

Development of an Intelligent Polymerized Crystalline Colloidal Array Colorimetric Reagent

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We have developed a novel colorimetric reagent for the determination of Pb²⁺, pH, and temperature. This colorimetric reagent consists of a dispersion of ~100- μ m particles composed of an intelligent polymerized crystalline colloidal array (IPCCA). The IPCCA particles are composed of a hydrogel polymerized around a face-centered cubic (fcc) array of monodisperse, highly charged polystyrene colloidal particles. These IPCCA particles diffract visible light because the (111) planes of the fcc polystyrene colloidal particle array have an ~200-nm lattice constant. The IPCCA particles also contain a molecular recognition agent that actuates array volume changes as a result of changes in analyte concentration or temperature. This results in changes in the IPCCA lattice constants, which shifts the wavelength of light diffracted. We report here the use of these sensing materials in a liquid dispersion that can be poured into a sample solution. This diffraction measurement method is analogous to X-ray powder diffraction measurements. The diffraction wavelength is monitored at a defined angle relative to the incident light.

We have developed a new colorimetric reagent for the determination of Pb²⁺, pH, and temperature by using intelligent polymerized crystalline colloidal arrays (IPCCA). Crystalline colloidal arrays (CCAs) are arrays of monodisperse, highly charged colloidal particles that self-assemble to form periodic structures.^{1–13} These arrays are body-centered cubic (bcc) or face-centered cubic (fcc) lattices that Bragg diffract light in the IR, visible, and UV regions, just as atomic crystals diffract X-rays

meeting the Bragg condition.^{9,14} By polymerizing a hydrogel network around the CCA, we are able to lock the CCA ordering into a soft elastic hydrogel.¹⁵ Hydrogels are soft materials, mainly composed of water, that can undergo volume-phase transitions in which their volume shrinks or swells in response to certain stimuli.^{15–26} By attaching a chemical recognition element to these hydrogels,^{27–39} we have fabricated IPCCA that shrink or swell in response to the concentration of particular analytes within ~1 min of exposure to analyte.^{15,19–21} This change in the hydrogel volume

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alters the periodicity of the CCA, resulting in a shift in the diffracted wavelength.

Because the hydrogel volume is a function of analyte concentration, the color of light diffracted from the hydrogel is a function of analyte concentration. We report here the further development of our sensing technology by the fabrication of sensing IPCCCA particles for the determination of temperature, pH, and Pb²⁺. This new hydrogel particle dispersion acts as a colorimetric reagent.

EXPERIMENTAL SECTION

Highly charged, monodisperse polystyrene colloids were prepared via emulsion polymerization, as described elsewhere.¹³ The CCAs used in this study were 8 wt % suspensions of ~120-nm-diameter polystyrene particles. These colloidal particles were cleaned via dialysis (Spectra/Por Dialysis Membranes, Polyvinylidene Fluoride 2 000 000 MWCO) followed by shaking with ion-exchange resin (Bio-Rad mixed bed, AG501-X8). Once excess ions were removed, the suspension became iridescent as a result of Bragg diffraction from the CCA. The procedure for fabrication of the PCCA was similar to that previously reported.¹⁹ We used acrylamide (AMD, Fluka) or *N*-isopropylacrylamide (NIPAM, Acros Organics) as polymerizable monomers, *N,N*-methylenebisacrylamide (bis-AMD, Fluka) as a polymerizable cross-linker, and 2,2-diethoxyacetophenone (DEAP, Acros Organics) as a UV photoinitiator. We added the monomer, cross-linker, and initiator to solutions of diffracting colloidal particles and injected the resulting solutions into cells consisting of two quartz plates separated by a spacer of thicknesses between 25 and 500 μm . The cells were exposed to UV light from a Blak Ray (365 nm) mercury lamp for up to 1 h. The polymerized CCA hydrogels were removed from the quartz cells and were allowed to equilibrate with large quantities of water for several hours.

