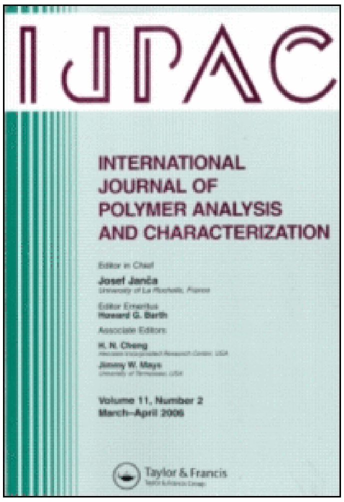

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Organophosphate Nerve Agent Sensor Based on Polystyrene Core and Zirconium Shell Colloidal Crystal Array

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Organophosphate Nerve Agent Sensor Based on Polystyrene Core and Zirconium Shell Colloidal Crystal Array

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Abstract: A simple method has been developed for the preparation of colloidal crystal arrays (CCA) based on a polystyrene (PS) core and a zirconia (ZrO_2) shell sensing microgel that detects the organophosphorus compound paraoxon at parts per million (ppm) concentrations in aqueous solution. The molecular recognition agent for the sensor is zirconia (ZrO_2), which has a chemical affinity for paraoxon. Colloidal crystal arrays based on these core-shell particles were prepared by a simple gasket method at room temperature. Transmission electron microscopy (TEM) and energy dispersive analysis of X-rays (EDX) was used to characterize the PS-coated ZrO_2 particles. TEM analysis confirmed two distinct morphologies of the core-shell particles, while the EDX analysis confirmed the presence of zirconium around the polystyrene core. TEM confirmed the presence of ZrO_2 clusters on polystyrene spheres. Reflectance measurements were carried out for varying concentrations of paraoxon from 0.024 to 0.096 ppm, showing a characteristic shift in the reflectance peak from 468 to 488 nm. The shift in the peak wavelength from right to left suggested that the distance between the crystals or lattice spacing increased due to addition of paraoxon. Thus, it acts as a chemical sensor for paraoxon since zirconia has an affinity for the phosphate group of paraoxon. It was demonstrated that by increasing paraoxon

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concentration, the reflectance peak increased linearly, making CCA a potential candidate for future chemical sensor applications.

Keywords: Colloid crystal array; Nerve agent sensor; Polystyrene core and zirconium shell

INTRODUCTION

Coating colloidal particles with shells probably represents the simplest and most versatile strategy for increasing the diversity and complexity of a colloidal system.[1] First of all, formation of shells on colloidal particles may dramatically alter the charge, functionality, and reactivity of their surfaces and thus enhance the stability and compatibility of the core particles.[2] In principle, the properties (optical, mechanical, electrical, rheological, and catalytic) of core-shell particles can be tailored in a controllable fashion by independently altering the composition, dimension, and structure of the cores or shells.[1–3] Second, formation of shells on cores provides a natural vehicle to incorporate different materials into the same structure and thus to obtain hybrid, multifunctional colloidal particles.[4] As a result of their unique features, core-shell particles have recently found use in a variety of applications. For example, core-shell particles with their shells optically matched to the suspending fluid have been explored as a good model system to investigate issues related to colloidal interaction and stabilization.[5] Core-shell particles have also been demonstrated as a new class of building blocks to fabricate colloidal crystals with photonic properties different from those based on conventional plain particles.[6]

There is currently an urgent need for efficient, rapid detection of organophosphorus (OP) compounds. OP compounds are prominently used by the agricultural industry worldwide in the form of pesticides and insecticides. These OPs have been detected in streams draining from urban watersheds and in agricultural watersheds.[7] OPs also occur in the form of chemical warfare nerve agents such as sarin, cyclosarin, VX, and tabun. These compounds are potent irreversible inhibitors of nerve system function[8,9] and are thus quite toxic. As a result of acute toxicity of these OP neurotoxins, environmental monitoring of the presence of these compounds in food and groundwater is an important step in keeping these compounds below the harmful level for humans and animals.

