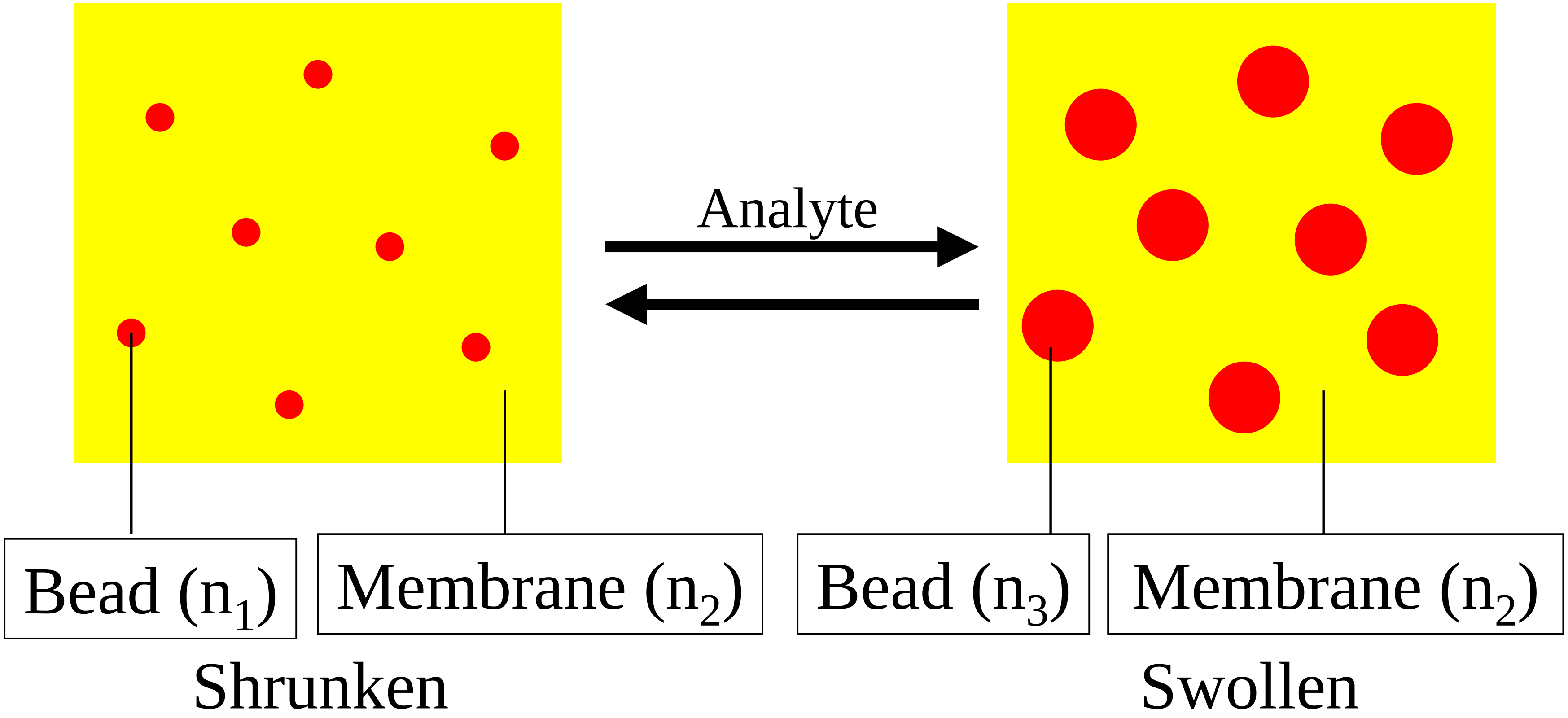
Compound Specific Imprinted Microspheres for Optical Sensing

Barry K. Lavine, Department of Chemistry,
Oklahoma State University

Concept Behind a New Technology For Chemical Sensing

- Chemical sensing is based on changes in the optical properties of the membrane that accompany swelling.



Advantages of Chemical Sensing Based on Swellable Polymers

- very stable
- swelling and shrinking has minimal effect on the size of the hydrogel and does not generate enough force to affect adhesion of the hydrogel on the substrate
- high selectivity (derivatization or molecular imprinting)
- fast response time
- easy to construct
- low cost
- can be operated at near IR wavelength for fiber optic sensing

Polymer Swelling

$$q_m^{5/3} = \left(\frac{V_0}{\nu_e}\right)\left[\left(\frac{1}{4}\right)\left(\frac{i}{V_u}\right)^2\left(\frac{1}{S*}\right) + \frac{\left(\frac{1}{2} - X_1\right)}{\upsilon_1}\right]$$