Pb²⁺ Sensor. The Pb²⁺-sensitive hydrogel was produced by a procedure similar to that of Holtz et al.¹⁹ We used the polymerizable chelator, 4-acryloylamidobenzo-18-crown-6 (AAB18C6, Aldrich) to chelate Pb²⁺. The IPCCCA was made by dissolving 2.38 mmol of AMD, 0.108 mmol of bis(AMD), 0.267 mmol of AAB18C6, and 0.0072 mmol of DEAP in 3 mL of an 8 wt % dispersion of diffracting polystyrene colloids. The mixture was injected into a quartz cell with a 250- μm spacer, photopolymerized, and allowed to equilibrate with deionized water for 24 h.

pH Sensor. The pH-sensitive hydrogel was fabricated by dissolving 2.11 mmol of AMD, 0.097 mmol of bis(AMD), and 0.0072 mmol of DEAP in 3 mL of an 8 wt % dispersion of diffracting polystyrene colloids. This CCA mixture was injected into a quartz cell with a 250- μm spacer and photopolymerized. After allowing the resulting PCCA to equilibrate in water overnight, it was placed for 3 min into a solution of 1 M NaOH containing 10% (wt/wt) of *N,N,N,N*-tetramethylethylenediamine (TEMED, Sigma). This IPCCCA was then extensively washed with deionized water. The base hydrolysis resulted in an ionic gel in which some amides were hydrolyzed to carboxylates.

Temperature Sensor. The temperature-sensitive hydrogel was made by dissolving 1.37 mmol of NIPAM, 0.07 mmol of bis-AMD, and 0.0072 mmol of DEAP in 3 mL of an 8 wt % dispersion of diffracting polystyrene colloids. This solution was injected into a quartz cell equipped with a 250 μm spacer and was photopolymerized in an ice bath.

Preparation of Gel Fragments and Spectral Measurements. The hydrogels were fragmented into ~25–250 μm particles using a tissue homogenizer (Biospec Products Inc., model 985370). The diffraction from the IPCCCA hydrogel fragments was measured in backscattering using a CCD array UV–Vis spectrophotometer (Spectral Instruments, 400 Series) in which the source and the detector were collinear. The particles were washed for reuse by rinsing with deionized water. The water was removed by filtering the liquid through a 5.0- μm nylon syringe filter, which trapped the particulate dispersion. Water was then flushed through this filter in the opposite direction to deposit the IPCCCA gel fragments into a sample vial. This washing procedure was repeated five times between sample determinations.

RESULTS AND DISCUSSIONS

The face-centered CCA diffracts visible light in a manner similar to how atomic crystals diffract X-rays (Figure 1). Our IPCCCA diffraction almost obeys Bragg's law,^{9,14}

$$m\lambda_B = 2nd \sin \theta_B \quad (1)$$

where m is the order of diffraction, λ_B is the diffracted wavelength in a vacuum, n is the refractive index of the system, d is the spacing between the diffracting planes, and θ_B is the glancing angle between the incident light propagation direction and the diffracting planes.

We adjust our IPCCCA fcc (111) plane spacing such that it dominates the visible diffraction.⁴⁰ A dispersion of these IPCCCA particles in solution will show a random orientation of the fcc (111) planes of the individual IPCCCA particles. These randomly oriented hydrogel particles will show a powder diffraction pattern.^{41,42} Illumination with collimated monochromatic light that meets the Bragg condition results in diffraction along an angle that satisfies the Bragg condition; the light will be diffracted along the surface of a cone at an angle $180^\circ - 2\theta_B$ to the incident beam (Figure 1). Similar phenomena will occur for polychromatic light incident on these randomly oriented crystallites. Polychromatic light meeting the Bragg condition will be dispersed with the longest wavelength meeting the Bragg condition diffracted at the largest angle ($\theta_B = 90^\circ$, Figure 1). In Figure 1 at $\sin \theta_B = 1$ ($180^\circ - 2\theta_B = 0^\circ$), we obtain a back-diffracted red beam (the longest wavelength). As $\sin \theta$ decreases, the diffraction shifts to shorter wavelengths.