At present, there are several techniques available for determining the concentration of OPs in solution. Gas chromatography/mass spectrometry (GC/MS) and high-performance liquid chromatography (HPLC) techniques are commonly used[10] and show detection limits in the nanomolar concentration range.[11] However, these techniques are time-consuming and require extensive sample preparation in order to detect environmentally significant levels of OPs. Molecularly imprinted polymers (MIPs) that have very high selectivity towards OP substrates have also been developed. These sol-gel films utilize silanes, which are functionalized to create OP-binding templates. Detection of the sequestered OP molecule is achieved using fluorescence or electrochemistry.[12] The layer-by-layer deposition technique is also used to generate ultrathin films with molecular order and stability for detection of paraoxon. This technique overcomes some of the disadvantages of the other methods of ultrathin film assembly (see above). One major advantage of this technique is that it has significant industrial applications in the biosensing field. The layer-by-layer deposition method involves the alternate adsorption of oppositely charged macromolecules and biomacromolecules.[13,14] This approach provides a simple method to develop films of 5–500 nm thickness that possess high strength[14]; however, field detection appears impractical.

To overcome these disadvantages, enzymatic biosensors have been designed for speed detection, high efficiency, and sensitivity. The biomaterials currently in use for organophosphate detection are enzymes, antibodies, and cells. Zirconia is an inorganic oxide with thermal stability, chemical inertness, and lack of toxicity.[15–18] Researchers have demonstrated that zirconia has a strong affinity for the phosphoric group. This affinity has been used to prepare multiple films by self-assembly[19–21] or as a DNA probe[22,23] in which the phosphate group at 5′ end was attached to develop a DNA biosensor. To date, little work has been carried out using colloidal crystal arrays as a sensor for paraoxon, since a physical adsorption method and interaction of zirconium with the phosphate group of paraoxon have yet to be explored.

In an effort to detect paraoxon, we have designed a novel crystalline colloidal array based on a polystyrene core and a zirconia shell. In this work, we utilized Zr as the molecular recognition agent for paraoxon, an OP. Zirconia is an inorganic compound that has a strong affinity for the phosphate group of paraoxon. Our OP chemical sensor is based on a colloidal crystal array (CCA) with a photonic crystal sensing technology. These CCAs utilize an array of colloidal particles fabricated from core-shell morphology that interact with light in the visible spectral region based on Bragg diffraction.

EXPERIMENTAL SECTION

For the preparation of the CCA, we obtained OptiBind polystyrene micro-particles (PS-MPs, Seradyn Inc., Indiana, USA), which are uniformly sized (210 nm) stable colloids and are characterized to be monodispersed spheres. The specific gravity of polystyrene is 1.05 g/mL, and the carboxyl content is 0.210 meq/g. Polystyrene colloid spheres were centrifuged seven times at 15,000 rpm. The final product is an iridescent opal-colored colloidal microgel. The gel mainly reflects light in the green to red regions. The particles are supplied as nominal 10% solids after dilution with distilled water from the original polymerization mixture. The stability of the colloids (i.e., maintaining spheres as separate particles) requires minimum amounts of surface negative charge. All reagents were purchased from Sigma-Aldrich unless otherwise noted.

Preparation of Core-Shell Particles

In the process, 1.0 g of PS particles was mixed with 0.75 g of zirconium oxycholoride and diluted to 20 mL with distilled water. This mixture was stirred for 8 h in a 100 mL three-necked flask equipped with a magnetic stirrer and a nitrogen inlet system. It was then centrifuged at 20,000 rpm for 1 h. The core-shell particles were separated from the bottom sediment part and top supernatant layer was discarded.

Preparation of CCA

To prepare CCA, 1.5 μL of PS particles was dropped over a 0.2 mm thick double-sided gasket, followed by 0.5 μL of paraoxon of 0.024 ppm. The double-sided sticky gasket was left as such at room temperature for 6 h to record the spectra. In this manner, varying concentrations of paraoxon at 0.048 and 0.096 ppm were employed to prepare CCA, while the amount of PS particles was kept constant.

Measurement of Reflectance

The reflectance was monitored using an Ocean Optics USB 4000. Stock solutions were made by dissolving 50, 100, and 200 mg of paraoxon in 20 mL volumetric flasks and diluting up to the mark with nano pure water with vigorous shaking. Serial dilutions were then performed using nano pure water to prepare varying concentrations of paraoxon ranging from 0.024 to 0.096 ppm. With the gasket on the analyzer stand, one by one the samples with varying concentrations of paraoxon were analyzed.