q_m is the swelling ratio

V_o is the volume of unswollen polymer

ν_e is the effective number of unit segments in the network

$\dfrac{i}{V_u}$ is the charge per polymer unit segment

$S*$ is the ionic strength of the bulk solution

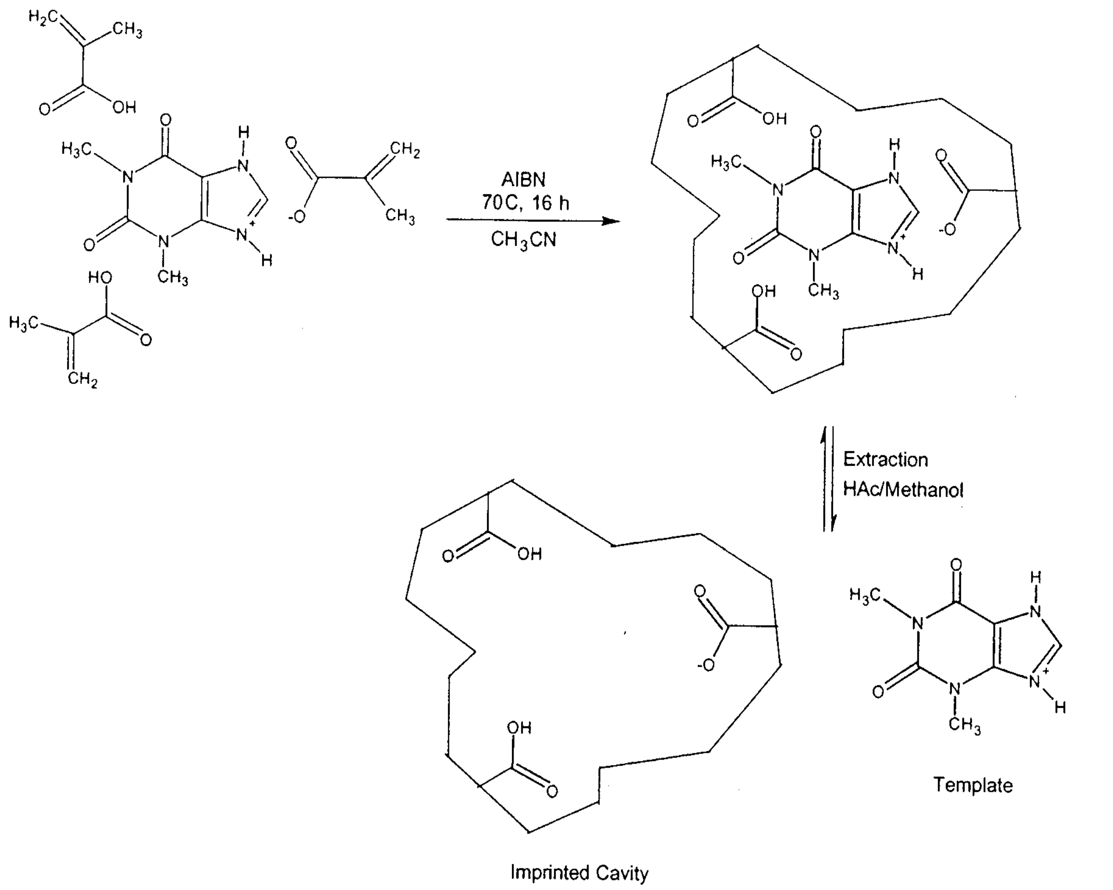
X_1 is the polymer-solvent interaction parameter

υ_1 is the molar volume of the polymer

Molecular Imprinting

- Functional and crosslinking monomers are copolymerized in the presence of the target analyte, which acts as a molecular template.
- Prior to polymerization, the functional monomers form a guest host complex with the imprint molecule, which is then fixed sterically upon polymerization.
- During the imprinting process, a crosslinked polymer with a 3-dimensional structure is formed.
- Removal of the template molecules from the polymer matrix reveals cavities that resemble the size and shape of the print molecules, with the spatial arrangements of the functional groups in each cavity complementary to that of the print molecule.

Example – Theophylline Imprinting



Molecular Imprinting

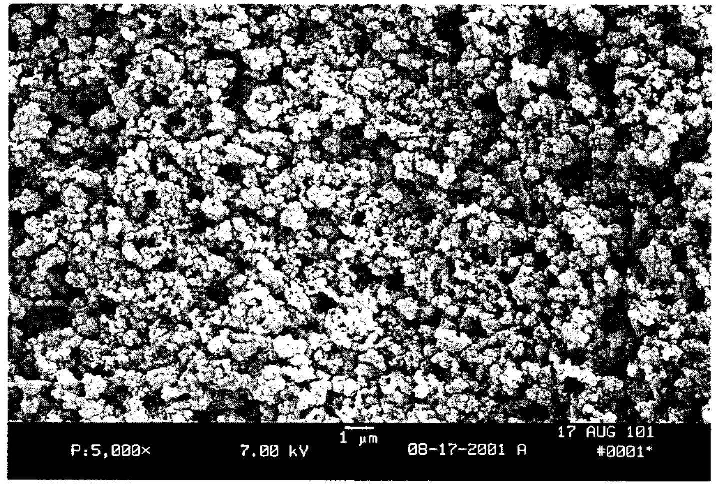
- The binding site towards the print molecule is due to ionic interactions and hydrogen bond formation between the analyte and the functional groups as well as the overall three-dimensional geometry of the site.
- The major advantage of molecular recognition based on molecular imprinting is that molecularly imprinted polymers are far more stable than biological materials such as antibodies that also provide molecular recognition.

Molecular Recognition Polymer

- Moderately crosslinked copolymer of N-isoproplylacrylamide (NIPA) and Methacrylic Acid (MAA) will selectively swell in the presence of template.
- Swelling is temperature dependent.
  - At low temperature, the polymer does not appreciably swell after binding to template because it is solvated by water
  - At higher temperature, the polymer shrinks and separates from water. It will selectively bind to template and swell.

