

NanoBio Sensors and Integrated Microsystems for Intelligent Food Packaging

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Outline

- *Intelligent Packaging Against Counterfeit Food and Pharmaceutical Products*
 - Scope of counterfeiting
 - Strategies against counterfeiting
 - Intelligent anti-counterfeit packaging
- *NanoBio Sensors*
 - Definition, Advantages, synthesis
 - Applications
- *Research on Sensor Based Anti-counterfeit Packaging*
 - Nanoparticles and nano-tagants: magnetic, conductive, photoluminescent
 - Conductive polymers
 - DNA markings



Counterfeit Foods and Pharmaceuticals

- *Counterfeiting is emerging as a brand-security issue for commodity consumables*
 - Deprives manufacturers of revenue
 - Harms brand integrity
 - In some cases, compromises safety
- *~ 5-7% of all goods sold worldwide are counterfeit → \$600B/yr¹*
- *Global counterfeit food threat is estimated at \$49 billion (2007)²*
- *In the European Union, seizures of fake foods and medicines were up by 80%³*
- *The most commonly counterfeited food products include nutraceuticals, coffee, baby formula, bottled water, soy sauce and other sauces*

1 IACC, <http://www.iacc.org/counterfeiting/counterfeiting.php>

2 PIRA, <http://www.pira.com/default.htm>

3 <http://www.sleeper.com/technologies/solution/brand-protection-and-anti-counterfeiting>

Forms of Food & Pharma Counterfeiting

- *Product substitution*
 - Diners ordering high-valued seafood replaced with low-valued counterpart
- *Ingredient substitution*
 - Medicine with less active ingredients or infant formula with reduced nutritional benefits
- *False labeling*
 - Fake country-of-origin labels, expiration date, or method of growing (e.g. organic foods)
- *Up-labeling*
 - Low quality version of a highly priced product (e.g. olive oil)
- *Down-labeling*
 - High-valued item declared as lower-valued to avoid import taxes

Risks Associated With Counterfeit Foods

- *Harm to public health*
 - Consumers can be exposed to poisons, toxins and allergens, or products of a reduced nutritional and medicinal value
- *Harm to producers*
 - Products can lead to recalls and economic loss
 - Undermine public trust in the global food system and in specific brands and product categories
- *Harm to homeland security*
 - Counterfeiting could potentially fund terrorist activities

Anti-Counterfeit Measures in the US

- *Counterfeit Alert Network – Created by FDA in February 2004*
 - A coalition of health professional and consumer groups
 - When there is a confirmed counterfeit case in the United States, FDA sends an alert to partners
 - The agency also sends partners a notice if a counterfeit incident is confirmed elsewhere in the world that could affect U.S. patients
- *Food and Drug Administration Amendments Act of 2007 (FDAAA) (Public Law 110-85) was signed into law on September 27, 2007*
 - Drug regulation

Strategies to Fight Counterfeiting

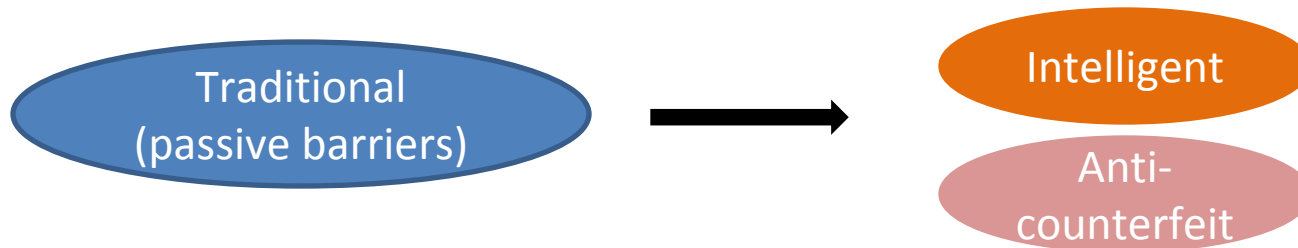
- *Interception*
 - Use investigators to find fake products and give leads on their origin to law enforcement
 - *Authentication*
 - Help consumers pick authentic goods by educating them regarding product authenticity features, such as special labels
 - *Secure Supply Chain*
 - Track goods through supply chain to prevent counterfeit goods from being introduced, through such technologies as UPC codes or RFID
- *Our research is focused on nanotechnologies for Authentication and Supply Chain Biosecurity strategies.*

Design Criteria for Anti-Counterfeit Technology

- *Easy for brand owners and their investigators to identify genuine goods*
 - Help consumers pick authentic goods
 - Overt: visible to the naked eye and to the consumer
 - Covert: not visible to the naked eye or consumer, but readily noticed in field with portable sensors
 - Forensic: lab analysis is required to determine authenticity
- *Difficult for counterfeiters and crooks to know how that is being done*

Intelligent Anti-Counterfeit Packaging

- *Roles of food packaging*
 - Protection and preservation from external contamination
 - Containment
 - Convenience
 - marketing and communication
 - Traceability
 - tamper indication
 - portion control
- *From traditional packaging to intelligent anti-counterfeit packaging*
 - Packaging with enhanced functions
 - Responds to concerns for food safety and counterfeit products



Intelligent Anti-Counterfeit Packaging

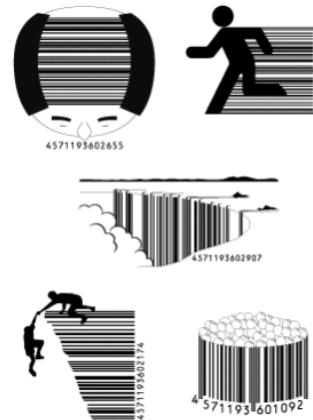
- [Active packaging](#): A packaging system that actively changes the condition of the package to extend shelf life or improve food safety or sensory properties, while maintaining the quality of the food
- [Intelligent packaging](#): A packaging system that is designed to monitor and communicate information about the packaged product
 - Functions of intelligent packaging
 - Track products
 - Sense environment
 - Communicate with people



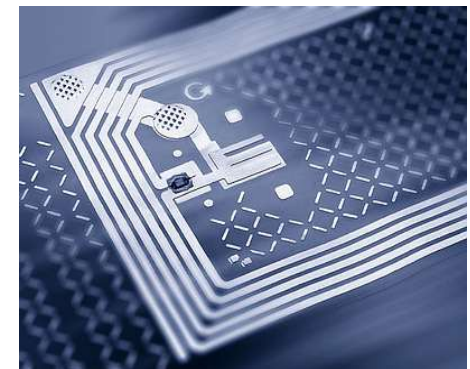
Intelligent Anti-Counterfeit Packaging

- *Two basic types of intelligent anti-counterfeit packaging*
 - Data carriers
 - Barcodes
 - Radio frequency identification tags (RFID)
 - Package indicators
 - Time-temperature indicators (TTIs)
 - Freshness indicators
 - Gas indicators
 - Sensors

Barcodes



RFID



Intelligent Anti-Counterfeit Packaging

- *Time-temperature indicators (TTIs)*
 - Ensure food safety
 - Use TTI labels to monitor critical control points (time and temperature) at specific locations
 - Based on mechanical, chemical, electrochemical, enzymatic or microbiological changes



TTI label (Ciba OnVu)

Intelligent Anti-Counterfeit Packaging

- *Freshness indicators*

- According to USDA, dates stamped on packages may not be safety dates.
- Ensure food safety
 - Provide direct product quality information resulting from microbial growth or chemical changes within a food product
 - Based on indicator color change in response to microbial metabolites produced during spoilage



Freshness indicator

Intelligent Anti-Counterfeit Packaging

- *Gas indicators*
 - Monitor the quality and safety of food products
 - Respond to changes in gas composition inside packages due to leaking or tampered packages, microbial spoilage (a kind of freshness indicators), etc
 - Most common gas indicators: oxygen indicators and carbon dioxide indicators



Oxygen Indicator Tablets (Tell-Tab)

Intelligent Anti-Counterfeit Packaging

- *Sensors*

- Sensors vs. indicators

- Sensors comprise receptor and transducer components, while indicators communicate information through direct visual change

- Gas sensors

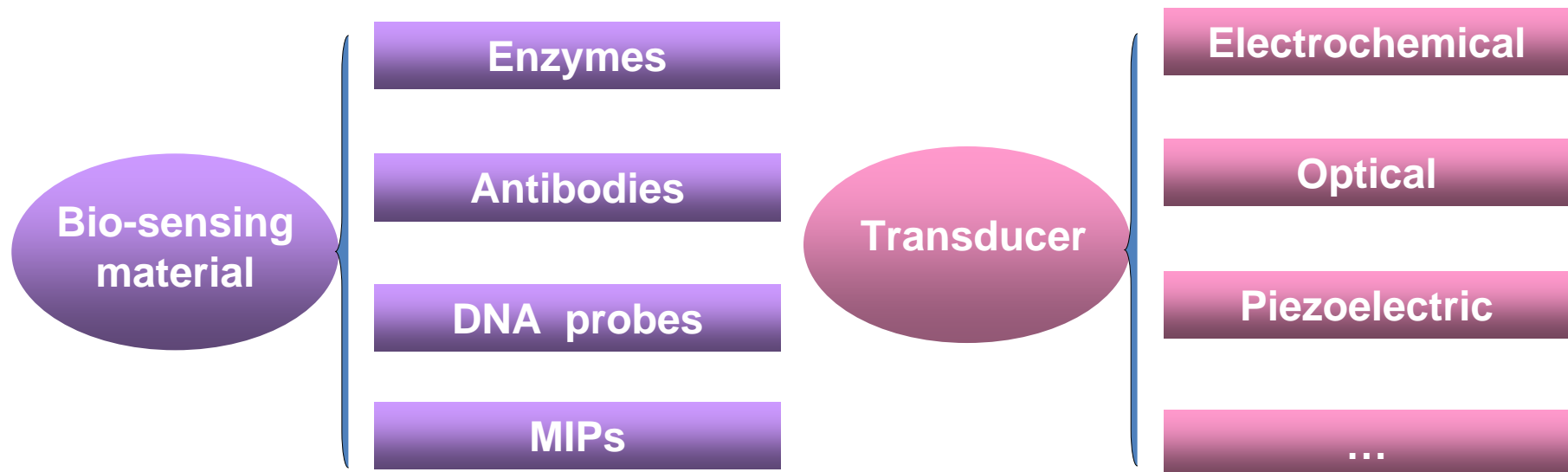
- Respond reversibly and quantitatively to the presence of a gaseous analyte by changing the physical parameters of sensors and are monitored by an external device
 - e.g. amperometric oxygen sensors (electrochemical sensors), fluorescence oxygen sensors (optical sensors) and piezoelectric crystal sensors



OxySense® OxySentry System

NanoBio Sensors

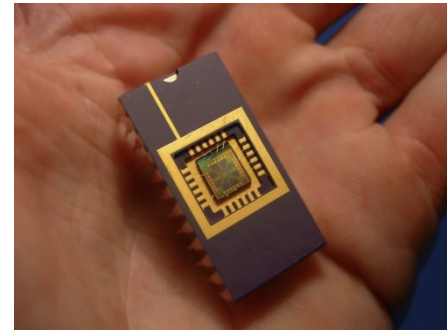
- *What is a NanoBio Sensor?*
 - Biosensor
 - Definition: a device or instrument comprising a biological sensing material coupled to a chemical or physical transducer which converts a biological, chemical, or biochemical signal into a quantifiable and processable electrical signal.