Transmission Electron Microscopy (TEM)

Transmission electron microscopy (TEM) was performed using a Tecnai 20 TEM (FEI Co.) with a LaB_6 filament. The specimen was dispersed in deionized water (without ultrasonication to preserve the ZrO_2 clusters on the PS spheres) and then spread on a 400-mesh lacey carbon-coated Cu grid. The exposure time was 1.6 s.

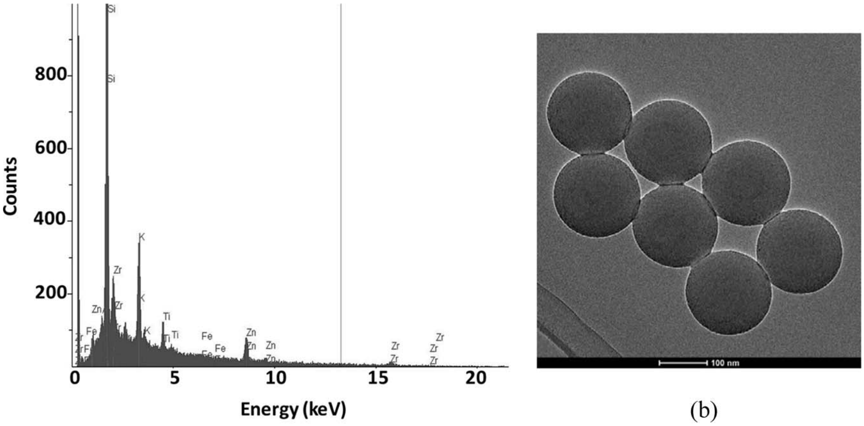
RESULTS AND DISCUSSION

In our experiments, polystyrene cores terminated in the sulfonate and carboxylate groups were employed as the cores for zirconia coating. In the process to prepare CCA we utilized 3:1 ratio of core-shell (wt/wt) particles and paraoxon as the analyte. We fabricated the crystalline colloidal array (CCA) by physically adsorbing zirconia onto polystyrene spheres with a weight ratio of 1:0.75 polystyrene core and zirconium oxycholoride as shell particles. Core-shell particles were repeatedly centrifuged to remove any unabsorbed zirconia particles at 20,000 rpm for 1 h. We prepared CCA out of these novel core-shell particles by the double-gasket method and allowed them to dry at room temperature.

Formation of zirconia polystyrene composite particles is supported by previous studies in which particles of zirconia were obtained by aging for 5 h. Chen et al. have shown through XRD and TEM analysis that zirconia particles are formed on polystyrene.[24] It was shown that the zirconia forms Zr–O bonds with the carboxylate of sulfate dopants present in the PS colloids. Furthermore, polystyrene latex particles were coated with zirconium compound, requiring positive charge. They used a minimal quantity of formamide and urea in order to maintain the system sufficiently acidic.[25] Presence of acidic groups can be observed from carboxylate ions and sulfate ions available from the original polystyrene particles. Furthermore, we can say that the complex transforms in oxycholoride in acidic condition. As in our report we used the aging process for the formation of zirconia particles on polystyrene colloids. However, we did not use formamide and urea due to the effect on the isoelectric point of zirconium compounds of interest.[26] An additional advantage of our method is that it can be applied quickly, as far as the preparation goes, compared to other methods.

The physical adsorption method was reported for the preparation of composite core-shell particles, where Gedanken et al.[25] prepared noble metal nanocrystals on silver, gold, palladium, and platinum. In the process they used acidic solutions of nitrates of silver and palladium with 10% polystyrene spheres dispersed in water and sonicated for 2 h in the presence of argon gas to expel dissolved oxygen/air. We used a similar concept for coating of polystyrene spheres with zirconia using the physical adsorption principle. Furthermore, if the temperature was increased during the adsorption of zirconia, a uniform coating was not obtained. It is reported that when gold and silver particles were deposited on silica spheres, it gave amorphous products, while coating onto PS gave crystalline products. The reason for this can be change in the surface property of silica and polystyrene and chemical interaction between particles. This leads to a better mechanism, as we observed heat exchange between polymer and metallic particles. Further, PS particles are capable of plastic deformation, due to which crystal growth can occur. Another possibility is that somehow the PS is functioning through chemical interaction as a kind of templating site for initial deposition of Zr particles. In our case, we prepared CCA at once and used it for the detection of paraoxon, showing superiority of our process in terms of time, rapidity, and ease.