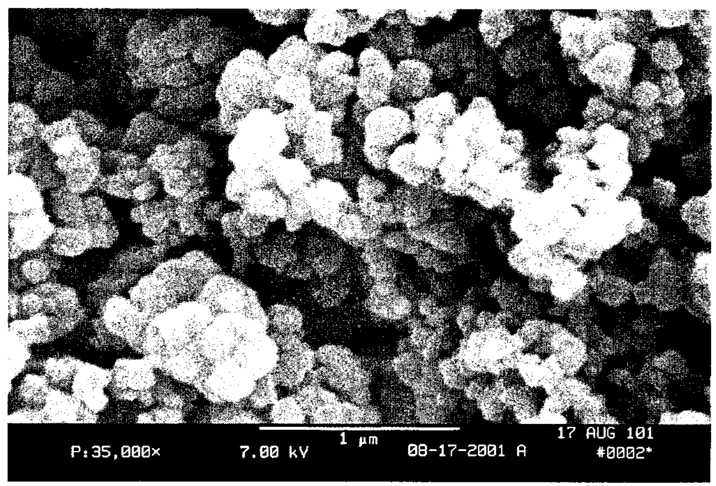
Theophylline Sensor

- Using dispersion polymerization, polymeric nanospheres were prepared that selectively bind theophylline.
- The particles were prepared by dispersion polymerization using a formulation consisting of 17mmol NIPA, 2mmol MAA, 1 mmol N,N'-methylenebisacrylamide (MBA) crosslinker and 1 mmol theophylline in acetonitrile.
- The solution was polymerized for 16 hours at 60^0C using azobisisobutryonitrile (2% w/w) as the free radical initiator.
- Polystyrene-acrylonitrile (20% w/w) was used as the stabilizer.



(A)

5,000



(B)

35,000

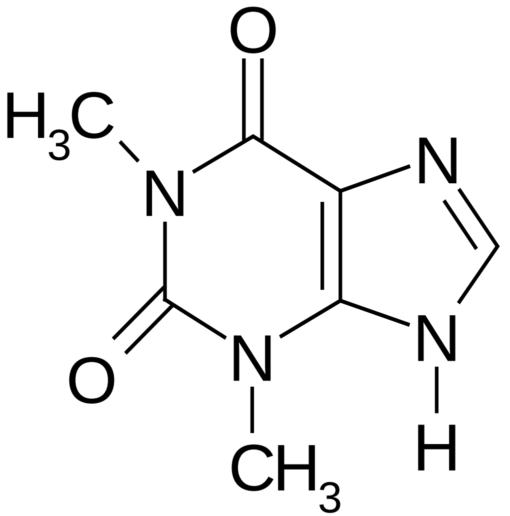
Theophylline Sensor

- The particles were suspended in polyvinyl alcohol hydrogel and exposed to varying concentrations of theophylline.
- In the absence of theophylline, the hydrogel is turbid because the refractive index of the particles differ from the refractive index of the hydrogel.
- In the presence of aqueous theophylline, the polymer will swell.
- The increased water content in the particles causes a decrease in particle refractive index bringing it closer to the refractive index of the hydrogel.
- This leads to a decrease in membrane turbidity.

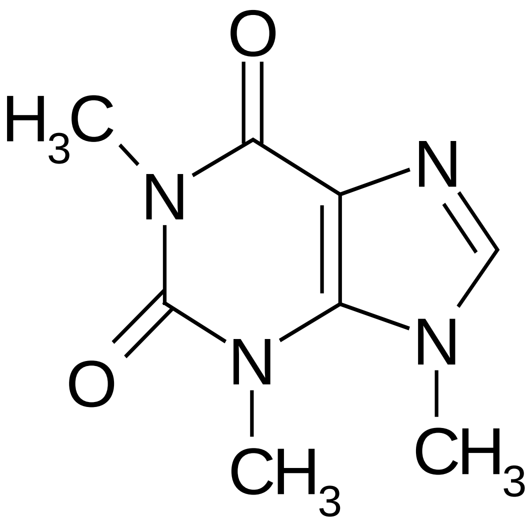
Sensor Response

- Response is both sensitive and selective
- The turbidity of the membrane measured as absorbance at 500 nm using a conventional spectrophotometer (Cary 5) and a cuvet with a custom built membrane holder (which is a Teflon spacer) decreased by 10% when it was exposed to $1x10^{-7}$ M theophylline.
- There was only a slight response to caffeine at concentrations as high as 1×10^{-3} M

