Glucose Sensing Intelligent Polymerized Crystalline Colloidal Arrays

Sanford A. Asher and John H. Holtz*
Department of Chemistry
University of Pittsburgh
Pittsburgh PA 15260
412-624-8570
Fax 412-624-0588
asher+@pitt.edu
** corresponding author*

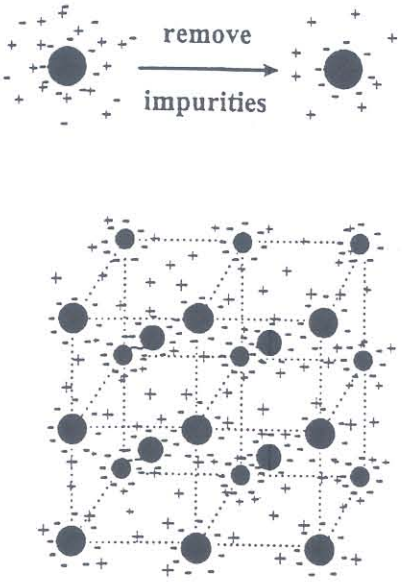
We have developed novel glucose and galactose sensing materials that report on analyte concentrations via diffraction of visible light from polymerized crystalline colloidal arrays (PCCAs)[1-4]. These PCCAs are mesoscopically periodic crystalline colloidal arrays (CCA) of spherical polystyrene colloids polymerized within thin intelligent polymer hydrogel films (Fig 1). CCAs are brightly colored; they efficiently diffract visible light meeting the Bragg condition. The intelligent hydrogel contains molecular recognition agents that cause the gel to swell (Fig. 2) in response to the presence of analyte[5-10].

CCAs self assemble from suspensions of highly charged, monodisperse colloidal particles (Fig. 1).[4,11-14] At low ionic strengths, the colloidal particles repel each other, and the system assumes a minimum energy configuration, which is usually a body- or face-centered cubic lattice. The colloidal particles may be composed of inorganic materials such as silica, or organic polymers such as poly(methylmethacrylate), polystyrene, or poly(N-isopropyl acrylamide).[2, 15,16]

The periodicity of the CCA is on the order of ~200 nm, so the CCA diffracts visible light. The diffraction is in the "dynamical diffraction regime", and almost obeys Bragg's law: [11,17]

$$m\lambda = 2\, n\, d \sin\theta$$

where m is the order of diffraction, λ is the diffracted wavelength in vacuum, n is the refractive index of the system (solvent, hydrogel and colloids), d is the spacing between the diffracting planes (for the CCAs here, the 110 planes of a BCC lattice), and θ is the glancing angle between the incident light propagation direction and the diffracting planes.

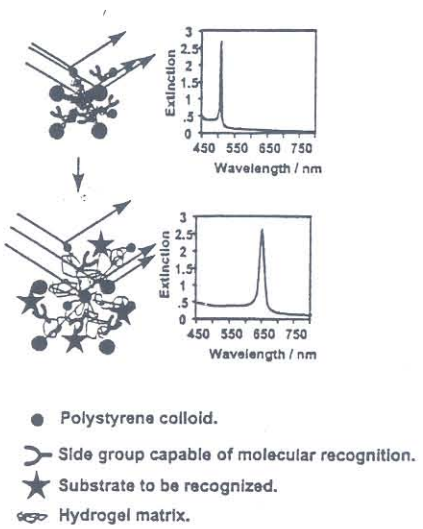


▼ *Figure 1. Self assembly of a body centered cubic CCA. At low ionic strengths repulsion between monodisperse, highly charged colloidal particles forces the colloidal spheres into a minimum energy configuration, which is either a body or face centered cubic lattice.*

We polymerize the CCA into an acrylamide hydrogel film to form a PCCA[1-3] by dissolving non-ionic polymerizable monomers, crosslinkers and photoinitiators into the liquid CCA, and then photopolymerizing the mixture to make a thin, diffracting PCCA film. These PCCA films have applications as tunable filters, optical switches and non-linear optical devices.[2-4,18,19]

To make an intelligent polymerized crystalline colloidal array (IPCCA) sensor, we incorporate glucose oxidase or β-D-galactosidase as the chemical recognition elements. We attach glucose oxidase or galactose oxidase by coupling some of the acrylamide amide groups in the PCCA to biotin functional groups. This biotinylated hydrogel then binds avidinated glucose oxidase, and β-D-galactosidase.

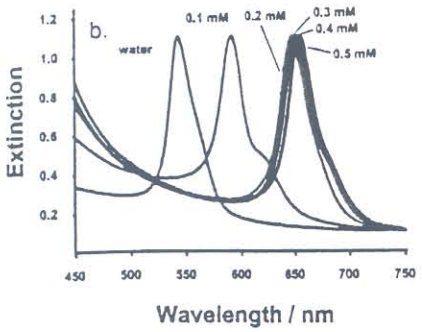
Figure 3 and 4 show the response of the glucose and galactose sensors, to varying concentrations of glucose or galactose. For example, 0.1 mM glucose concentrations causes the diffraction to shift from yellow at 550 nm to red at 600 nm. The IPCCA continues to shift to diffract in the deep red for glucose concentrations of 0.2 mM; the shifts saturate for concentrations above 0.2 mM for this sensor. In the case of the galactose sensor the shifts occur for concentrations even beyond 0.3 mM galactose. Neither sensor responds to sucrose, or to the substrate of the other enzyme (glucose or galactose).