By fixing the angle between the incident beam and the detector, we monitor only a small diffraction angular width. Figure 1 illustrates a system in which the detector and the incident beam are collinear, such that $\sin \theta_B = 1$. When these particulates shrink or swell in response to environmental changes, the diffraction wavelength shifts.

For our IPCCCA dispersion, in addition to diffraction from the fcc (111) planes, we also expect to observe diffraction of visible light from the fcc (200) planes at a somewhat smaller wavelength.⁹ However, we see only weak diffraction from the (200) planes.⁴³

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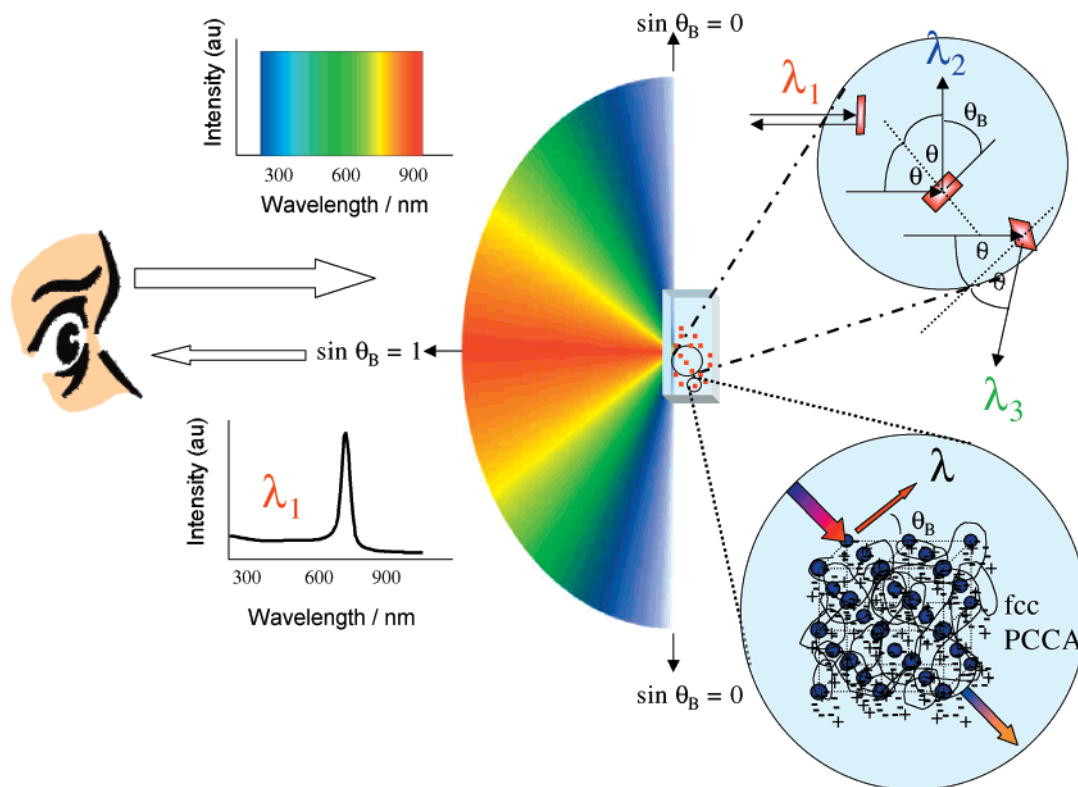


Figure 1. Diffraction from IPCCCA particle dispersion. These randomly oriented diffracting particles consist of fcc PCCA and give rise to rings of diffracted light similar to those obtained by X-ray powder diffraction. Back-diffracted light, which has the longest diffraction wavelength, results from planes whose normal is parallel to the incident beam. Note that θ_B is the Bragg glancing incident angle.