NanoBio Sensors

- *Advantages of a biosensor*

- High selectivity
- High sensitivity to targets
- Portability
- Reduced detection time
- Ability for continuous monitoring of a specific analyte
- Minimization of the perturbation of the sample
- Relatively lower cost compared with some conventional methods or devices



- *Applications*

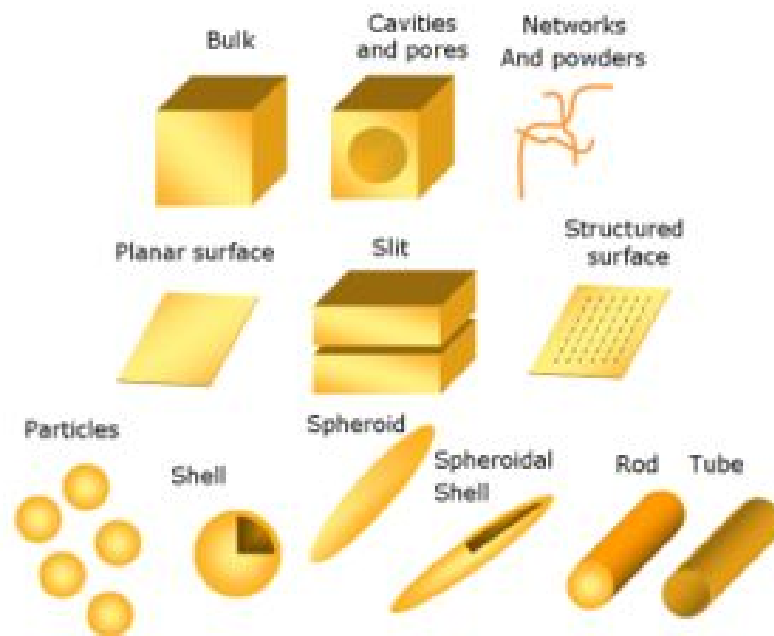
- Biodefense and countermeasures, clinical diagnosis, detection of biological and chemical agents in agriculture, food and drug industries, environment monitoring, and control

NanoBio Sensors

- *Diagnostic tools*
 - Rapid results, highly sensitive, simple to use, inexpensive, and portable
 - Nanomaterials
 - Nanoparticles, nanotubes, nanowires/nanorods, etc
 - High aspect ratios and large surface area result in unique properties
 - Nanoscale transducers
 - Nanochips
 - Increase the contribution immobilized biomolecules has on the measured properties
 - Enhances sensitivity

NanoBio Sensors

- *Nanostructures with optical, electronic and catalytic properties*
 - Characteristics of nanostructures depend on their size and shape



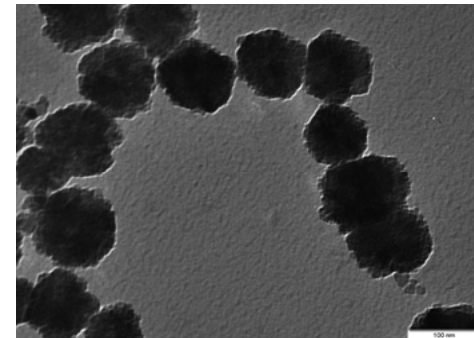
Nanostructure geometries

Nanotechnologies in Food Packaging

- *Barrier and mechanical properties*
 - e.g. Use clay nanoparticles to improve plastic packaging for food products
 - e.g. Nanolayer of aluminum coats interior of food packages
- *Detection of pathogens*
 - *Smart packaging with food safety and quality benefits*
 - e.g. Antimicrobial packaging
 - *The market for nanotechnology in food packaging was expected to reach \$360 million in 2008*

Synthesis and Assembly of NanoBio Sensors

- *Natural and biogenic nanomaterials*
- *Chemically synthesized nanomaterials*
 - Solvent extraction/evaporation
 - Crystallization
 - Self-assembly
 - Layer-by-layer deposition
 - Microbial synthesis
 - Biomass reactions
- *Nanotransducers*
 - Use sputtered or evaporated metal films to fabricate a nanoband electrode
 - Etching of thin wires to generate one individual nanoelectrode
 - Depositing metallic layers through nanoporous polymeric membranes to provide arrays of nanoelectrodes (E-beam lithography)
 - Self-assembly: bottom-up approach

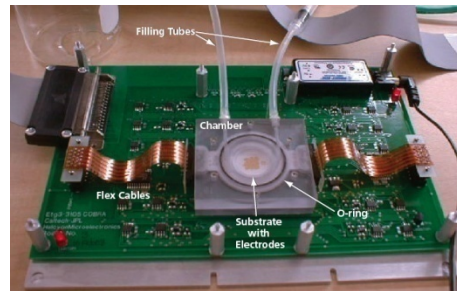


Applications of NanoBio Sensors

- *Detection of pathogens, chemicals, and toxins in food*
 - Real-time and label-free detection of toxic chemicals
 - Nano-Biosensors for detection of food-borne pathogens
- *Detection of gases*
 - Luminescent nanobeads for optical sensing and imaging of dissolved oxygen (Borisov and Klimant, 2009)
 - PDMA nanoparticles based fluorescent ratiometric nanosensors for dissolved oxygen (Cao et al., 2004)

NanoBio Sensors

- *DNA biochips*
 - DNA and carbon nanotube serve as sensing element and transducer, respectively, to detect odors and tastes
- *Electronic tongue nanosensors*
 - Detect substances in parts per trillion (ppt), could trigger color changes in food packages when food is spoiled



- *Limitations of NanoBio Sensors for food packaging*
 - High development and production cost
 - Safety considerations from industry and consumers
 - Strict industry specifications
 - Relatively limited demand

NanoBio Sensors for Intelligent Anti-Counterfeit Packaging

- *Our Research Objective*

- Develop a three-tiered sensor system against counterfeiting (SAC)
 - Tier 1: consumers
 - Tier 3: Manufacturers
 - Tier 2: transport, customs agents and retailers
- Identify and evaluate the most viable short and long term solutions regarding anti-counterfeit
- Evaluate and develop the most promising technologies
- Specifically explore the development of tier 3 SAC systems

- *Innovations*

- Nanoelectronic sensing
 - Advantages: Quantum effect, low noise, high accuracy
- Nano taggant or tracer for covert product authenticity identification

Tier 1 Sensor Based Anti-counterfeit Packaging

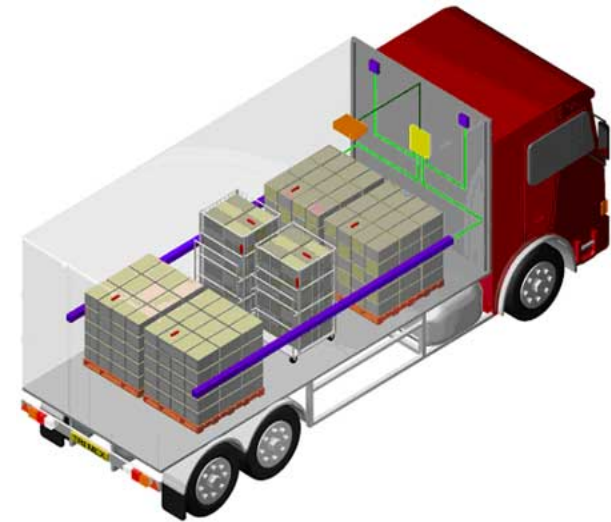
- *Public recognition features visible to the naked eye*
 - Commercially available technologies
 - Water markers
 - Holograms, stickers, shrink sleeves, hotstamping, packing films, masters, scratches, pouch, tapes
 - Holograms have been copied by many counterfeiters
 - Integrity indicators and sensors
 - Conductive polymer based amperometric sensors
 - Luminescent oxygen nanosensors
 - Fluorescent ratiometric nanosensors



Holographic packing films

Tier 2 Sensor Based Anti-counterfeit Packaging

- *RFID-based deformation sensors*
 - Piezoelectric sensors
 - Electrochromic sensors
 - Organic-based chemical sensors
 - Invisible pigments which can be detected with a simple hand-held device
- *Concerns of RFID chips*
 - Cost
 - At risk of being reprogrammed
 - Labels can be diverted and put on counterfeits

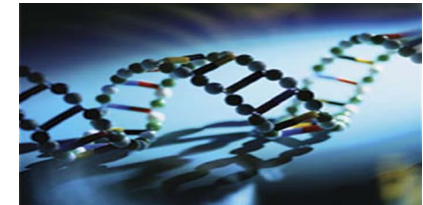


RFID enabled truck

Tier 3 Sensor Based Anti-counterfeit Packaging

- *Nanotechnology based sensor systems*

- DNA markings
- Nano-particle taggants
 - Multicolored nanoparticles added to the product
- Nano-crystal semi-conductor pigments
- Sensors
 - NMR sensor
 - Dynamic sensor systems stimulated by a u



DNA

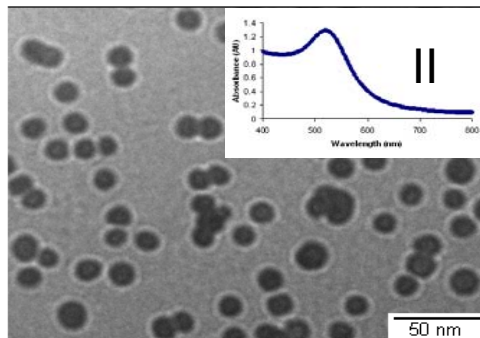


Quantum dot

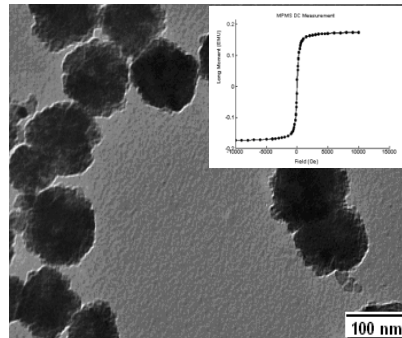
- *Molecularly imprinted polymer (MIP) based sensor systems*

- Molecular templates used as authentication signature

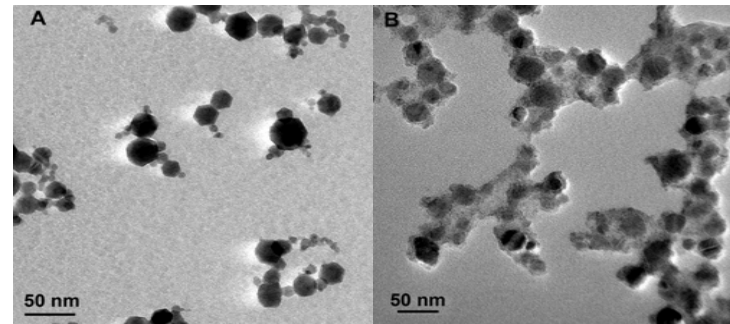
Tier 3: Nanoparticles as Taggants



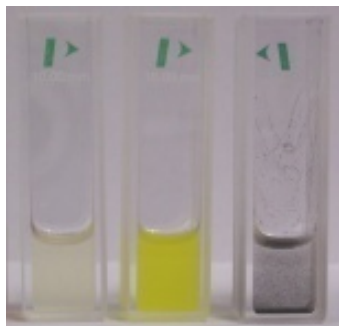
TEM image of AuNPs ~15 nm in diameter. Inset: Absorbance spectrum of AuNPs at 520 nm.



TEM image of MNPs ~100 nm in diameter. Inset: magnetic hysteresis, saturation at 74.6 emu/g.



TEM images of (left) unmodified Fe₂O₃ nanoparticles and (right) electrically active magnetic nanoparticles.



Nanoparticle tracers (~10 nm) in solution. Left to right: ZnS, CdS, and PbS.

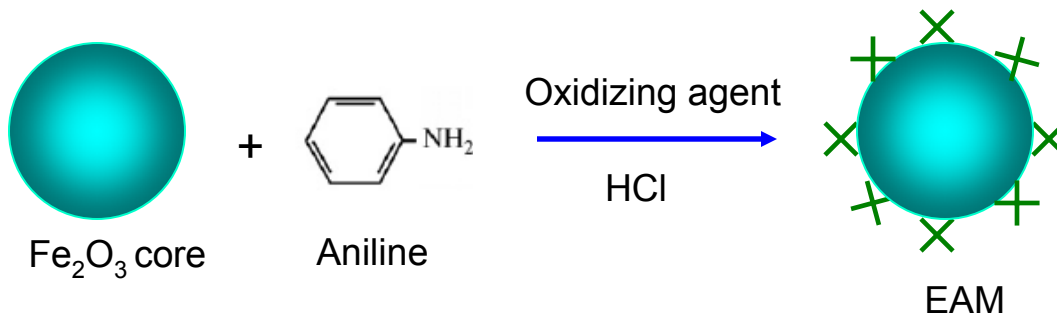


TEM image of biogenic AuNPs ~30nm (left) and AuNPs in solution (right). Inset: absorption spectra of AuNP in solution over time.