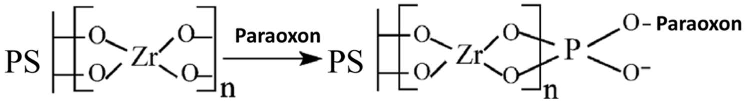
Energy dispersive X-ray (EDX) analysis of the CCA particles indicates the presence of zirconium as shown in Figure 1, along with its respective TEM image. Based on the EDX analysis, the percent elemental composition of the polystyrene zirconia CCAs was found to be: Al (47.1%), Zr (26.2%), K (13.5%), Zn (7.7%), Ti (4.1%), Al (0.9%), and Fe (0.5%). However, the EDX analysis does not show the presence of oxygen ($Z = 8$), as its atomic number is below 11, making it a difficult element to detect using EDX. As shown in several references,[18,27] zirconia is attracted to the carboxyl and sulfate groups present on the PS, thus forming a matrix of Zr–O bonds throughout the surface of the



(a)

(b)

Figure 1. (a) EDX spectrum of PS-zirconia CCA, (b) TEM image of PS-zirconia colloids.



Scheme 1.

colloids. Figure 1(b) shows the presence of Zr atoms on PS spheres in the TEM image.

We used a simple method to prepare these novel core-shell particles as a sensor for paraoxon. Zirconia is an inorganic oxide with thermal stability, chemical inertness, lack of toxicity, and affinity for the groups containing oxygen,[18] so it is an ideal candidate material for immobilization of biomolecules with oxygen groups as contained in phosphates. Because ZrO_2 has affinity for phosphoric group,[27] the scheme can be outlined as shown in Scheme 1.

There exists an interaction between the phosphoric group of paraoxon and zirconia particles at the molecular level that switches reflectance as the concentration of paraoxon is varied. Our chemical sensor uses a three-dimensional periodic CCA of highly negatively charged polystyrene spheres and positively charged zirconium spheres. The periodic array formed through the electrostatic self-assembly of charged colloidal spheres. It is well known that submicron periodic-ordered structures called crystalline colloidal arrays (CCA) self-assemble from monodispersed colloidal spheres containing surface functional groups that ionize in solutions. If the spheres are dispersed in a polar medium such as water, the surface groups ionize to form spherical particles that are surrounded by a diffuse counterion cloud. If the solution medium is pure and contains few other ionic species, the repulsive interactions between spherical particles can be significant over macroscopic distances greater than 1 μm. Figure 2 shows the reflectance response of CCA upon exposure to paraoxon solution of different concentrations. Monodomain CCA arrays efficiently Bragg diffract light. We have developed novel composite/core-shell particles composed of polystyrene core and zirconium shell formed according to physical adsorption theory. The Bragg diffraction at 468 nm occurs when prepared with initial 0.024 to 0.096 ppm paraoxon and almost follows the classical Bragg's law:

$$m\lambda = 2nd\sin\theta$$

where λ is the wavelength of light in vacuum, n is the refractive index of the system, d is the plane spacing, and θ is the Bragg glancing angle.

Figure 3 shows that CCA diffraction wavelength monotonically increases with increasing paraoxon concentrations between 0.024 and

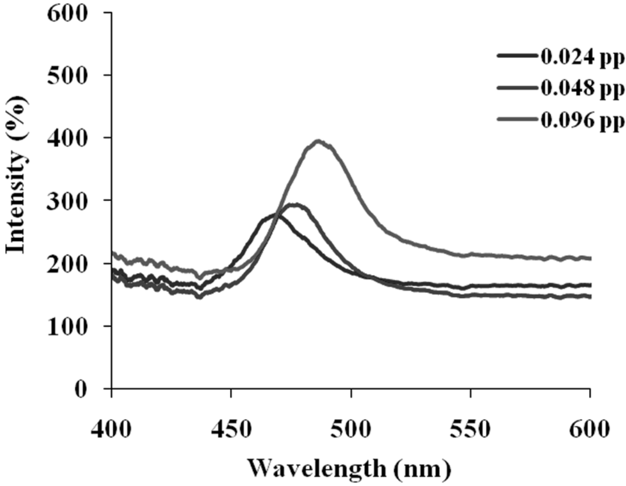


Figure 2. Reflectance spectra of PS-zirconia CCA exposed to different concentrations of paraoxon.