Theophylline and Caffeine

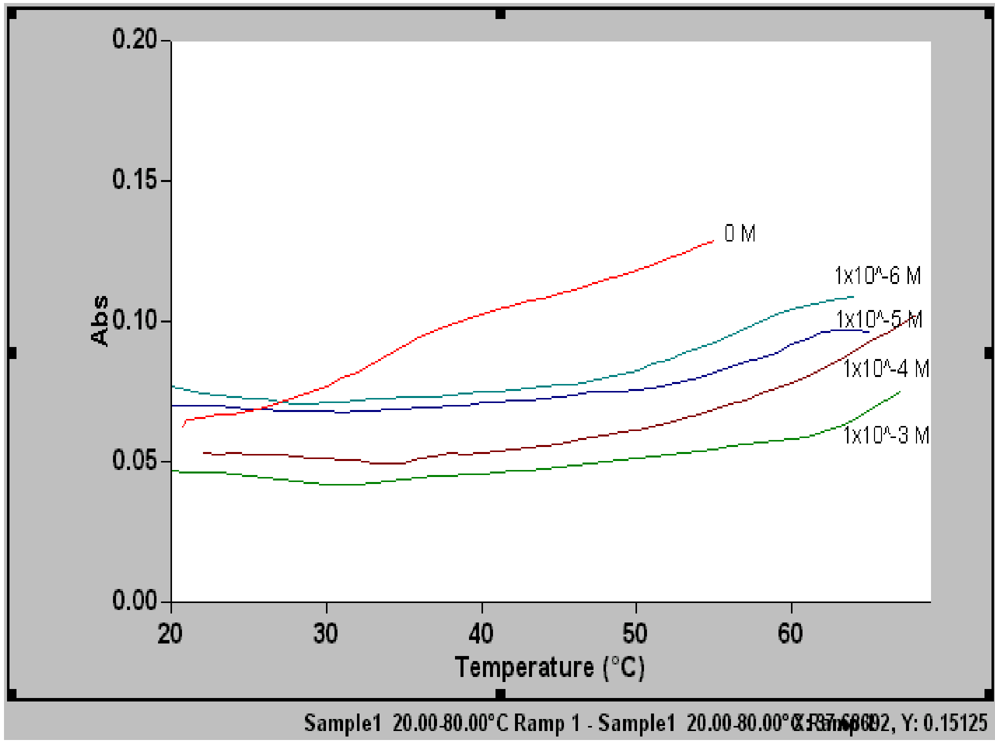


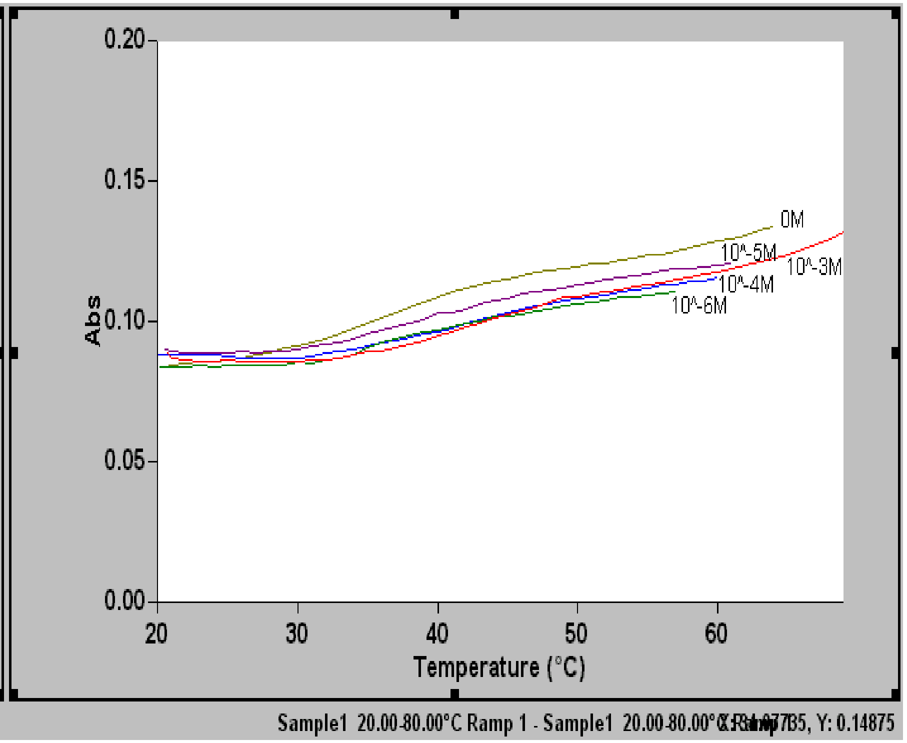
Theophylline



Caffeine

Absorbance versus Temperature

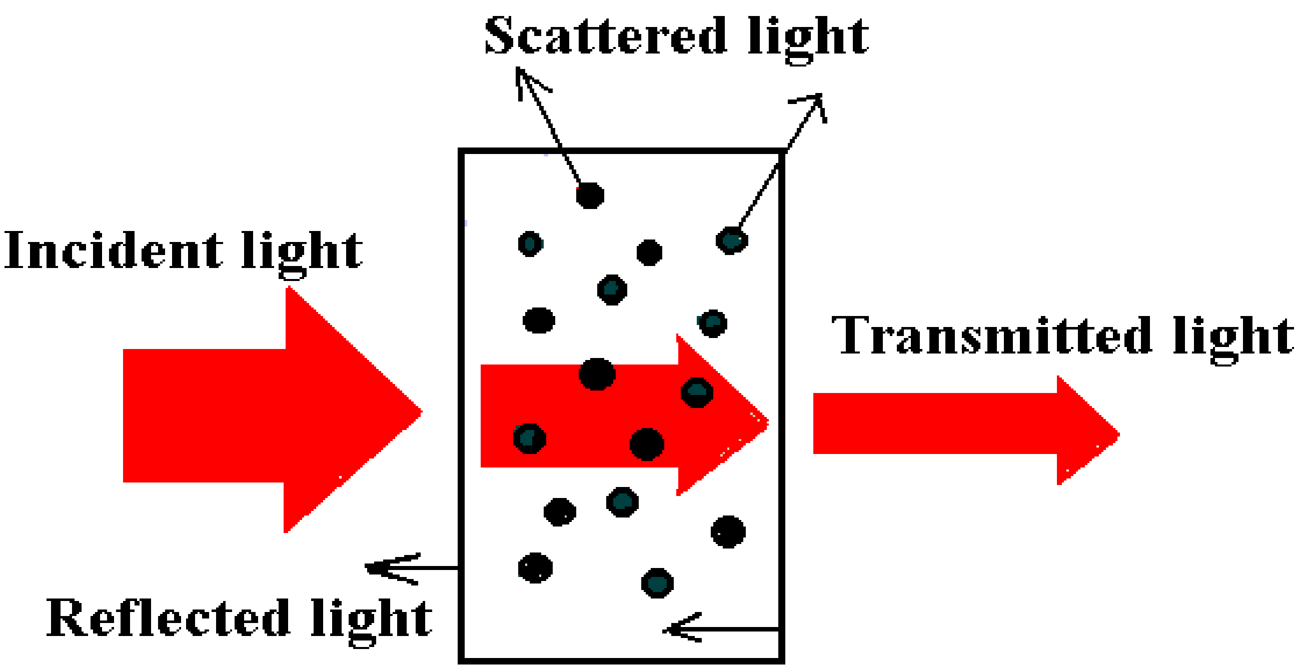




Theophylline standards

Caffeine standards

Scattered and Transmitted Light



•Because swelling leads to an increase in the percentage of water in the polymer, the refractive index of the particles will decrease as they swell.

•This brings them closer to the refractive index of the hydrogel membrane, leading to a decrease in turbidity (amount of light scattered/reflected by the particles).

Reflection:

$$R = (n_2 - n_1)^2 / (n_2 + n_1)^2$$

Turbidity:

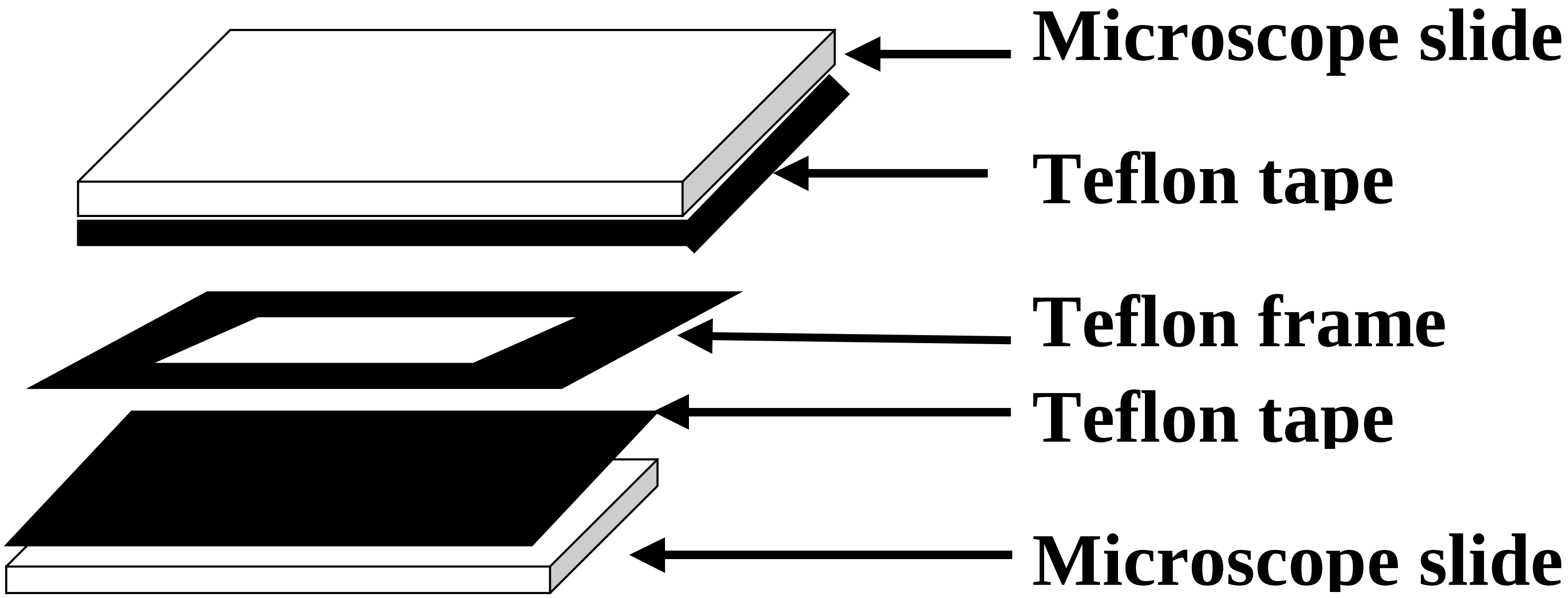
$$\tau = (2.303 / b) \log I_0 / I$$

$$I_{scat} = (1+\cos^2\theta) I_0 8\pi^4\alpha^2 / \lambda^4 p^2$$

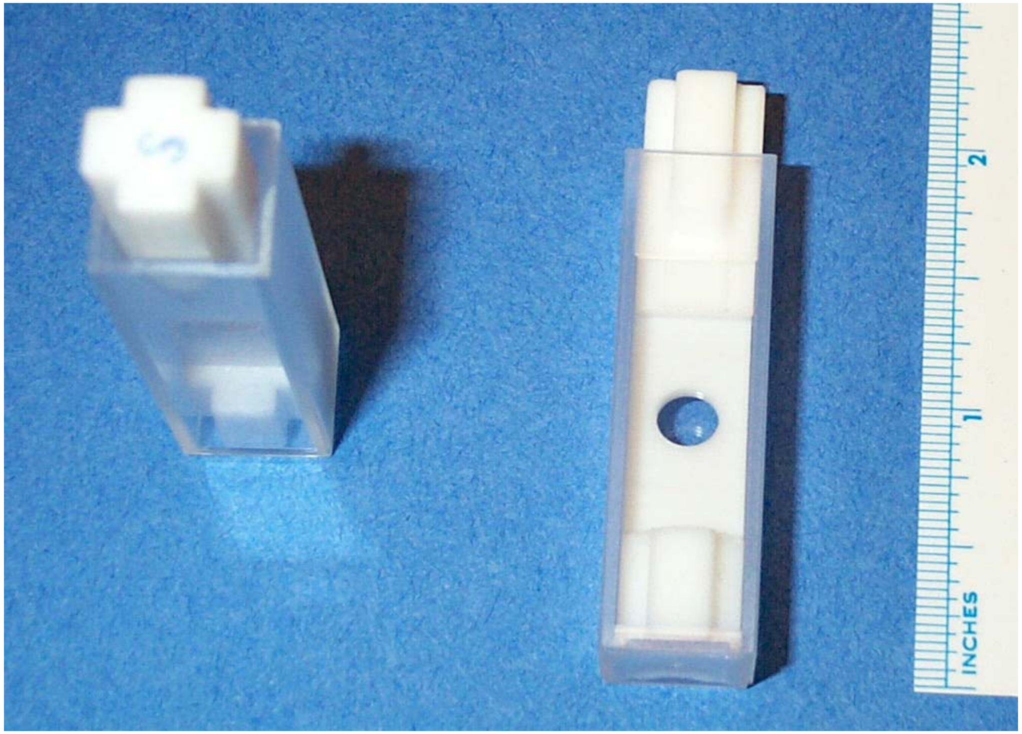
Absorption:

$$A = \log P_0 / P = \varepsilon bc$$

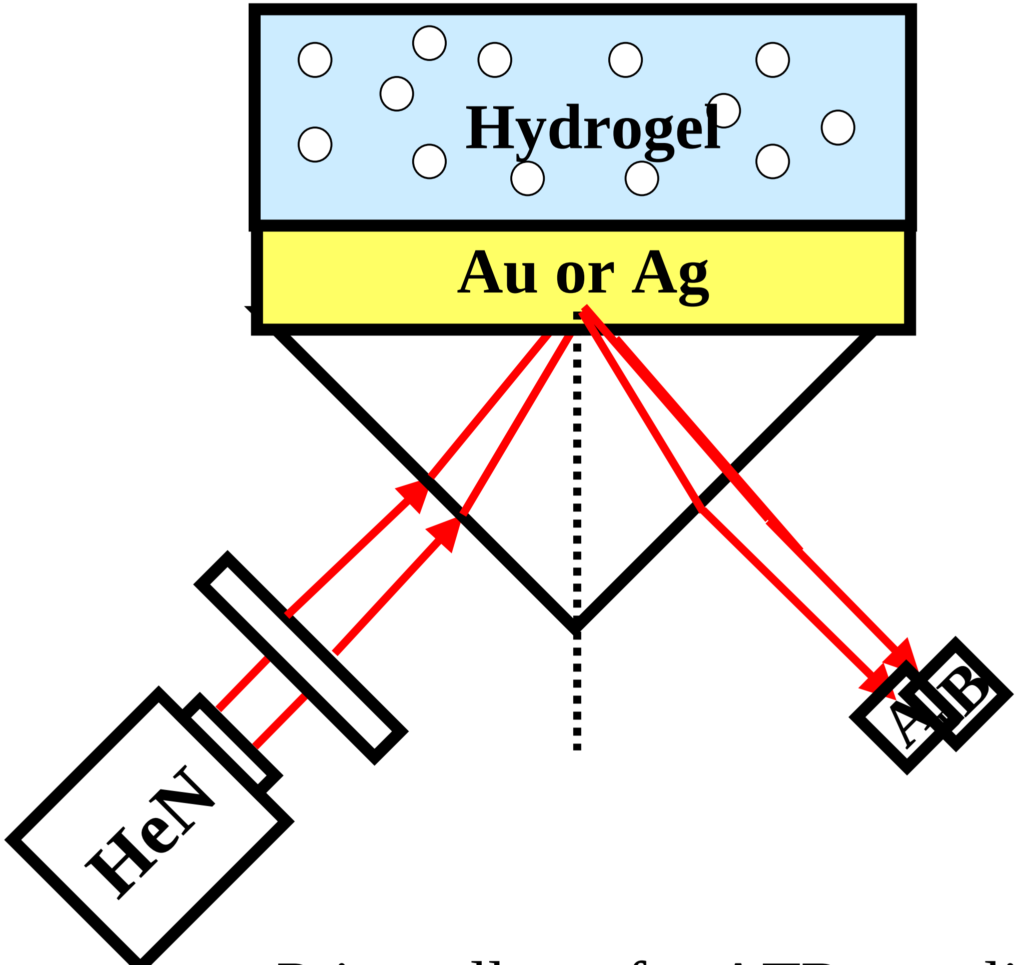
Making Hydrogel Membranes



Membrane Holders



Surface Plasmon Resonance Spectroscopy