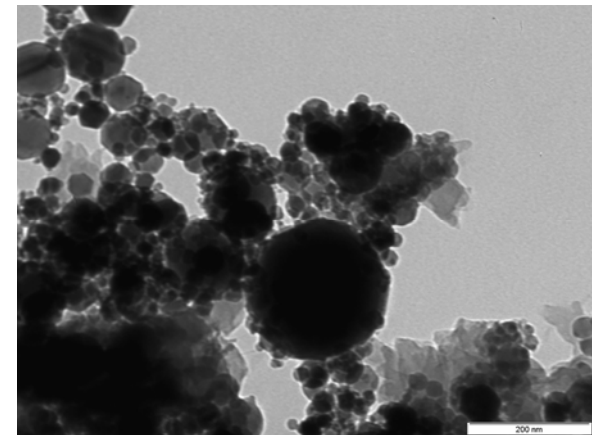
Unique combination of nano-taggants can be embedded in packaging materials for overt and covert signatures for identification. Taggants can be read with a simple photo-detector or voltmeter.

Electrically Active Magnetic (EAM) Nanoparticles

General approach:



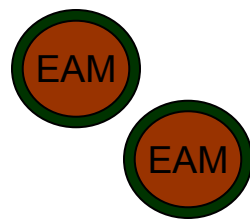
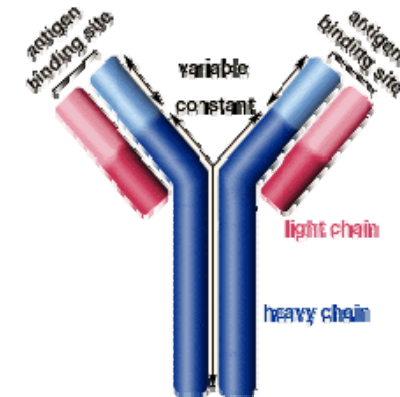
- Unique electronic structure and flexible electrical properties of protonated polyaniline
- Magnetic properties from the core
- Simple and low cost of preparation
- Excellent environmental stability



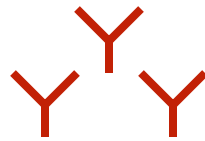
TEM image of EAM Nanoparticles

Nano-EAM to Detect Targets

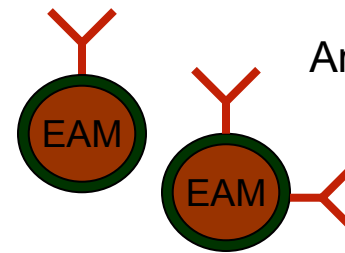
- **Method: Physical adsorption** (Muhammad Tahir et.al, 2003)
 - Combine EAM nanoparticles with monoclonal antibodies to target (e.g. E.coli O257:H7)
- **Conditions**
 - Room temperature incubation
 - Time – 1 hr



+



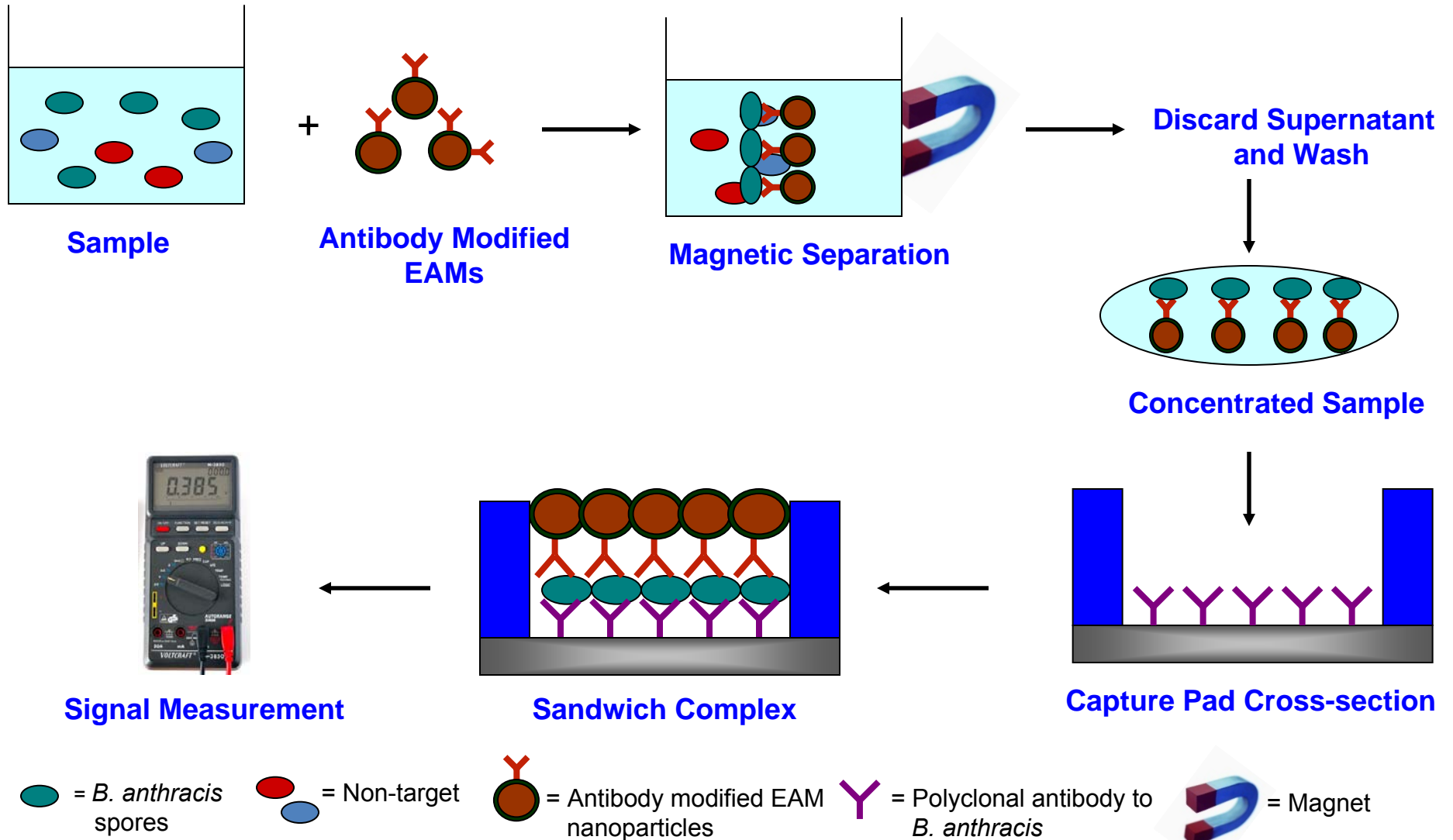
Monoclonal antibody to
B. anthracis



Antibody Modified EAMs

Antibody structure

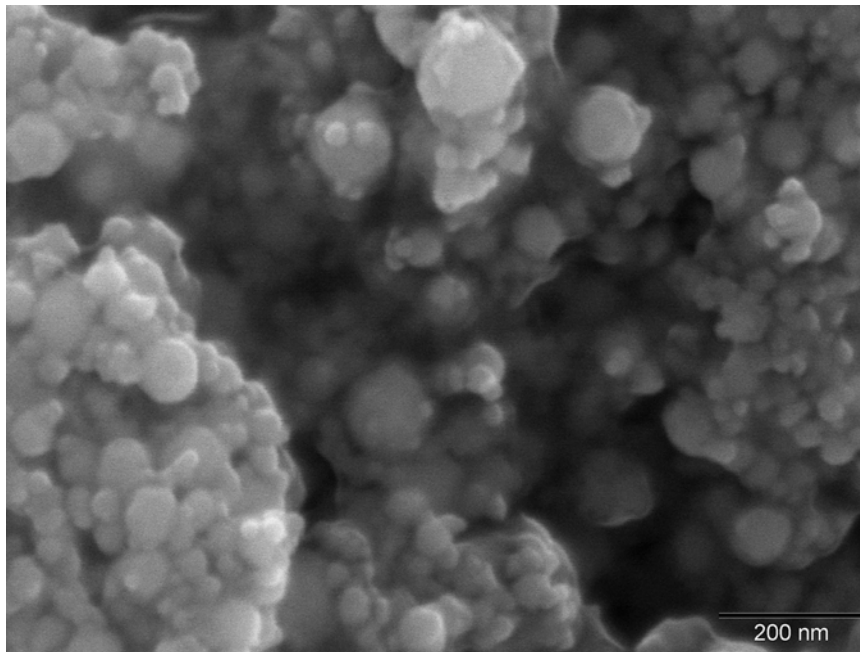
Detection Scheme



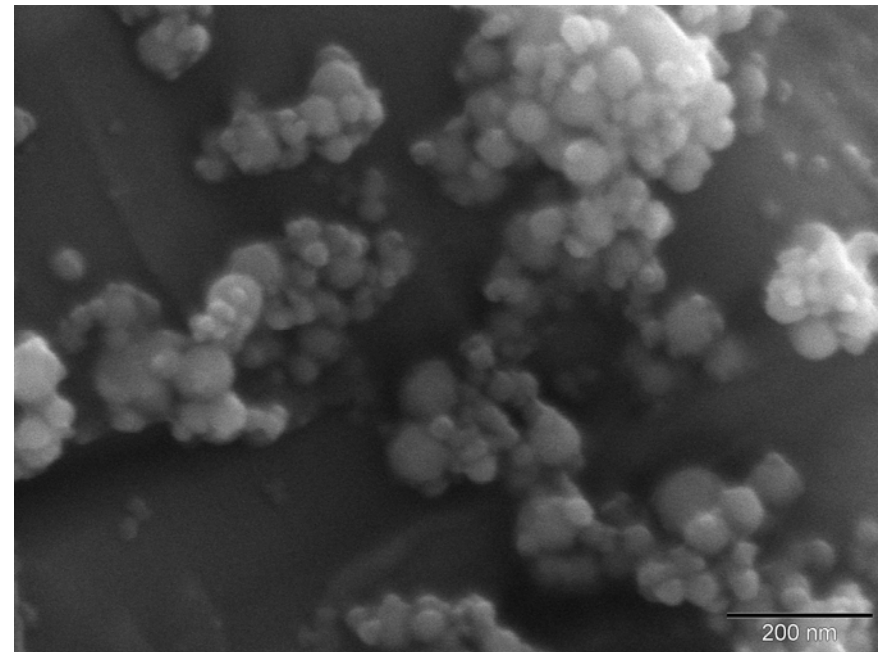


Characterization of EAM

Scanning Electron Microscopy Images



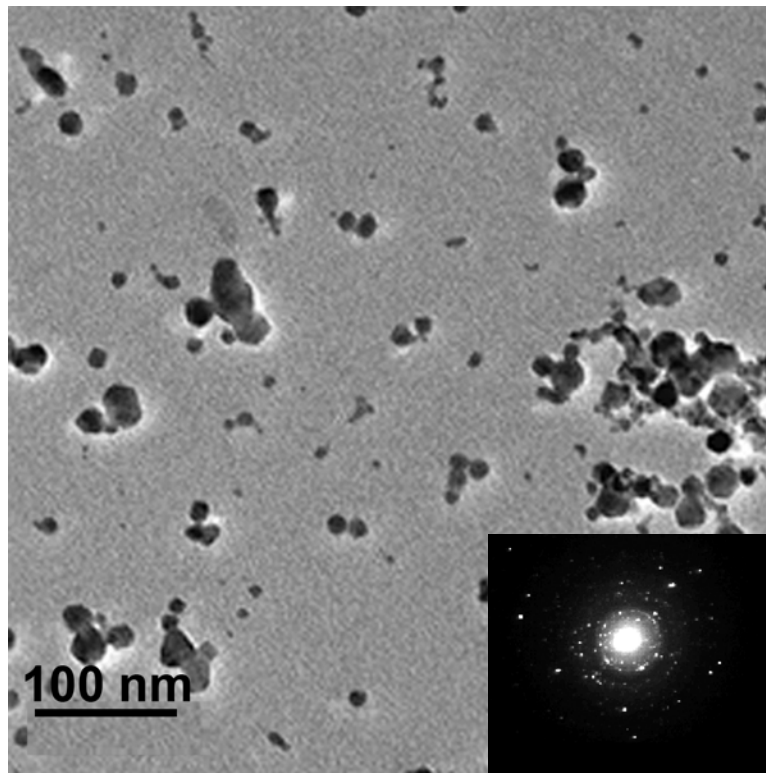
EAM Nanoparticles (1:0.4)