Temperature Sensing. Hydrogels containing NIPAM show a temperature-induced reversible volume-phase transition from a swollen to a collapsed state.^{16,22–26,44–46} We previously demonstrated that a NIPAM IPCCCA single crystal could be used as a temperature sensor.¹⁶ NIPAM IPCCCA are swollen in cold water, but when the temperature increases they undergo a reversible volume-phase transition to a collapsed state, with a resulting diffraction blue-shift.

(43) Diffraction from the (200) planes has not previously been observed from IPCCCA single crystals, because in all previous cases, the IPCCCA crystals were grown such that the IPCCCA sample container walls were parallel to the IPCCCA fcc (111) planes. The (200) planes are oriented 54° from the (111) planes. Thus, it was difficult to couple light at a sufficiently high θ_B to observe 200 diffraction. The 200 diffraction occurs in the UV if light is incident normal to the (111) planes. We expect to observe visible wavelength diffraction from our randomly oriented IPCCCA dispersion. Perfect fcc crystallites should show similar 111 and 200 diffraction efficiency, because they have the same structure factor.⁹ For $\sin \theta_B = 1$ (back-diffraction), if the (111) planes diffract at 600 nm, the (200) planes will diffract at 519 nm, because the ratio of their plane spacings is 0.866.^{9,35} However, we observe only weak diffraction from the (200) planes. This weak 200 diffraction appears to result from stacking faults within the IPCCCA fcc array. We carefully measured the diffraction from a macroscopic single IPCCCA crystal within a water tank in order to almost refractive index match the crystal. We oriented the crystal to meet the Bragg condition for the (111) and (200) planes for 514.5-nm light and observed diffraction from both planes. However, the diffraction efficiency from the (200) planes was much less than from the (111) planes (~ 10 -fold). Stacking faults will locally convert the fcc structure to an hcp structure that does not have an analogous set of diffracting planes. Thus, stacking faults will cause the (200) planes to locally disappear, which will dramatically decrease the 200 diffraction efficiency.

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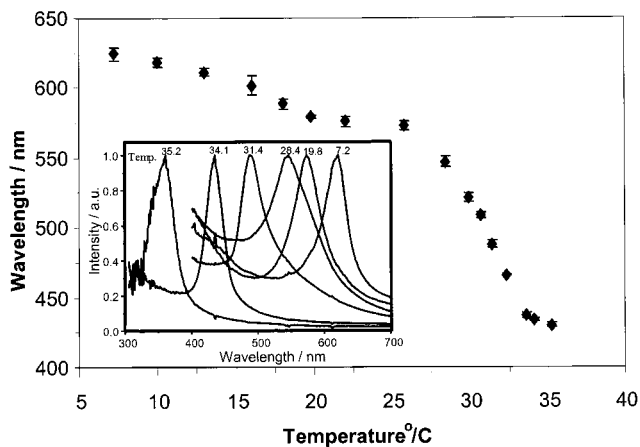


Figure 2. Temperature dependence of diffraction spectrum of NIPAM IPCCCA particle dispersion. The diffraction is measured in back-scattering. As the temperature increases, the wavelength blue-shifts.

Figure 2 shows that NIPAM IPCCCA particles show similar diffraction temperature dependences. At 7.2°C , the particles diffract 625-nm light, but at 35.2°C , the particles diffract 425-nm light. Between 7.2 and 25°C , the diffraction shifts from 625 to 560 nm, which gives a sensitivity of $\sim 3\text{ nm}/^\circ\text{C}$. A more sensitive response occurs around the phase-transition temperature, with an 80-nm shift occurring over the 6° range of 27.5 – 33.5°C , giving a sensitivity of $\sim 17\text{ nm}/^\circ\text{C}$. Because the likely error for determining the diffraction wavelength maximum is $\sim 1\text{ nm}$, these IPCCCA particles should be able to resolve temperature differences of $< 0.05^\circ\text{C}$ around the phase-transition temperature.

