Nanoparticle Synthesis and Assembly for Biological Diagnostics

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Templated Synthesis of Nanoparticles
Magnetic Nanoparticle Assembly

Support: Division of Infection Diseases, Eli Lilly Inc
NASA Institute for Nanoelectronics and Computing at Purdue
1.0 Biological Sensing: Immunoassays

Nanoparticles are currently used as optical and chemical transducers in many commercially available diagnostic kits.

Antibodies are commonly used molecular receptors for these diagnostic assays:

\[
Ab + Ag \xrightarrow{K_{on}} Ab - Ag \xleftarrow{K_{off}} Ab + Ag
\]

\[
K_{eq} = \frac{K_{on}}{K_{off}} = \frac{[Ab - Ag]}{[Ab][Ag]} = \frac{10^6 - 10^8}{10^{-4} - 1} = 10^6 - 10^9
\]

Sensitivity is determined by both \(K_{eq}\) and kinetics.
1.0 Biological Sensing: Immunogold Assays

Dipstick assays use *antibody coated colloidal gold* to detect antigens

“...The dipstick assay is aimed at the rapid detection of *Brucella*-specific IgM antibodies in human serum or whole blood samples from patients in the early stage of the disease. The dipstick assay is based on the binding of specific IgM antibodies to a lipopolysaccharide fraction ... The assay utilize stabilized non-enzymatic detection reagents that *can be stored without the need for refrigeration for at least two years without losing reactivity*. The result of the dipstick assay is *obtained after three hours and no special equipment is required to perform the assay*.”

KIT - Royal Tropical Institute of the Netherlands. Assay format include Brucellosis, Leprosy, Leptospirosis, Leishmaniasis, and Typhoid Fever
1.0 Biological Sensing: Magnetic Materials in Sensing

Magnetic Separation

Giant Magnetoresistance

Magnetic Tweezers

Prestvik WS etc. Scientific and Clinical Applications of Magnetic Carriers; Baselt DR. etc. Biosensors & Bioelectronics, 13, 371; Gosse and Croquette Biophysical Journal. 82, 3314
The optical properties of colloidal dispersions have been studied for several hundred years and have been described by rigorous theory:

Rayleigh Scattering

\[ Q_{sca} = \frac{8\lambda^4}{3} \left| \frac{m^2 - 1}{m^2 + 2} \right|^2 \propto \frac{1}{\lambda^4} \]

Adsorption

\[ Q_{ads} = 4\lambda \text{Im} \left| \frac{m^2 - 1}{m^2 + 2} \right|^2 \propto \frac{1}{\lambda} \text{ simple metals} \]

Surface modes can be excited where \( Q_{ads} \) will become very large.
2.1 Physical Basis for Optical Properties of Nanoparticles
2.2 Physical Basis for Magnetic Properties of Nanoparticles

**Ferromagnetic Material B-H Curve**


**Paramagnetism**

Nonhysteretic B-H curve but
Iron saturates at 1.6 T while ferrites will normally saturate between 200-500 mT
2.2 Physical Basis for Magnetic Properties of Nanoparticles

Ferromagnetism is based on pinning of magnetic domain walls that are 10-100 nm in scale. This can not happen in nanoparticles

<table>
<thead>
<tr>
<th>Material</th>
<th>$D_{\text{crit}}$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co</td>
<td>70</td>
</tr>
<tr>
<td>Fe</td>
<td>14</td>
</tr>
<tr>
<td>Ni</td>
<td>55</td>
</tr>
<tr>
<td>Fe$_3$O$_4$</td>
<td>128</td>
</tr>
<tr>
<td>$\gamma$-Fe$_2$O$_3$</td>
<td>166</td>
</tr>
</tbody>
</table>

The experimental criteria for superparamagnetism are: 1) the magnetization curve exhibits no hysteresis, and 2) the magnetization curves at different temperatures must superimpose in a plot of M vs H/T.
3.0 Nanoparticle Synthesis: Traditional vs. New Methods

**Traditional aqueous formation** of colloids depends on reduction of metal salts (Reike metals)

\[ MX_n + nK \rightarrow M^* + nKX \]

Gas phase synthesis of colloidal particles has been demonstrated using vapor trapping, sputtering, and carbon arching.

New aqueous chemistries have used **self-assembly** and sonochemistry
3.1 Nanoparticle Synthesis: Templated Synthesis

3.1 Nanoparticle Synthesis: Templated Synthesis


3.3 Nanoparticle Assembly: Templated Self-Assembly

Form an emulsion from nanoparticles and initiator:

Size emulsion using extrusion:

Remove the solvent and adsorb hydrophobic monomer and surface groups:

Crosslink:

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3.3 Nanoparticle Assembly: Microparticle Ultrastructure

Transmission electron micrographs of the microparticles

3.3 Nanoparticle Assembly: Superparamagnetic Behavior

Magnetization and density measurements indicate the microparticles are > 70% by volume magnetite.

Magnetization of superparamagnetic Dynal M-280 beads is 10 emu/g and theoretical magnetization of ferromagnetic materials is 150 emu/g.
3.3 Nanoparticle Assembly: Surface Chemistry

**XPS Analysis of Surface Chemistry**

<table>
<thead>
<tr>
<th>AA:RN-10</th>
<th>-CH₂CH₂ (%)</th>
<th>-CH₂CO₂H (%)</th>
<th>-CH₂CH₂O (%)</th>
<th>COOH/CCO</th>
<th>C/Fe</th>
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<tbody>
<tr>
<td>4:1</td>
<td>26</td>
<td>26</td>
<td>22</td>
<td>2.3</td>
<td>2.5</td>
</tr>
<tr>
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<td>25</td>
<td>26</td>
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<tr>
<td>1:1.5</td>
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<tr>
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<td>16</td>
<td>30</td>
<td>1.1</td>
<td>2.7</td>
</tr>
</tbody>
</table>

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3.3 Nanoparticle Assembly: Surface Chemistry

Antibody Immobilization Produces Highly Active Materials

**Sandwich Immunoassay**

![Graph showing absorbance (Abs) for different samples S1, S2, S3, S4, and Seradyn.](image)

- HRP anti-mouse IgG
- HRP anti-rabbit IgG

HRP Transducer

Antibody 1

Antibody 2
3.3 Nanoparticle Assembly: Two Particle Mixed Structures

- 5nm Au + 20 nm Fe3O4
- 5 nm Fe3O4 + 5 nm Au
- 10 nm Fe3O4 + 10 nm Au

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3.3 Nanoparticle Assembly: Two Particle Mixed Structures

10nm Au + 20 nm Fe₃O₄

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4.1 Applications: Single Molecule Measurements

These homogeneous microparticles with high magnetic moments provide new opportunities for biophysical and analytical measurements:

Single molecule force measurements have been made between protein A and an antibody demonstrating force differentiation for the first time.
4.2 Applications: Multiplexed Magnetophoreic Assays

Average 0.66 um
C.V. 33%

1: 0.73 um, 20%
4: 0.70 um, 19%
6: 0.64 um, 15%
8: 0.61 um, 14%
5.0 Conclusions

- Although nanoparticles are commonly used in diagnostic technologies it appears that there are excellent opportunities for new developments.
- Templated synthesis promises to allow multifunctional microparticles to be created.
- Magnetically active microparticles produced by templated synthesis promise new modes of sensing with higher sensitivities and specificities.
- Optically active microparticles promise to allow assays to be highly multiplexed allowing 100’s analytes to be sensed simultaneously.
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Questions & Answers
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