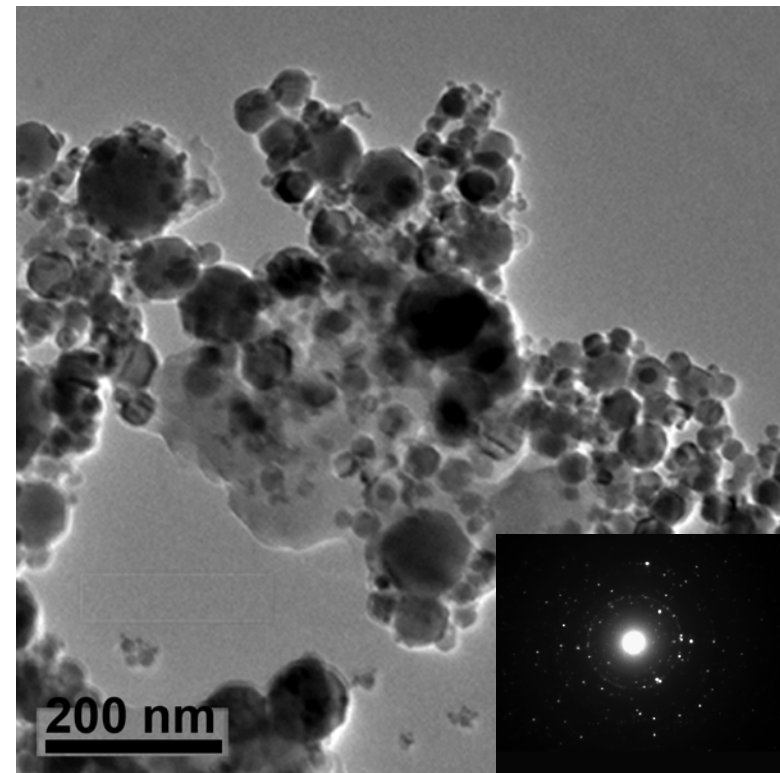
Iron oxide Nanoparticles

Characterization of EAM

Transmission Electron Microscopy and Electron Diffraction Images



1:0.1 EAM NPs



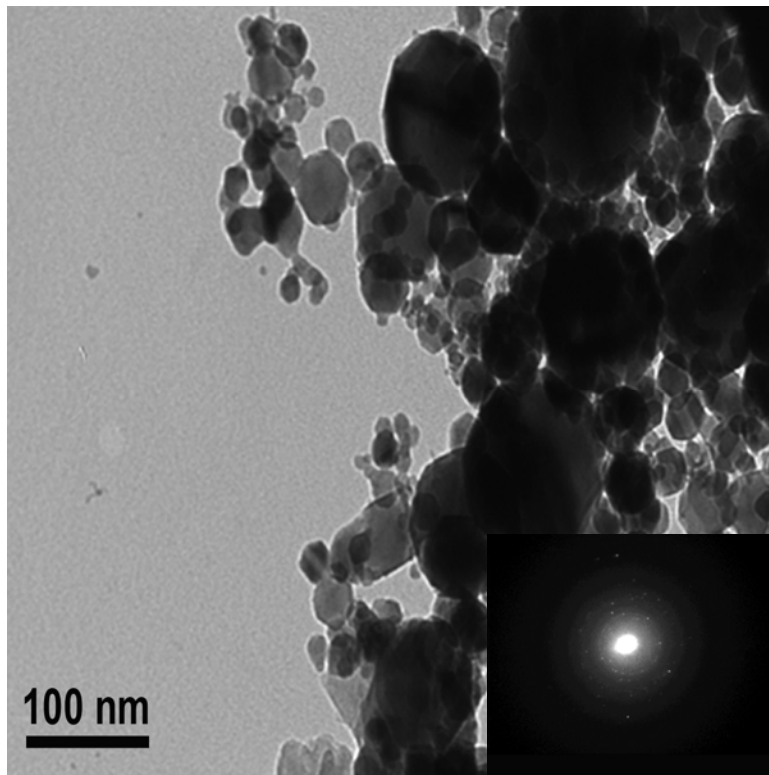
1:0.4 EAM NPs

XRD shows EAM is crystalline

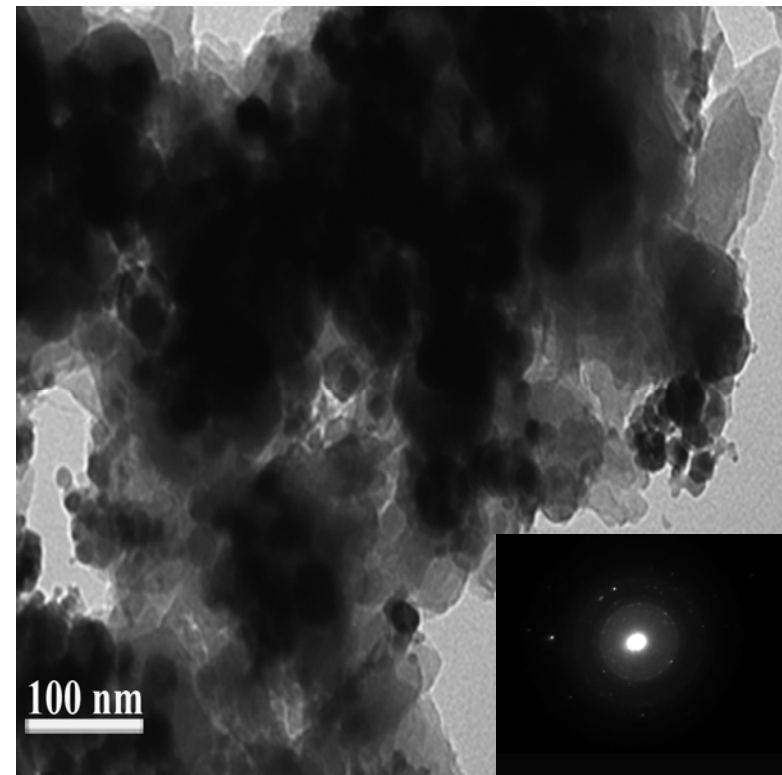


Characterization of EAM

Transmission Electron Microscopy and Electron Diffraction Images



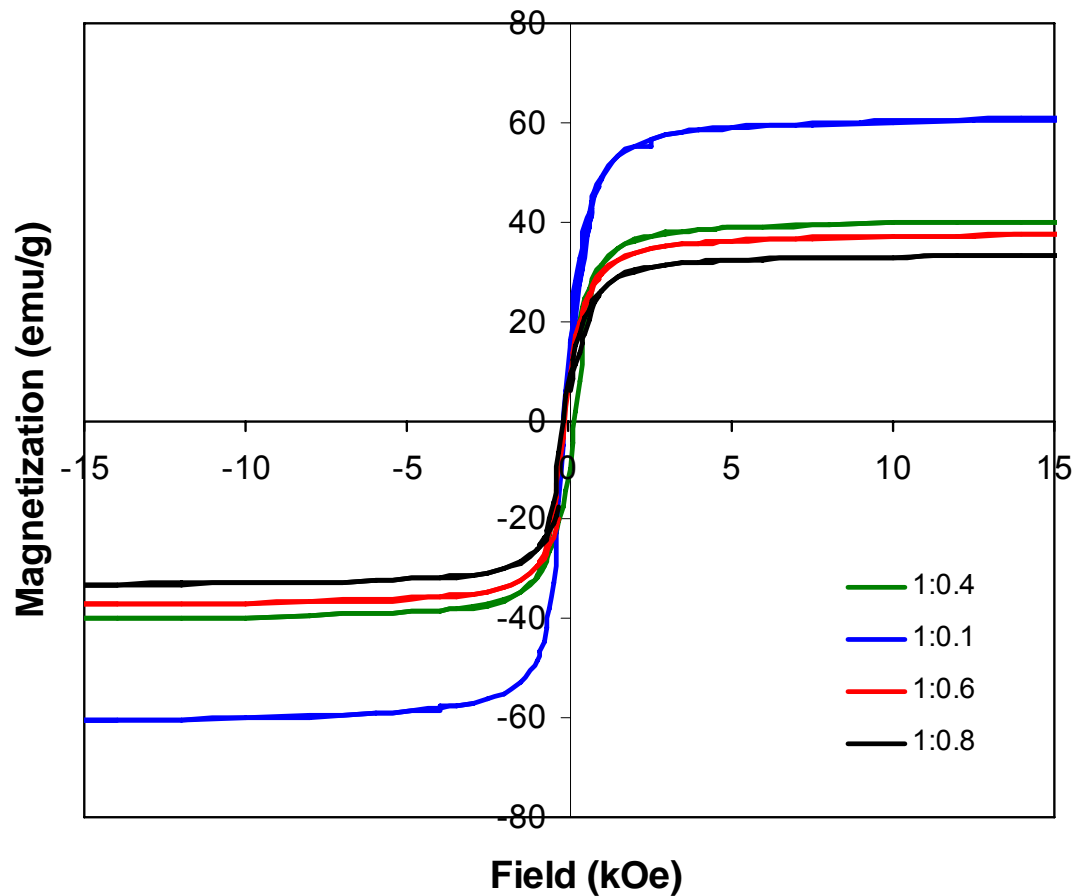
1:0.6 EAM NPs



1:0.8 EAM NPs

Magnetic Measurements

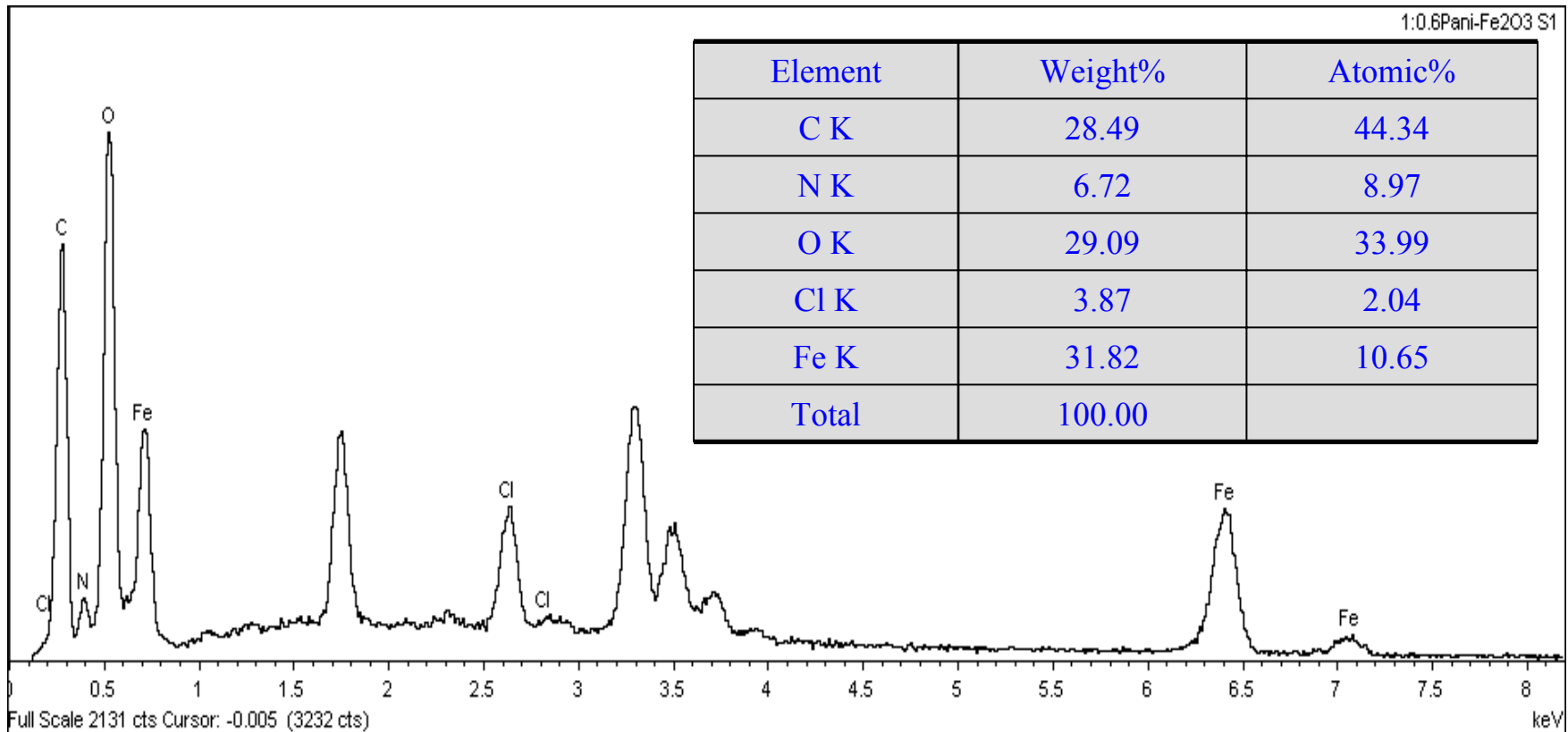
Experimental hysteresis measurements at 300K



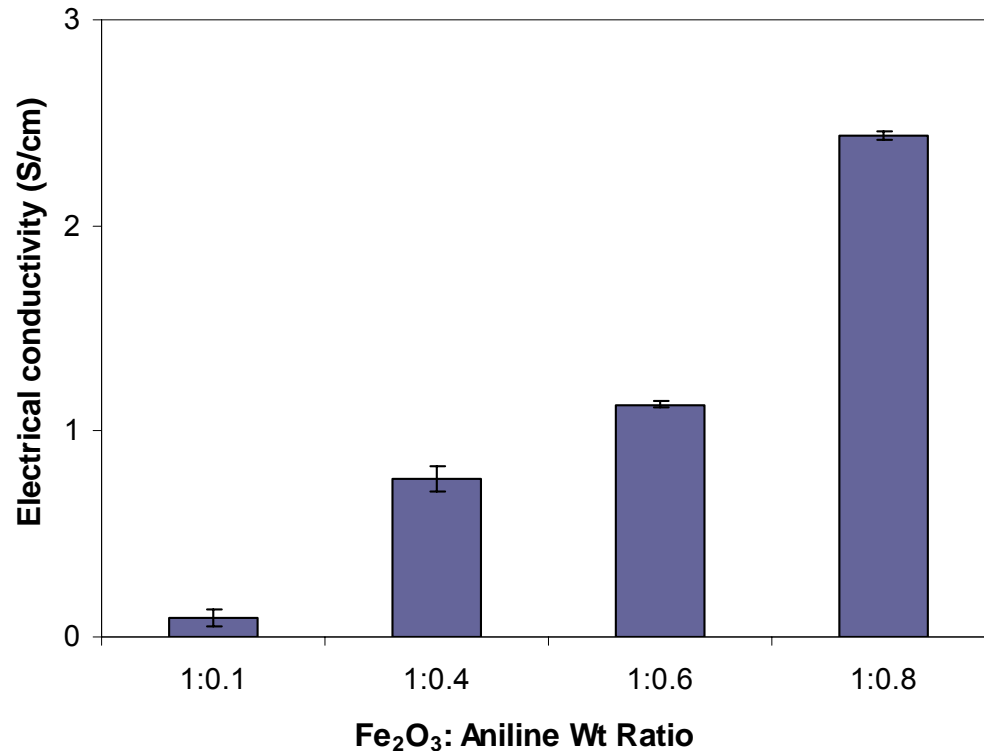
γ -Fe₂O₃ nanoparticles: aniline monomer weight ratio was varied as 1:0.1, 1:0.4, 1:0.6, and 1:0.8.

Energy Dispersive Spectroscopy

1:0.6 EAM Nanoparticle



Electrical Conductivity



Four point probe measurements in compressed pellets of 2000 microns in thickness.

