au with size of 10-15 nm (also found by TEM, see Figure 5B) has been achieved. Fluctuations in the measured value of \( \zeta \) during the experiment were not observed; therefore the measurements were time independent in the present study, which confirms the stability of GGH colloid. Bottom up (sol-gel) green synthesis of Au-NPs modified Gibbs reagent has been developed to prevent the aggregation of the particles during synthesis. To synthesised nanoparticles exhibited high degree of stability (due to the high measured \( \zeta \), i.e. steric effects > van der Waals forces). The GA
Fig. 4. TEM images of sensing elements, A: morphology of gold-modified Gibbs hybrid sensing element; B: morphology of gold-modified Gibbs hybrid sensing element after addition of buffered phenol analyte; C: lattice fringe of gold-modified Gibbs hybrid sensing element; D: TEM ring pattern of nanogold; E: corresponding X-ray diffraction (XRD) pattern of gold-modified Gibbs hybrid sensing element with standards for gold and Gibbs.
polymers are adsorbed onto the particle surface and cause van der Waals forces weakening. Significant steric repulsion prevents Au-NPs from being adhered and the particle surfaces are separated as a result.

The main goal during the synthesis phase was to control the particle size and its structure, followed by nanocharacterization of the nanoparticles. These particles were synthesised using a bottom-up colloidal chemistry approach. Au-NP size was controlled through incorporation of GA as the dispersing agent, which also aids wetting of the metal salt and distribution of the surface of the GGH composed of a continuous Au layer which was then deposited onto the Gibbs cluster successively.

3.6 Evaluation of GGH sensor performance

Colorimetric evaluation of the phenol sensor reagent was performed and color changes of different concentrations of phenol and Gibbs reagent in different dilutions at different intervals of time were captured (images not shown). Initial color was developed within one hour and changes in intensity of color were different in all wells with the passage of time.
Using the calibration grey-scale tablet, the minimum intensity value was set to zero and the maximum optical density set to 255, from which the optical density (OD) was calculated as:

\[
\text{OD} = \log\left(\frac{255-p}{255-x}\right)
\]

where ‘p’ stands for average background correction for the well-plate(s), ‘x’ for mean intensity of color converted to grey-scale. The measurements were recorded as relative optical density (ROD) values, where

\[
\text{ROD} = \frac{1}{\text{OD}}
\]

ROD of the 12 wells (numbered 1-12) at various intervals was calculated based upon the intensity of color development of each well, converted to grey-scale. Measured intensities from wells 13 to 16 were used for background correction and average background determination to calculate the p-value. Evaluation of the ROD from the different formulations at different intervals was examined, indicating that the variable which influenced the measured ROD the most was phenol concentration. The observed color change was almost instantaneous (~ 1 sec, various parameters are summarized in Table 1, including color intensity recorded every five minutes from zero minutes to twenty-four hours (data not shown)) was standardized to one hour. The calibration plot at one hour for different sensor formulations is shown in figure 7. It was found that optimal parameters were having an incubation time of five minutes for phenol to Gibbs reagent, at a molar ratio of 4:1 between Gibbs reagent to phenol and 1:1 molar ratio between Gibbs reagent and Au. Using various volumes of buffered phenol, the LOD was measured in terms of concentration as 0.1 \(\mu\)M, while the variation in measured ROD was 36%. The ROD values (determined as the (minimum measured intensity value / maximum intensity value) x 100)

![Fig. 7. The sensitivity study using various concentration ratios of Gibbs vs phenols, noting three formulations were selected to demonstrate the linearity of sensitivity.](http://www.intechopen.com)
indicate degree of sensitivity to subtle changes in color, after a fixed time period (optimised to either 5 or 60 min). The mode of sensor operation is schematically illustrated in figure 8.

Fig. 8. A: Schematic of likely operation of sensor and B: likely intermediate.

Accurate quantification of phenol is important because of its well documented health side effects such as stress to the kidneys, liver, heart, respiratory tract, mucous membranes and central nervous system upon acute exposure (Diz et al., 2006). In comparison to sol-gel derived Au-NPs, other investigations in the analysis of phenol (Metzger et al., 1998) or its derivatives (Neujahr, 1982) have been based on enzymes (Orupold et al., 1995) from species such as Trichosporon sporotrichoides. (Rella et al., 1996) Pseudomonas putida (Skládal et al., 2002) and Bacillus stearothermophilus (Buswell, 1975) coupled to Clark-type oxygen electrode (reviewed by Patel, 2002) for detection of phenol.