It is relatively easy to vary the phase-transition temperature by altering the hydrogel chemical composition. For example,

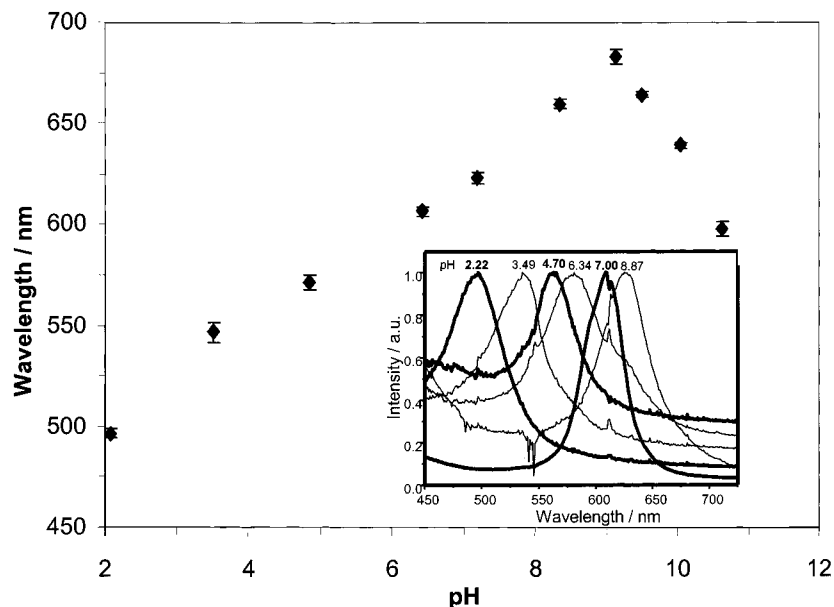


Figure 3. pH dependence of diffraction spectrum of IPCCAs particles between pH 2.22 and 11.11. From pH 2.22 to 9.6 the diffraction red-shifts with increasing pH. The diffraction is measured in back-scattering. Above pH ~ 10 , all carboxylates are ionized, and the pH increase only increases the ionic strength, which blue-shifts the diffraction.

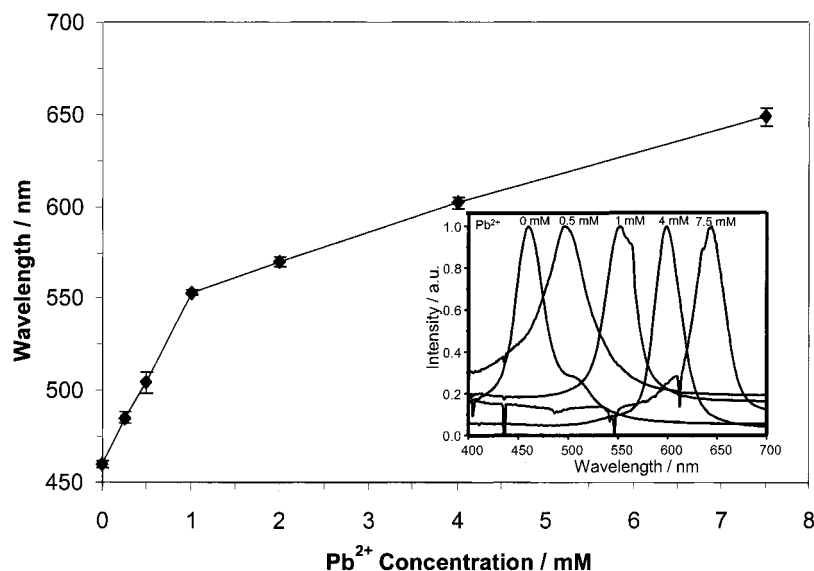


Figure 4. Pb^{2+} -concentration-dependence of diffraction spectrum of IPCCAs particles. The diffraction red-shifts as the Pb^{2+} concentration increases.