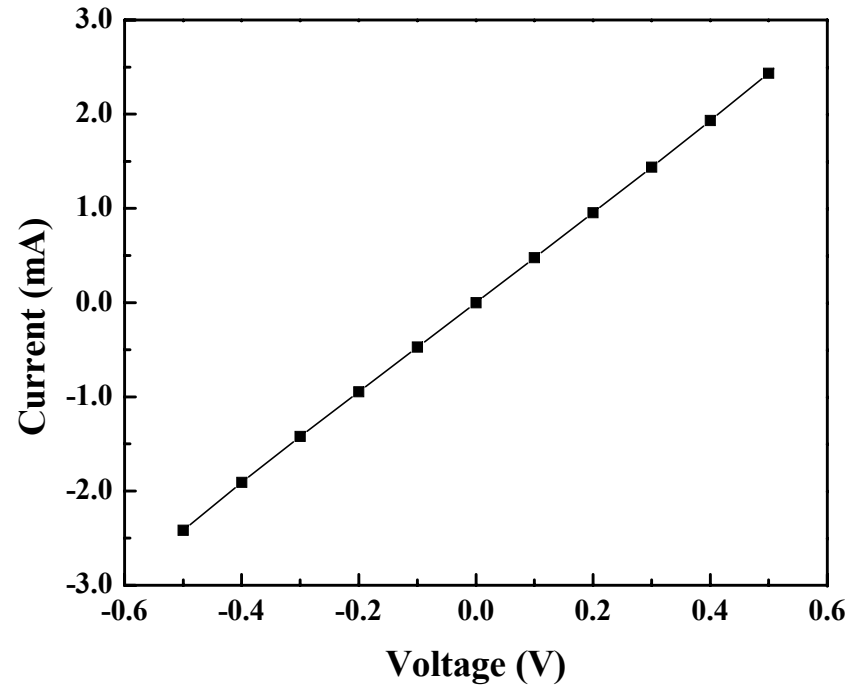
γ -Fe ₂ O ₃ : Aniline Wt. Ratio	Conductivity (S cm ⁻¹)
1:0.1	0.092
1:0.4	0.768
1:0.6	1.129
1:0.8	2.436
1:0.0	0.000017

Nano-EAM Characterization

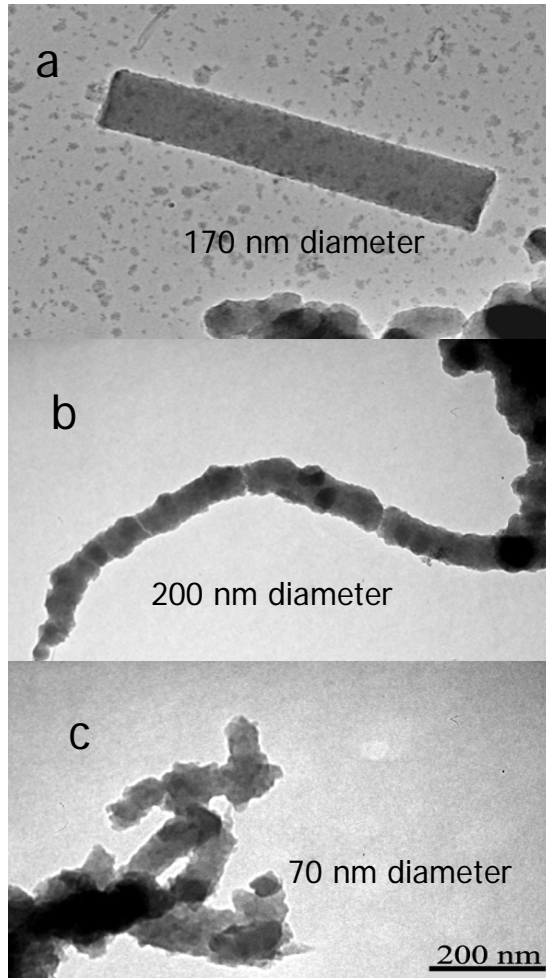
Nano-EAM follows ohmic behavior.

Ohm's law:

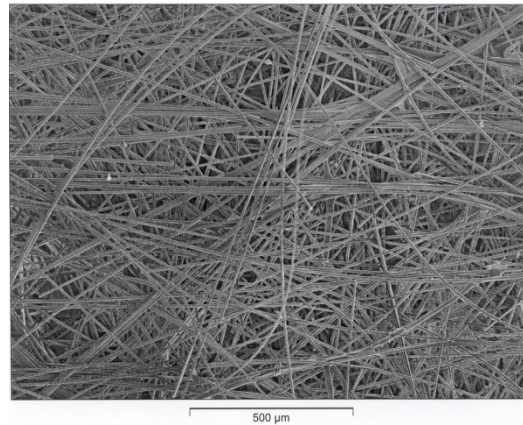
$$I = V/R$$



Tier 3: Conductive Organic Nanowires



(a, b, c) TEM images of synthesized polyaniline nanowires.



TEM image of synthesized polyaniline nanowire bundle.



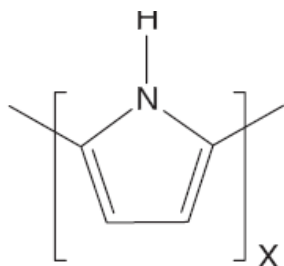
TEM images of carbon nanotube.

Nanowires can be embedded in packaging materials for overt and covert signatures for identification. Nanowires can be read with a simple voltmeter. These nanowires can be enhanced with luminescent particles and then they can be read with a simple photo-detector.