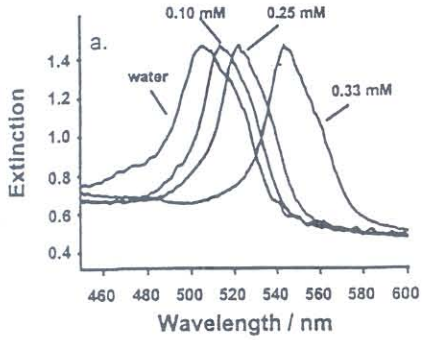
▼ *Figure 2. General motif for the Intelligent Polymerized Crystalline Colloidal Array (IPCCA) sensors. The CCA Bragg diffraction is a sensitive monitor of the hydrogel volume change induced by the interaction or binding of the molecular recognition agents to an analyte. In principle, any molecular recognition agent can be attached to the hydrogel*

As discussed below these shifts result from a steady state concentration of reduced enzyme where a competition occurs between the reduction of the enzyme by reaction with glucose and reoxidation of the enzyme by oxygen. At low levels of oxygen, where the enzyme oxidization rate is very small, the glucose sensor swells in sub-nanomolar concentrations of glucose. We observed a 30 nm diffraction wavelength shift in 10^{-10} M glucose, and an 8 nm wavelength shift in 10^{-12} M glucose.

These IPCCA hydrogels are crosslinked polymeric networks that are swollen in water. These hydrogel networks change volume as their environment changes; hydrogels swell into good solvents, and this tendency to



▼ *Figure 3. Response of an IPCCA containing glucose oxidase to glucose.*



▼ *Figure 4. Response of the IPCCA sensor containing galactosidase to galactose.*

swell is resisted by the elastic restoring force of the hydrogel network, which arises from the crosslinks. Hydrogels with covelently attached ionic groups swell in water, due to Donnan-type equilibria established by the mobile counterions of the fixed charges inside gel and by electrolytes in the gel or its bathing solution.[20-23] Tanaka has shown that the degree of swelling of a polyelectrolyte gel is proportional to the number of ionic side groups per polymer chain in the gel (a polymer chain is defined as the length of polymer between crosslinks).[21,22] In addition, repulsions between charged groups will further swell the gel at high charge densities.

The equilibrium hydrogel volume is determined by the sum of the free energy of mixing of the polymer chains with the solvent medium, the free energy of elasticity of the crosslinked network, and the ionic electrostatic energy due to the Donnan equilibrium and electrostatic repulsions between charged side groups on the polymer backbone:

$$\Delta F_{tot}=\Delta F_{mix}+\Delta F_{elas}+\Delta F_{ion}$$

Glucose oxidase converts glucose to gluconic acid in a two-step process. In the first step, glucose is converted to gluconic acid, and the enzyme is reduced. In the second step, the enzyme is reconverted to its oxidized form by oxygen in the solution, producing H_2O_2 as a by-product:[23,24]

$$\text{EnzymeH(ox)} + \text{Glucose} \rightarrow \text{Enzyme}^{1-}\text{(red)} + \text{Gluconic Acid} + H^+$$

$$H^+ + \text{Enzyme}^{1-}\text{(red)} + O_2 \rightarrow H_2O_2 + \text{Enzyme(ox)}.$$

When the glucose oxidase reacts with glucose the enzyme flavin prosthetic group becomes reduced and negatively charged[24,25]. The hydrogel then expands, primarily due to an increased osmotic pressure from a Donnan-type potential arising from mobile counterions.

In the absence of oxidizer, the sensor should eventually reach its maximum volume even if the bath solution contains only one stoichiometric amount of glucose relative to the glucose oxidase in the gel. In principle, we could detect diffraction from a 1 $(\mu m)^3$ IPCCA sensor. Since the glucose oxidase concentration in our IPCCA is ~ 10^{-4} M, and since we can detect a ~ 10 nm shift in diffraction, this suggests a detection limit of less than ~ 10^6 molecules of glucose which would be required to fully reduce the glucose oxidase in this $1\mu m^3$ IPCCA

Conclusions

These IPCCAs are a new motif for fabricating sensors. The utility of this motif is limited only by the availability of suitable molecular recognition agents. We previously demonstrated, that the IPCCA can be coated onto optical fibers such that they can be used to fabricate optrodes to remotely measure analytes.

These sensors have the great advantage of having a response which is easily detectable by the human eye. The sensitivity and detection range of these sensors can be tailored by controlling the gel composition, as well as the molecular recognition agents used. We are now modifying this glucose sensor for use at the high physiological ionic strengths of biological fluids such as blood.

Acknowledgments.

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