adding acrylic acid to the hydrogel backbone shifts the phase transition temperature to $\sim 50^\circ\text{C}$.²⁵ Thus, we should be able to create a set of very sensitive and accurate colorimetric reagent temperature sensors useful in the temperature range of $0\text{--}60^\circ\text{C}$. Further, it is possible to use these particles to detect temperature gradients. For example, a dispersion of particles could be placed in a vessel of water and a telescope could be used to direct light into the tank and to image the light diffracted from a certain volume element.

pH Sensing. We recently developed an IPCCAs sensing film that could determine pH and ionic strength.²¹ This IPCCAs is a hydrolyzed acrylamide hydrogel in which some amide groups are converted to carboxylic acids. The pH dependence is due to the acid–base equilibria of the carboxyl groups. As the carboxyl groups ionize, anions become localized on the hydrogel. This

causes a Donnan potential, which changes the chemical potential of water and causes an osmotic pressure, which swells the IPCCAs and causes the diffraction to red-shift.

It is important to note that both the pH-sensitive hydrogel particles and the Pb^{2+} -sensitive particles are made from acrylamide, and the hydrogel volume is not sensitive to temperature, in contrast to the temperature-dependent sensors made from *n*-isopropyl acrylamide. We observed less than a 2-nm shift in diffraction over the temperature range of $10\text{--}50^\circ\text{C}$ for these acrylamide hydrogel particles.

Figure 3 shows the pH dependence of diffraction for the IPCCAs particle dispersion. The diffraction monotonically red-shifts from 500 to 677 nm between pH 2 and 9.6, whereupon it blue-shifts from 677 to 590 nm between pH 9.6 and 11.11. The blue shift results from the decrease in osmotic pressure due to the increased

solution ionic strength at the high pH values.^{19–21,44–46} The response is relatively linear up to pH 9.6, with a sensitivity of ~23 nm/pH unit. Given a 1-nm resolution, we can determine pH with a 0.05 pH unit resolution in deionized water.

For solutions with a defined ionic strength, we can calibrate our IPCCCA sensor particles to determine pH. In fact, we have already shown that we can determine ionic strength independently by utilizing IPCCCA with ionic groups that do not undergo pH titrations. Thus, we can use two separate IPCCCA colorimetric reagents in parallel. We would determine ionic strength and then use an ionic-strength-calibrated pH-sensing IPCCCA to determine the solution pH.

Pb²⁺ Sensing. The Pb²⁺-sensitive IPCCCA particles utilize a crown ether to selectively bind Pb²⁺. The Pb²⁺ binding immobilizes charge on the IPCCCA, which causes swelling, just as for the pH sensor. Figure 4 shows the response of the IPCCCA particles to variation of Pb²⁺ concentration. The response of the IPCCCA particles to Pb²⁺ is approximately linear up to 1 mM, with a sensitivity of 90 nm/mM Pb²⁺, but between 1 mM and 7.5mM Pb²⁺ the sensitivity is 14 nm/mM Pb²⁺. In the low concentration range, with a 1-nm diffraction peak resolution, we should be able to easily resolve 10 μ M concentrations of Pb²⁺. Upon washing the IPCCCA particles with water, they shrink back to their original diffraction as the Pb²⁺ ions exchange out of the crown ether binding sites.

The detection sensitivity of these IPCCCA sensors depends on their composition. For example, the more elastic the sensor, the more sensitive. One of the parameters determining the IPCCCA

elasticity is their hydrogel cross-linking. The less cross-linking, the more elastic. Thus, we can increase sensitivity by decreasing hydrogel cross-linking. We have synthesized IPCCCA with 10-fold less cross-linking, which show much higher sensitivities and lower detection limits. We will report on these IPCCCA in a future publication.

CONCLUSION

These IPCCCA particle dispersions act as new colorimetric reagents that can be poured into a sample for the determination of temperature, pH and Pb²⁺, and in the future for other analytes. These IPCCCA can be modified for detecting other analytes by altering their molecular recognition elements. The chemical-sensing materials are easy to use and should be inexpensive. These IPCCCA particles can also be used for remote sensing of temperature and analytes by using a telescope to illuminate and image the diffracted light.

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