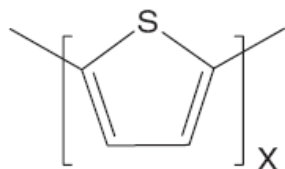


Conductive Organic Nanowires

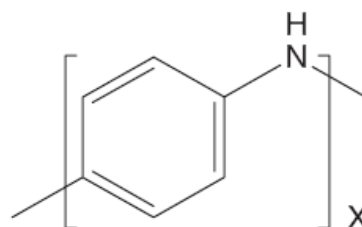
- π – conjugated system
- *electrochemical properties*
 - High electrical conductivity
 - High electron affinity, low ionization potential
 - Optical properties
- *Intrinsic electronic conductivity: 10^{-14} to 10^2 S cm⁻¹*



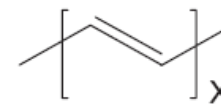
Polypyrrole



Poly(Thiophene)



Poly(Aniline)



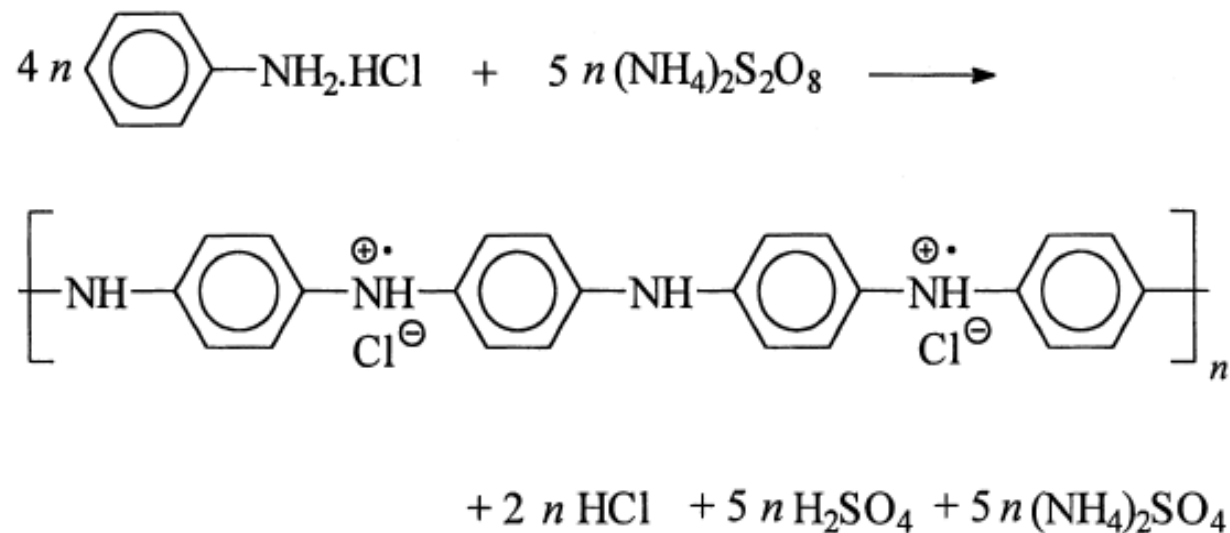
Poly(Acetylene)



Polyaniline Synthesis

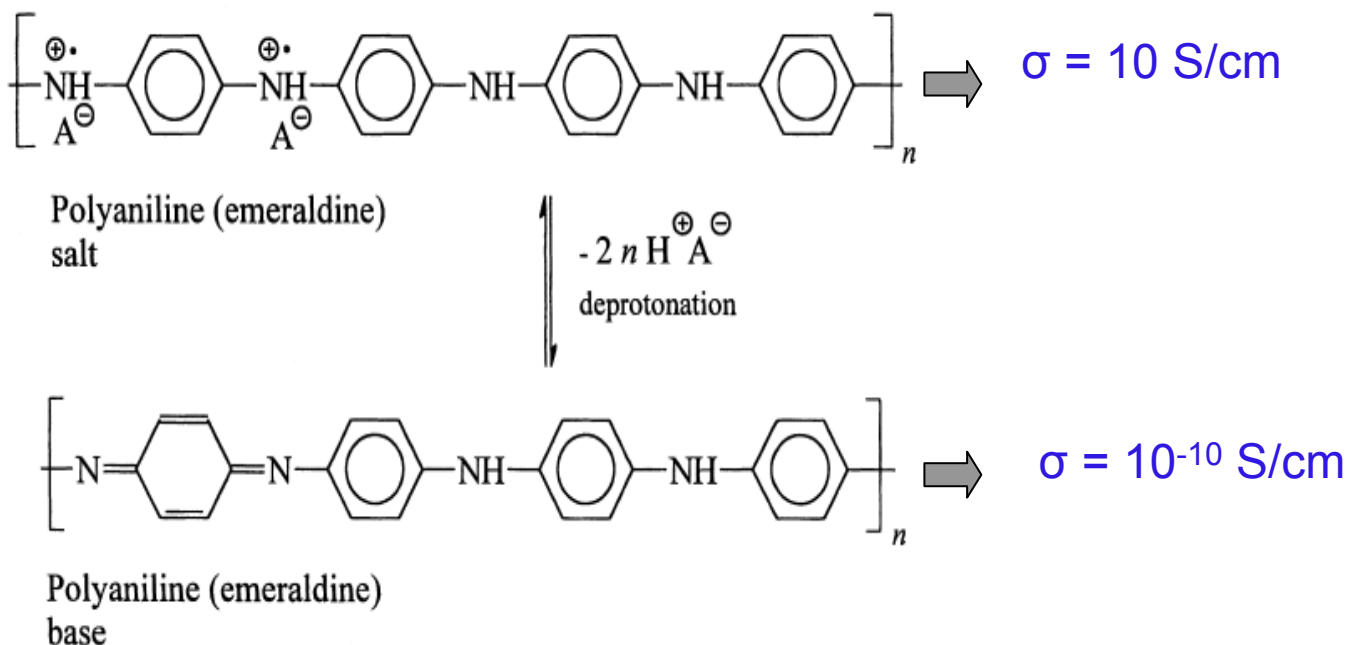
- *Aniline monomer*

- Chemical oxidation in acidic aqueous environment



Polyaniline Electronic Conduction

- *Origin of electrical conduction ~ doping*
- *Electronic structure and the electrical properties of polyaniline can be reversibly switched*





Polyaniline Nanowires

- *Suitable immobilization matrix for biomolecules*
 - Physical adsorption
 - Entrapment
 - Cross-linking
 - Covalent bonding
- *Direct deposition on electrode surfaces*
- *Efficient transfer of electric charge from biochemical reactions to electronic circuits*
- *Room temperature stability*
- *Easy and versatile synthetic procedure*

Tier 3: Molecularly Imprinted Polymers (MIPs)

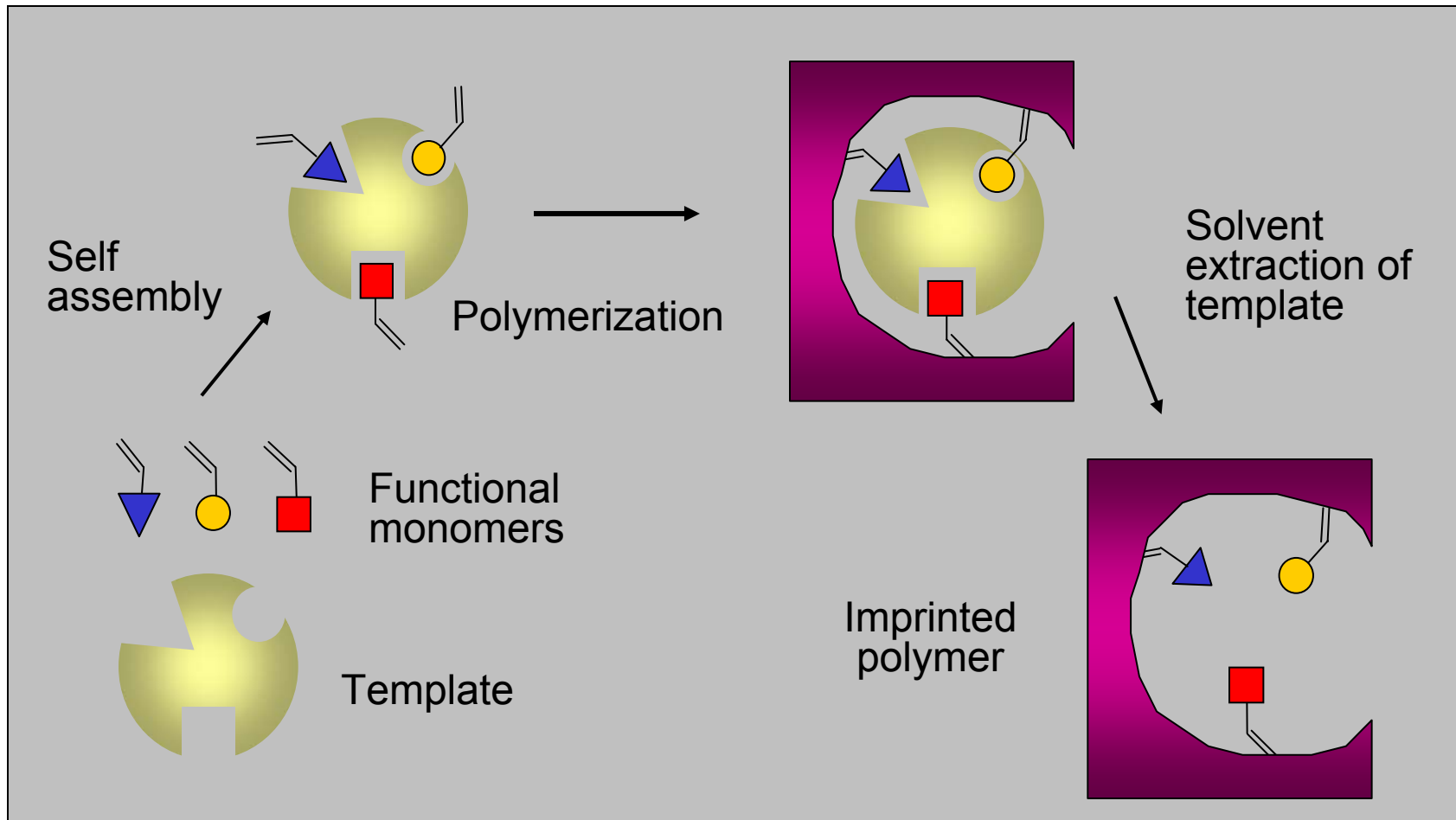
- *Technique of creating recognition sites in polymeric materials*
- *Functional monomers self-assemble around the template and get crosslinked to each other*
 - **molecularly imprinted polymer (MIP)**
- *Template molecule is removed from the matrix leaving behind a cavity complementary in size and shape to the template.*
- *Obtained cavity work as a selective binding site for a specific template molecule*

Advantages of MIPs

- *Stable and robust*
- *Able to withstand extreme temperatures and pHs*
- *Can be produced for almost any molecule*
- *Low cost to produce*
- *Long shelf life*



Overview of Molecular Imprinting



3 Imprinting Approaches

- *Covalent*
 - reversible covalent bond is formed between a print molecule and functional monomers/groups
 - chemical linkages include boronic esters, Schiff bases and ketals (functional group of a carbon bonded to two OR groups -- O is oxygen and R represents any alkyl group)
- *Non-covalent – prevalent in biological materials*
 - Hydrogen bond
 - ionic interaction
 - hydrophobic interactions
- *Semi-covalent*
 - reversible covalent bond in the imprinting step
 - non-covalent interactions in the rebinding step



Typical Imprinting Mixture

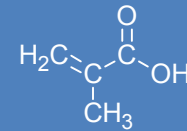
- *Print molecule*
- *One or more functional monomers*
- *Cross-linking monomer*
- *Porogenic solvent*
- *Initiator - to induce the polymerization*
 - UV
 - heat

Imprinting Process

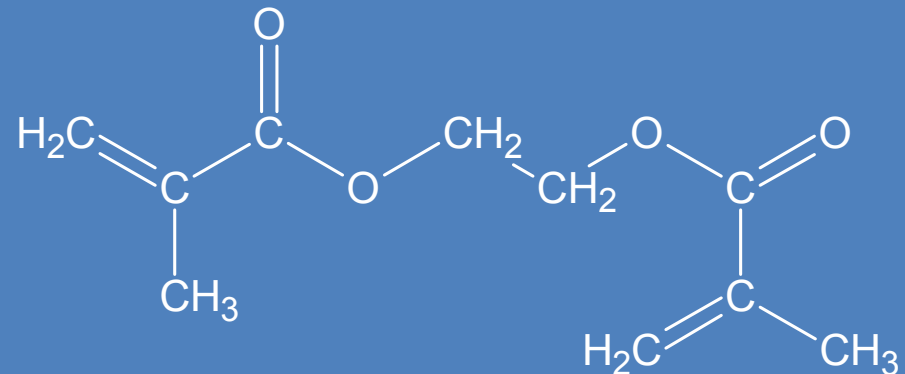
- *Functional monomers → form a stable complex with the print molecule prior to, and during, the cross-linking process*
- *Polymer monolith obtained is normally ground and sieved to the desired particle size*
- *Print molecule removed by simple solvent extraction*
- *Imprinted polymer particles are then ready for rebinding of the target molecule used as the template*
- *Structure and functionality of the print molecule determine selection of appropriate functional monomer*

Example

- *Methacrylic acid (MAA)*
– *functional monomer*
- *Ethylene glycol dimethacrylate (EGDMA)* – *crosslinking monomer*



EGDMA



Example, cont'd.