0.096 ppm; the sensor displays a 10 nm shift over a concentration range from 0.024 to 0.048 to 0.096 ppm. As we can see from Figure 3, with increase in concentration of paraoxon from 0.024 to 0.048 to 0.096 ppm, the characteristic change of reflectance peak shifts from 468 to 478 to 488 nm respectively. The shift in the CCA diffraction induced by

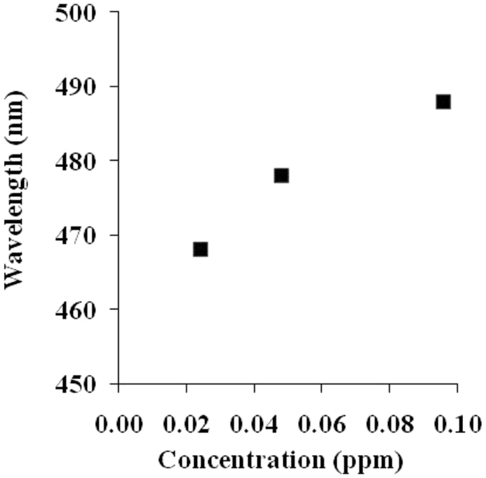


Figure 3. Effect of paraoxon concentration on the shift in reflectance wavelength.

0.024 ppm analyte is observed at 468 nm. At higher concentrations of paraoxon, the particles begin to increase in size because addition of more paraoxon increases overall crystal size and lattice spacing induces shift in diffraction wavelength. This could be explained by the fact that addition of paraoxon increases distance in lattice spacing and thereby increases peak shift, which follows the standard law of Bragg diffraction. The increase in shift of peak wavelength can be explained by the fact that as we increase the concentration of the paraoxon, particle size of the colloidal crystal array increases, which increases the distance between crystals, and peak wavelength increases as per Bragg's law ($m\lambda = 2nd\sin\theta$).

The Asher group[28] has reported an intelligent polymerized crystalline colloidal array (IPCCA) photonic crystal sensing material that reversibly senses the organophosphate compound methyl paraoxon at micromolar concentration. They created a periodic array of colloidal crystal particles and embedded them in a poly-2-hydroxyethylacrylate hydrogel. They utilized enzymes as molecular recognition agents. This group further explored polymerized crystalline colloidal arrays (PCCA) based on cross-linked microgel where the enzyme acetyl cholinesterase (AChE) is the molecular recognition agent.[29] Leblanc and coworkers[30] reported the layer-by-layer deposition method for the detection of organophosphorus compounds. They created several bilayers of chitosan (CS) and poly (thiophene-3-acetic acid) (PTTA) to produce a stable supramolecular ultrathin film. They used the principle of strong electrostatic segment-segment repulsion, which adopts an extended conformation. CS adsorbs strongly on negatively charged surfaces, and the adsorbed CS layer adopts a flat conformation that provides a stable film on which the PTAA can be adsorbed. All these above-mentioned processes require skilled supervision. Coating of polystyrene colloids core with zirconia shell is carried out using a physical adsorption method for detection of varying concentrations of paraoxon.

CONCLUSION

We prepared a novel material that can sense ppm-level concentrations of paraoxon. We are developing this as a sensor for use in detecting OPs in the field that is prompt, easy, fast, and reliable. We have demonstrated that polystyrene core particles terminated in sulfonate or carboxyl group could be directly coated with a uniform shell of zirconia using a simple adsorption method in the weight ratio of 1:0.75 respectively. The presence of zirconia in polystyrene particles can be evaluated by EDX and TEM analysis showing a peak for Zr and distinct morphology respectively. TEM analysis clearly shows the presence of discrete Zr atoms on PS spheres. CCA can be prepared using these novel polystyrene core-zirconia shell particles. This fact suggested that these core-shell particles can be used as chemical sensors for organophosphorus compound detection. These core-shell particles further could be used as chemical sensors for the nerve agents and pesticides that may have physicochemical interaction with zirconia. Our process is superior in terms of ease of fabrication/preparation, rapid in terms of detection, safe in terms of handling polymer, fast in terms of detection, and well suited for industrial applications as an onsite detection tool requiring minimal skilled personnel supervision.

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