

# Modeling Saliva/Apatite Interfacial Phenomena with Surface Plasmon Resonance (SPR) Spectroscopy

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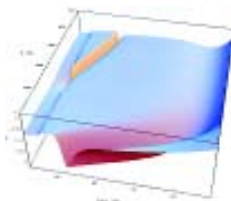


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## ABSTRACT

**Objectives:** The objective of this research was to evaluate the utility of a novel optical sensor to observe molecular-level sorption and growth/dissolution events at calcium phosphate mineral surfaces. **Methods:** Carboxylated thiols were used to prepare self-assembled monolayers (SAMs) to facilitate formation of mineral layers over a spectroscopically-active gold substrate. Changes in mineral thickness were calculated from Surface Plasmon Resonance (SPR) minima positions during exposure of the SAM to supersaturated solution. Temperature, pH and ion activity product (IAP) were carefully controlled throughout each experiment. **Results:** Changes in film thickness observed during SAM assembly were consistent with the known chain-length, orientation, and hydration of the thiol. Film changes upon exposure of the interface to mineralizing solution were also observed and attributed, on the basis of solution IAP, to the growth of various mineral phases, including OCP and HAP. Preparation of HAP was greatly facilitated by thermal conversion of precursor phases, such as OCP, vs. direct formation of HAP from dilute solution.

Figure 1 (above): Calculated response surface of the LASFN9/Au/C12 thiol-based sensor for HAP growth. Note the linearity of the system up to ca. 80 nm.



## INTRODUCTION / PURPOSE

The study of molecular-level events associated with nucleation, growth, and dissolution events at calcium phosphate mineral surfaces has proven to be experimentally challenging. Although Surface Plasmon Resonance (SPR) Spectroscopy has proven to be a valuable in molecular recognition analysis, the technique has not been successfully employed in the study of the mineral interface. The purpose of this effort was to create and evaluate an SPR-based optical sensor with a carboxylated self assembled monolayer to template the growth of mineral films atop an SPR-based thickness sensor. Such a sensor should prove useful in the study of (1) monolayer sorption and displacement phenomena at mineral surfaces, and (2) crystal nucleation and remineralization/demineralization in the presence of various surface complexes. Candidates of interest include plaque proteins, polyphosphates, and microbially-produced polysaccharides.

## MATERIALS AND METHODS

**Sensor Construction:** Figure 2 shows the SPR sensor in the Kretschman geometry. Schott LASFN9® glass was chosen to maximize the range of detection. Excitation was at 625 nm via LED to minimize optical interference. Template formation was effected via exposure of the bare gold surface to a 1 mM solution of 11-mercaptoundecanoic acid (Aldrich) in ethanol for 24 hours. National Instruments Labview® software was used for data collection and analysis. SPR minima were calculated via first moment analysis to utilize a broad set of the raw data. Although commercial SPR systems (Biacore) were available internally, a custom system was assembled for improved temperature control, solvent compatibility, and extended range. The temperature of the cell was controlled to  $\pm 0.2^\circ$  via a Cole-Parmer PID controller with a Teflon®-coated J-type thermocouple (placed in the liquid superstrate) and two 60 W cartridge heaters (McMaster-Carr) which were located in the cell block. Although film thicknesses (d) can be calculated directly, Mathematica® software was used to transform the appropriate  $q_{\min}$  vs. d Fresnel equation to a simple 3<sup>rd</sup>-order polynomial expression for convenience.

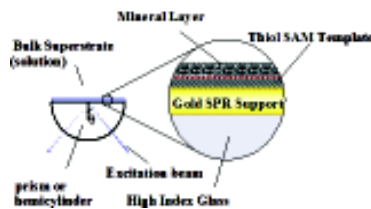


Figure 2: Composition profile of the SPR-based thickness sensor in the Kretschman geometry. A thin (ca. 2 nm) layer of Cr metal between the glass and the 50 nm Au support layer was required for adhesion.

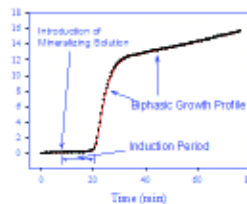


Figure 3: Film nucleation and growth atop the sensor. Collection parameters were  $T=37^\circ\text{C}$ ,  $\text{pH}=7$ , ionic strength  $=0.15$  &  $\text{pIAP}_{\text{OCP}}=88$ .

## RESULTS

Nucleation and growth of calcium phosphate mineral films was observed under various conditions. Figure 3 shows film growth at in a pH 7 solution. Nucleation and growth of calcium phosphate mineral films was observed under various conditions. Figure 3 shows film growth at in a pH 7 solution containing 4 mM  $\text{Ca}^{2+}$ /4 mM  $\text{PO}_4^{3-}$  (see figure caption for details). Induction times were a strong function of temperature, with a magnitude of approximately 12 minutes at  $37^\circ\text{C}$ . The cause of the sharp discontinuity in the growth profile at about 10 minutes is unknown but may correspond to a phase change as per evolution of IR spectroscopy (ATR mode) after about 10 minutes of growth. ATR spectra of films in the 20 nm+ thickness range are consistent with the OCP mineral phase. Grateful acknowledgement is made to H.L. Dean for SEM images and to the University of Tennessee and the National Science Foundation for financial support via an RSEC fellowship for J. Steehler.

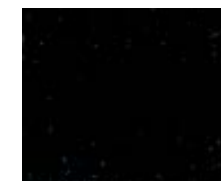


Figure 4: SEM image of a film of ca. 15 nm (avg.) @ 3500X.

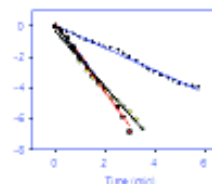


Figure 5: Response of annealed (blue – partially converted to HAP) vs. raw films to pH 5 acid challenge.

## CONCLUSION

Ca-P Mineral films can readily be prepared on a SPR-active optical sensor. The technique permits the real-time observation of mineral film growth and is sufficiently sensitive enough to be useful in the study of (1) monolayer sorption/displacement phenomena, and (2) nucleation and remineralization and demineralization in the presence of various surface complexes.