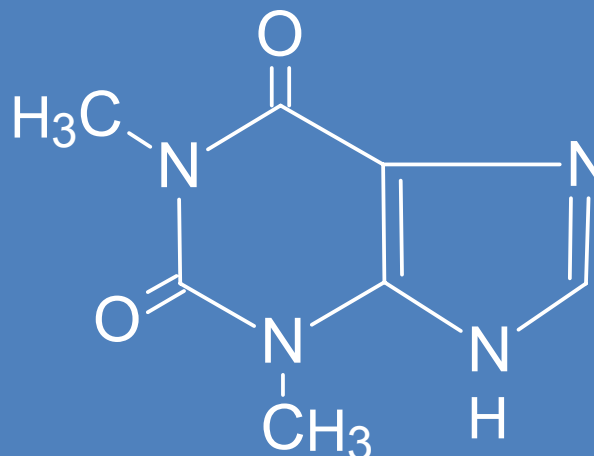
- *Dimethylformamide*
- *2,2'-Azobisisobutyronitrile (AIBN)*
- *3-(trimethoxysilyl)propyl methacrylate (3-MPS)*
- *Indium tin oxide coated glass slide*
- *Silicon wafer (p-type)*



Example, cont'd.

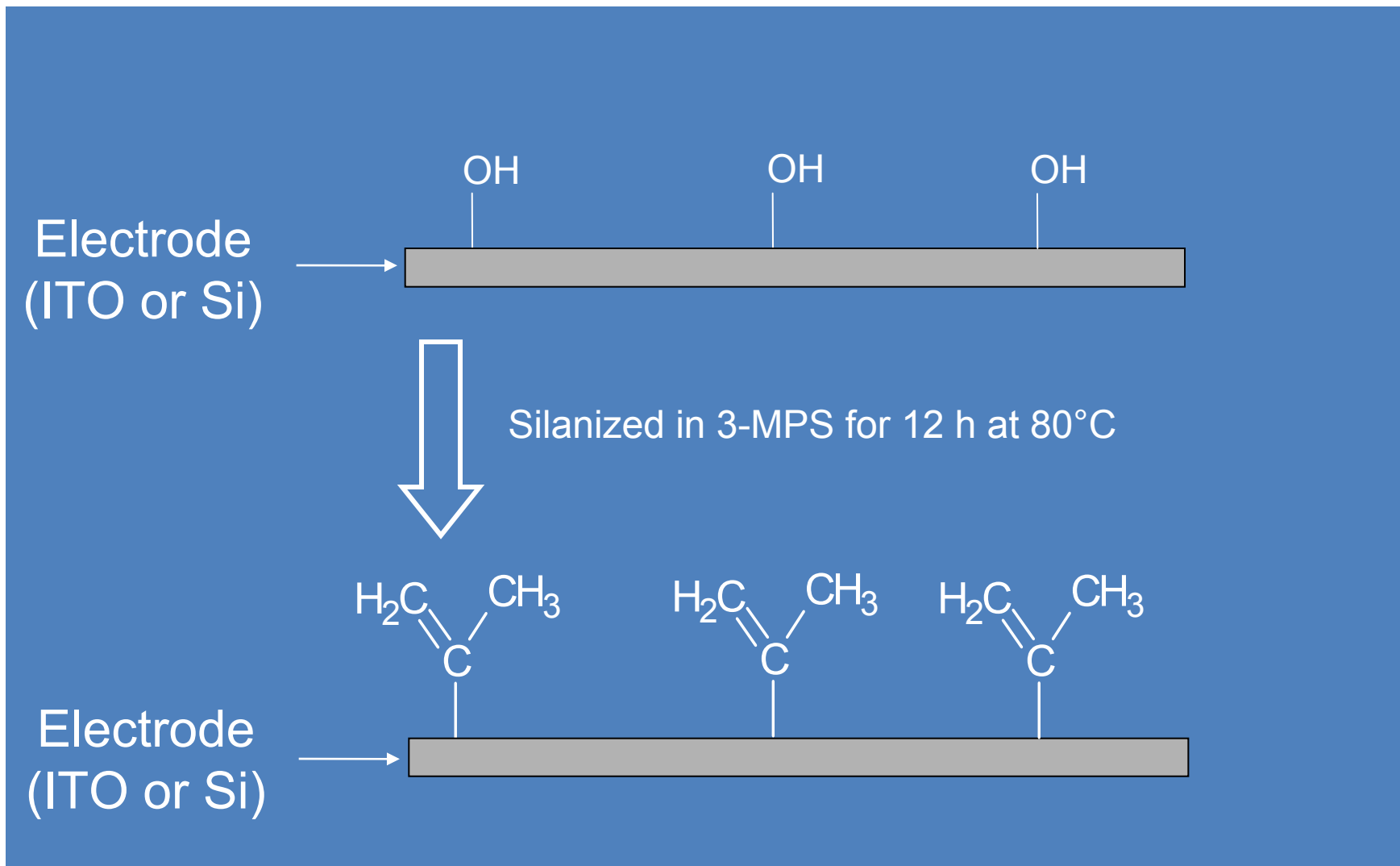
- *Theophylline*

- Bronchodilator drug, small molecule
- Needs to be monitored for treating asthma and other airways diseases
- Level in blood is 5-15 $\mu\text{g}/\text{ml}$ to relieve symptoms
- Drug delivery and monitoring – administering and measuring the amount of drug over a period of time





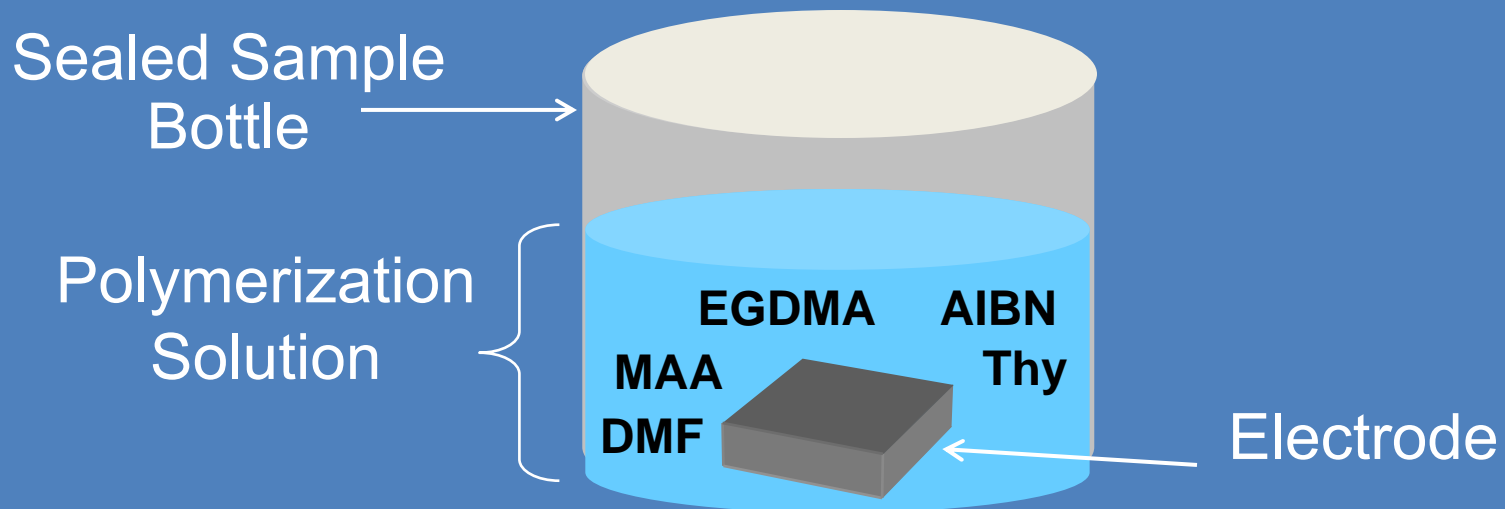
Surface Modification: Silanization





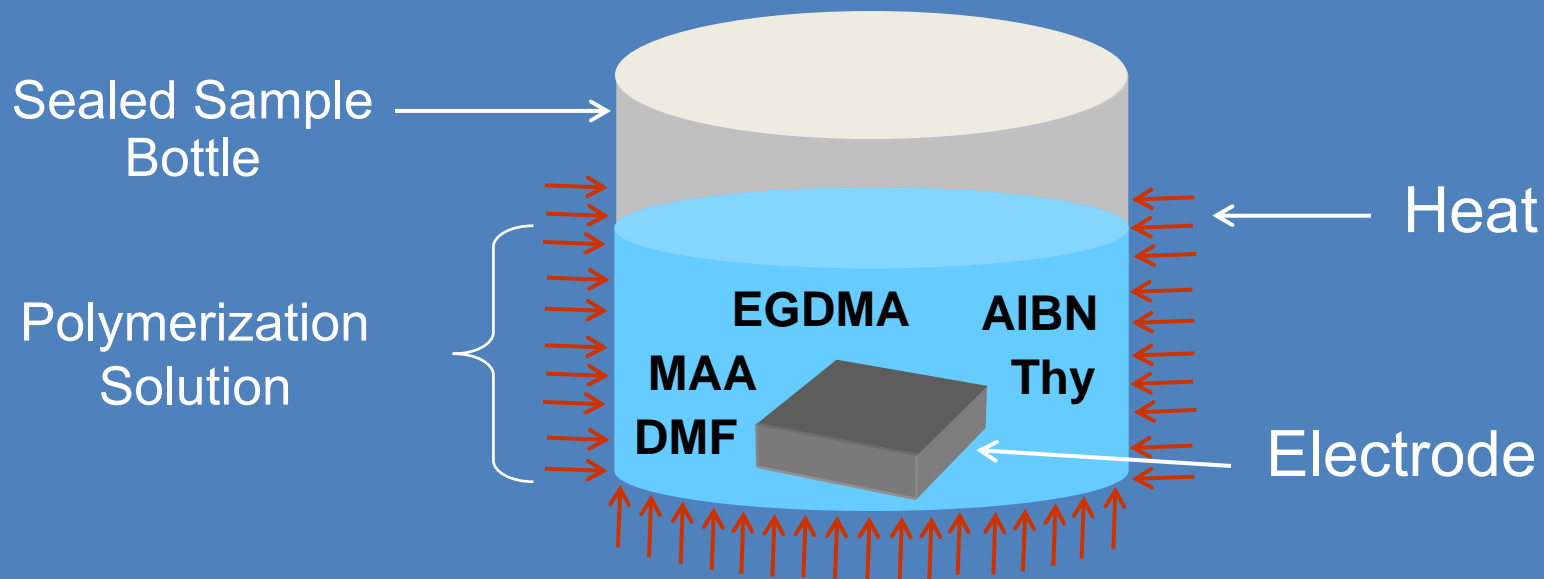
MIP Formation: Self Assembly

- *Combine polymerization solutions*
 - Combine MAA, EGDMA, DMF, AIBN, and template
 - Remove oxygen in anaerobic glove box