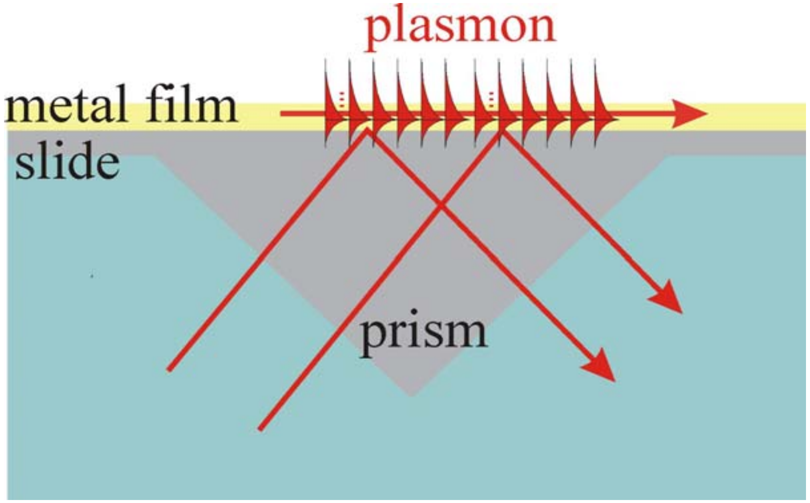


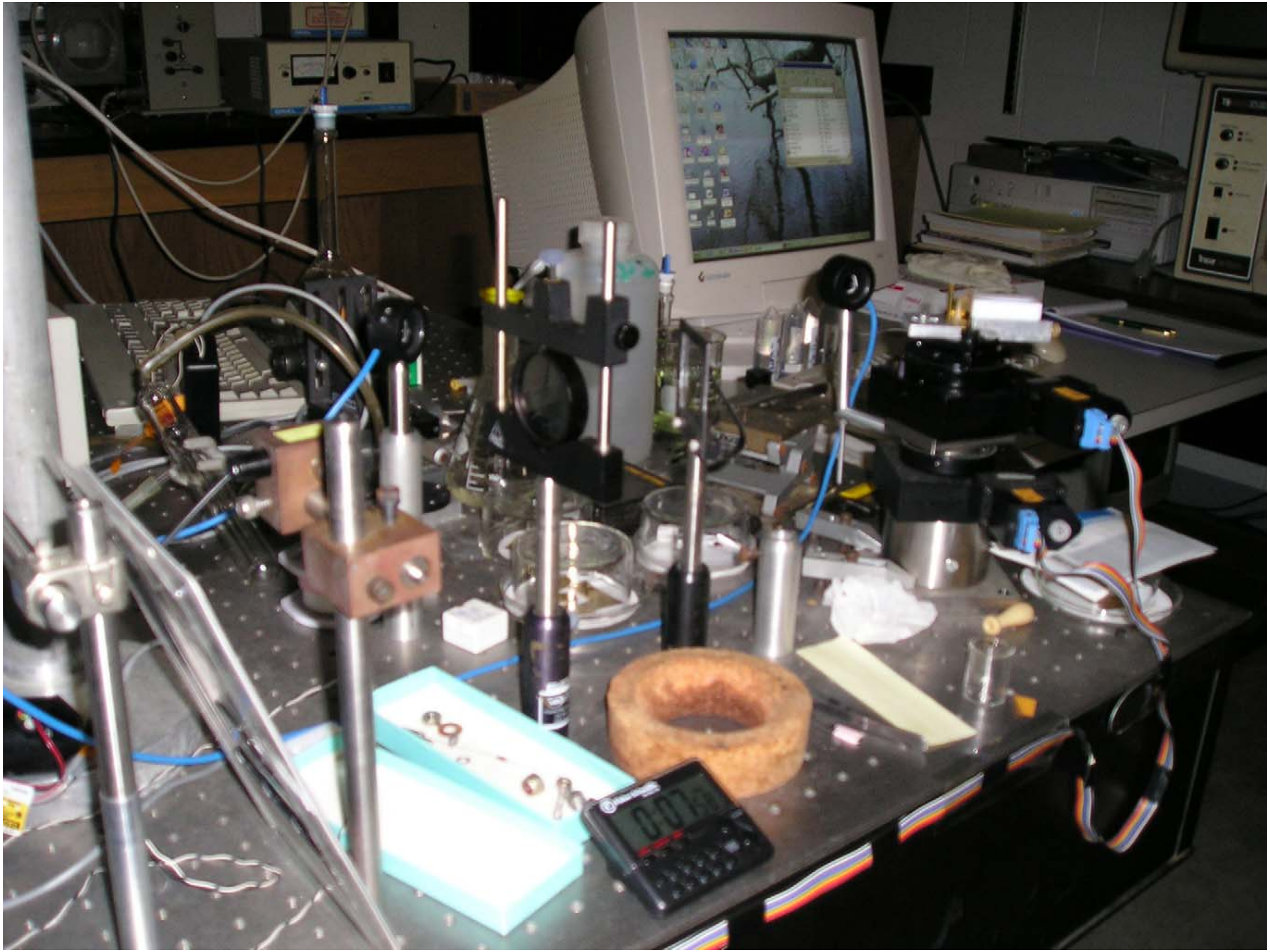
We are currently monitoring changes in the swelling of the theophylline imprinted particles using SPR