Procedures in preparing a sensor require advanced techniques of immobilization of bacteria (or substrate enzyme) to increase the stability of the final biosensor (reviewed by Warsinke et al., 1999). The roles of enzymes (derived from various plant tissues, spores or bacteria) are as substrate-specific biocatalysts (Wollenberger et al., 1999). Where phenol, is the substrate this involves electron transfer between the enzyme and substrate (phenol + 10 O₂ + 2H⁺ → catechol + tyrosinase + 10 O₂ → phthalate quinine) and generation of current, which in turn is related to consumption of oxygen during the oxidation process in a Clark-type electrode (reviewed by Scheller et al., 1988). Thus amperometric tyrosinase-modified imprinted or carbon electrode sensors have been extensively tested in determination of dissolved oxygen and indirectly phenol including some derivatives of phenol (reviewed by Riedel et al., 2002). This can be exploited by utilizing of enzymes from microorganisms which utilise phenol as a fuel source (such as laccase, Y. Liu et al., 2006) or enzymes, which can accept electrons to generate energy directly such as with Shewanella oneidensis via extracellular electron transfer mechanisms (H. Liu et al., 2010) have wider applications in biofuels and energy generation using biocatalysts (reviewed by Ragauskas et al., 2006). In our study, a colorimetric system was used toward detection of phenol (and not generation of energy) by using Gibbs reagent as the detecting/sensing element; because of its high detection limit and formation of indophenol colored complex when in contact with phenol (Raybin, 1945). Sol-based GGH sensors have overcome the problems of enzymatic or bacterial sensors because of their ease of preparation, low cost, high sensitivity, stability and operation under wide temperature, pH ranges. The 0.1 µM LOD compares favorably with other designs and is summarized in Table 2.
<table>
<thead>
<tr>
<th>Type</th>
<th>LOD (µM)</th>
<th>Linear range (µM)</th>
<th>Response time (s)</th>
<th>Notes or Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resonance-enhanced multiphoton ionization (REMPI) time-of-flight (ToF) mass spectrometry</td>
<td>1 ng L⁻¹</td>
<td>0.1 µg L⁻¹ -10 mg L⁻¹</td>
<td>-</td>
<td>Alimpiev et al., 1995</td>
</tr>
<tr>
<td>Electrode with enzyme</td>
<td>&lt; 0.1 mg/L</td>
<td>0.1-1.0 mg/L</td>
<td>&lt; 300</td>
<td>Rainina et al., 1996</td>
</tr>
<tr>
<td>Amperometric biosensor</td>
<td>12</td>
<td>-</td>
<td>-</td>
<td>Table 1 of Lindgren et al., 1997</td>
</tr>
<tr>
<td>Electrode with enzyme *</td>
<td>0.015 **</td>
<td>0.03 - 100</td>
<td>-</td>
<td>Table 3 of Nistor et al., 1999</td>
</tr>
<tr>
<td>Electrode with enzyme</td>
<td>0.2 nM</td>
<td>1.5 × 10⁻⁹ - 3.5 × 10⁴ mol/L</td>
<td>&lt; 4</td>
<td>Z. Liu et al., 2000</td>
</tr>
<tr>
<td>Enzyme-based calorimetric sensor</td>
<td>1 mM</td>
<td>-</td>
<td>&lt; 300</td>
<td>Wolf et al., 2000</td>
</tr>
<tr>
<td>Amperometric biosensor</td>
<td>6.1 nM</td>
<td>4- 48</td>
<td>&lt; 5</td>
<td>S. Liu et al., 2003</td>
</tr>
<tr>
<td>Gas Chromatography-Mass Spectrometry</td>
<td>10 pg</td>
<td>1 -12 ng</td>
<td>8 - 10</td>
<td>E. Staples, 2004</td>
</tr>
<tr>
<td>Amperometric biosensor</td>
<td>0.05</td>
<td>0 - 1</td>
<td>50 - 250</td>
<td>Tingry et al., 2006</td>
</tr>
<tr>
<td>Amperometric biosensor</td>
<td>0.36 nM</td>
<td>3.6 × 10⁻⁹ - 4 × 10⁻⁸ M</td>
<td>-</td>
<td>Han et al., 2007</td>
</tr>
<tr>
<td>Amperometric biosensor</td>
<td>1-10</td>
<td>0.005 - 10</td>
<td>10 - 480</td>
<td>Table 3 of Bashir &amp; J. Liu, 2009</td>
</tr>
<tr>
<td>Amperometric / colorimetric biosensor</td>
<td>1</td>
<td>2.6 - 5.2</td>
<td>&lt; 5</td>
<td>Bashir &amp; J. Liu, 2009</td>
</tr>
<tr>
<td>Electrode with enzyme</td>
<td>0.005 -0.410</td>
<td>-</td>
<td>-</td>
<td>Table 1 of Alarcón et al., 2010</td>
</tr>
<tr>
<td>Screen printed electrode (SPE) with enzyme</td>
<td>0.14</td>
<td>0.05 - 1</td>
<td>~ 15</td>
<td>Alarcón et al., 2010</td>
</tr>
</tbody>
</table>