Polymerization

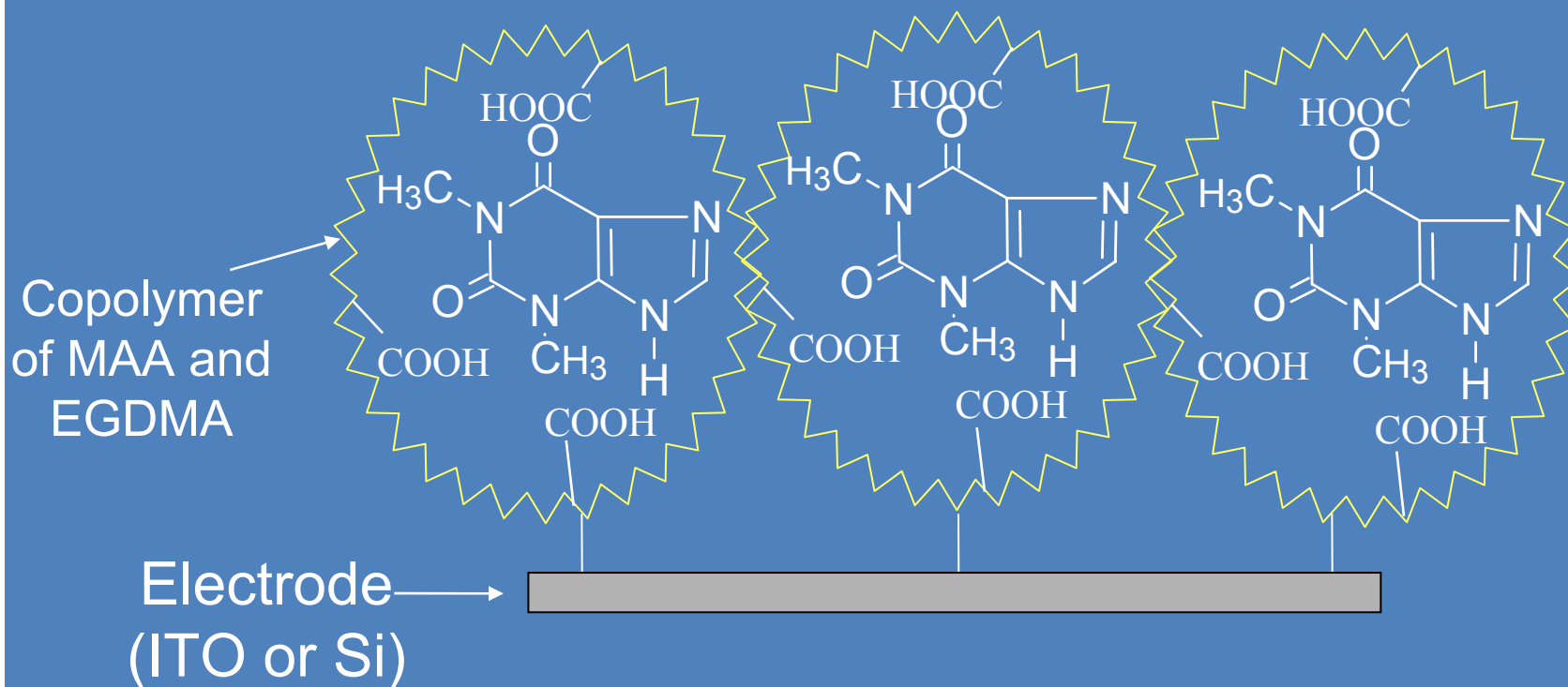
- *Polymerize in water bath for 12 h at 60°C*
- *Blank polymer was formed similarly to MIP except template not added*





Polymerization

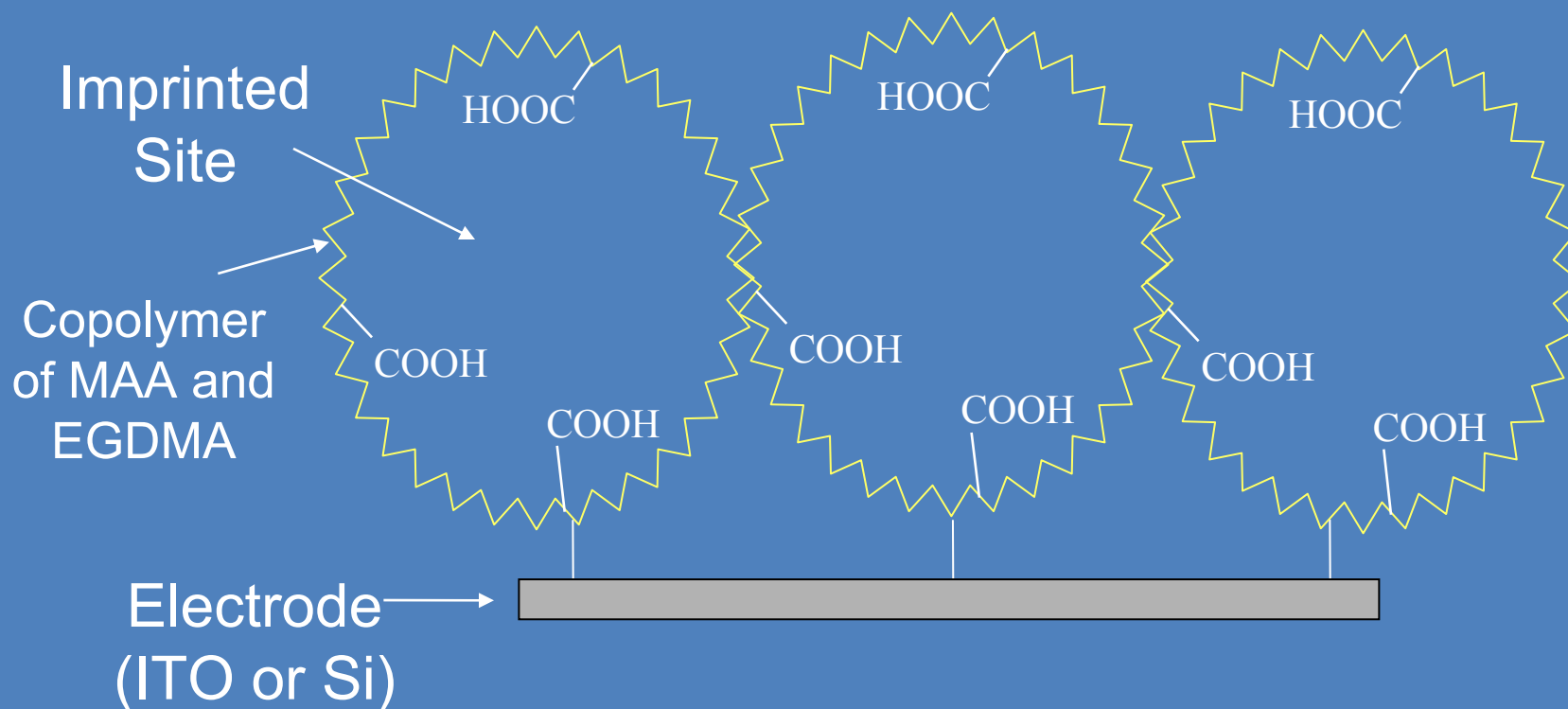
- *Break bulk copolymer, thin layer remains*





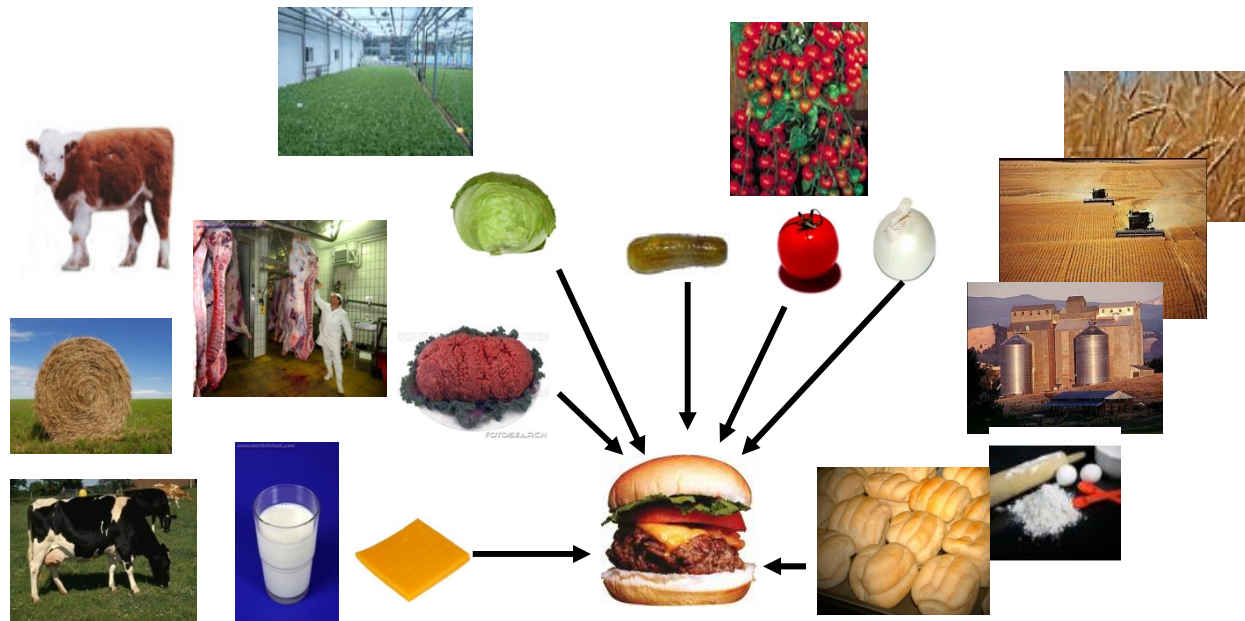
Template Extraction

- *Immerse in 9:1 v/v methanol/acetic acid for 1 h*



This system may now be used for product authentication.

Tier 3: Sensors for Supply Chain Biosecurity – “Highway Police Patrol”



Schematic food chain of a simple hamburger



Sensors for biosecurity management in global food and pharmaceutical trade – optimum type and location of surveillance mechanisms in the supply chain.

Future Research

- *Cost effective nanobio sensors*
- *Reusable designs*
- *Integrate sensors into packaging*
 - Meet the requirements for food safety
 - Possible use of naturally derived and biodegradable materials
- *Establish the three-tiered sensor system*
 - Minimize counterfeiting activities
 - Support the beneficial effects of Intellectual Property Rights and the concept of brands
 - Results in safe food and pharma products
 - Leads to a secure financial environment

Acknowledgment of Our Funding Sources



NATIONAL CENTER FOR
FOOD PROTECTION AND DEFENSE
A HOMELAND SECURITY CENTER OF EXCELLENCE



Acknowledgment of Research Group

- *Graduate students*
- *Postdocs*
- *Professorial assistants*
- *High school interns*

For a complete listing, refer to:

<http://www.egr.msu.edu/~alocilja>

Thank You
**for the opportunity to present our research
work and for your kind attention**

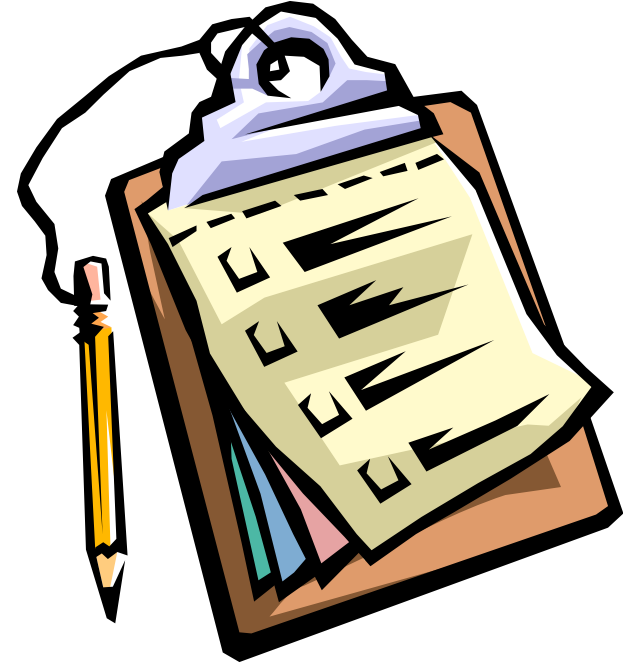
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*Please remember to turn
in your evaluation sheet...*