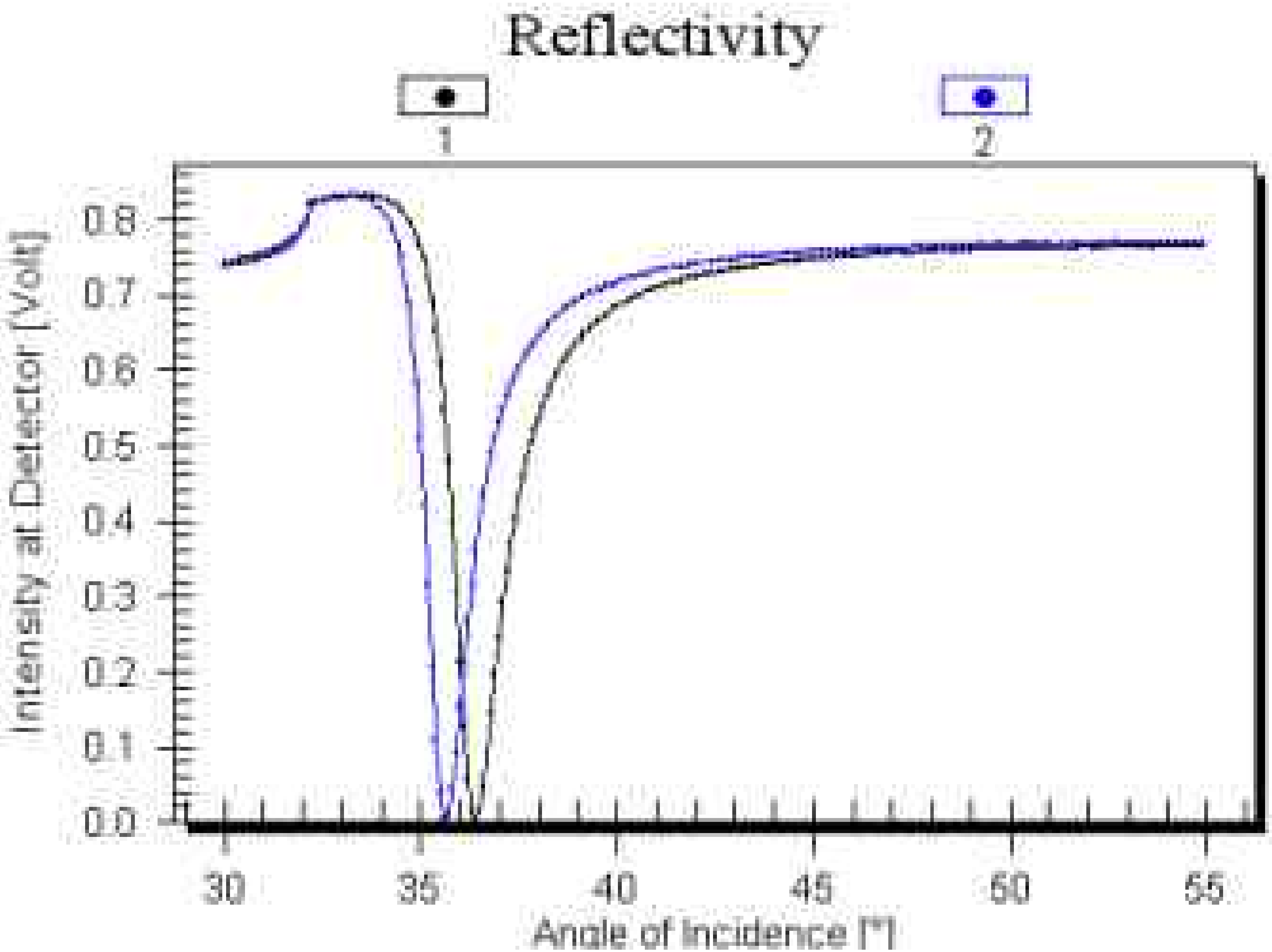
Prism allows for ATR coupling of the surface plasmon polartons.

SPR

- Surface plasmon is a coupled, localized transverse magnetic electromagnetic field / charge-density oscillation, which may propagate along an interface between two media.







Reflectivity
1
2
Intensity at Detector [Volt]
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
30
35
40
45
50
55
Angle of Incidence [°]

Preparation of Hydrogel Membrane

- Polymer microspheres are directly applied to the gold surface where they are held in place by electrostatic attraction.
- Encapsulation of the polymeric microspheres is achieved by micropipetting the hydrogel formulation onto the surface of the SPR substrate where it is distributed across the surface by a spatula prior to polymerization.

Summary of Results

Brewster angle computed using 15-point window Savitzky-Golay First Derivative Filter

Theophylline Concentration	**Brewster Angle**
Distilled Water	64.8 degrees
Caffeine (10^{-4} M)	64.8 degrees
10-^{7} M Theophylline	64.9 degrees
10^{-6} M Theophylline	65.1 degrees
10^{-5} M Theophylline	65.2 degrees

Future Work

- The swelling of the NIPA particles can be increased at room temperature by decreasing the amount of recognition monomer and template used in the formulation.
- A new SPR instrument will be constructed that has a sample chamber that will expose the gold to a larger amount of sample mitigating problems associated with alignment. Also, better stepping motors will be used to control the movement of the rotational stages allowing greater control over the change in the incident light angle.

Acknowledgements

- Dave Westover
- Environmental Protection Agency

  RD-830911101-0

This research is funded by
U.S. EPA - Science To Achieve Results (STAR) Program
Grant # RD-830911101-0