(*) The likely reaction is: phenol + enzyme → catechol; catechol + tyrosinase → o-quinone + H₂O; o-quinone + 2H⁺ + 2e⁻ → catechol (at electrode surface) (Yu et al, 2003), noting that phenol oxidized to o-quinone and is reduced to catechol (O₂→ 2H₂O) (Renedo et al., 2007)

(**) as catechol equivalents

Table 2. Literature review of sensors, their limit of detection (LOD in µM), the detection range (Linear range in µM) and the response time (in seconds), unless otherwise stated.

4. Conclusion

A GGH based sensor for the colorimetric detection of phenol was fabricated and evaluated. The sensor was successfully optimized using a trial-and-error approach of 16 formulations, involving a trapping and detecting element with AuNPs to enhance the SPR with phenol incubated at different times. The detector was based upon sol-gel technology utilizing trapping/detecting elements, which can be extended to other environmental pollutants or pharmaceutical organics, if sensor selectivity and sensitivity can be achieved.
Characterization revealed the nanostructure of the gold and GGH complex as spherical Au-NPs with size range of 10-15 nm, for individual NPs or 30-40 nm for metal clusters which were fabricated as linear cylindrical polymers of Gibbs reagents coated with clusters of Au-NPs (optimal molar ratio was 1:1 and 4:1 for Gibbs:phenol respectively). The stability of the sol sensor was measured in terms of zetapotential was shown to be -23.0 mV indicating stability of the sensor. Colorimetric based detecting elements were incorporated to bench test the performance of the sensor. The results indicate that once the intermediate color is formed was stable for at least for two weeks (the length of the study). The LOD for phenol (at room temperature) was 0.1 μM, with sensor sensitivity to small changes in color being 36% including a fast response time (< 1 sec, although the ROD values are based upon 60 minute incubation) as measured using colorimetric technique. The strategy outlined in the current chapter, although applied specifically to the detection of phenol, can similarly be applied to the detection of other environmental organic pollutants providing an appropriate detecting/sensing element can be found.

5. Acknowledgements

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6. Author contributions

C. Padidem conducted the synthesis, electrokinetic study and optical characterization of the sensor and co-wrote the first draft. S. Bashir and J. Liu conceived the concept and experiment design and co-supervised C. Padidem. In addition, he did the calibration of the optical study, some of the data analyses and generation of certain graphs, plots and schemes. J. Liu completed the rest of the experimental procedure (except where acknowledged elsewhere). She co-wrote the first draft, and completed the re-writing and editing. J. Liu also supervised the entire study, coordinated with the other investigators and in liaison with (e.g. Dr. Luo at) the Materials Characterization Facility and Microscopy Imaging Center at Texas A&M University-College Station, obtained the AFM and TEM images (see acknowledgment section) and oversaw the entire submission process.

7. References


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Sensor Enhancement Using Nanomaterials to Detect Pharmaceutical Residue: Nanointegration Using Phenol as Environmental Pollutant


Kutner W. & Dobiliohe K. (1992). Simultaneous cyclic voltammetry and electrochemical quartz-crystal microbalance study at the polymer film modified electrodes of


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A biosensor is a detecting device that combines a transducer with a biologically sensitive and selective component. Biosensors can measure compounds present in the environment, chemical processes, food and human body at low cost if compared with traditional analytical techniques. This book covers a wide range of aspects and issues related to biosensor technology, bringing together researchers from 12 different countries. The book consists of 20 chapters written by 69 authors and divided in three sections: Biosensors Technology and Materials, Biosensors for Health and Biosensors for Environment and Biosecurity.

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