Nanomaterials for Health Care
Modern, Ayurvedic, and Homeopathic NanoMedicines, and more.

Jayesh Bellare, students & collaborators
jb @ iitb.ac.in
Today’s healthcare situation: Drug or Device?

Biomedical devices are increasingly incorporating drugs.

and …

Drug dosage forms are increasingly becoming devices!

Nanostructured materials help.
Nanotechnology impact

1. Nanotechnology will affect all aspect of our life:
   Health care will be the major application!

2. Nanotechnology is the basis of alternative and traditional medicines
Nano + bio : not new

TEM image showing natural nanotechnology in magentotactic bacteria (from Dunin-Borkowski et al. 1998, © 1998 American Association for the Advancement of Science.)
Nanotechnology: so what’s new?

The four revolutions of nanotechnology:
1. Ability to see things smaller than seen with light microscope.
2. Ability to manipulate at this size scale
3. Political and governmental will
4. Business interest, commercial applications
Nanotechnology for Health Care

Joys and frustrations in translating innovations from bench to bedside

Jayesh Bellare
jb @ iitb.ac.in
Challenges:

1. Public perception: is it safe?
2. Uncertain regulatory systems
3. Cell-lines and Animal models
4. Large-animal testing (long-term intensive care)
5. Onerous CE/US-FDA approvals for human use
Powerful bio effects of nano:

1. Macrophage evasion
2. Cross blood-brain barrier
3. Slip through cell junctions
4. Bio-integrate (join to cells)
5. Deliver large aliquots (compared to molecular solution)
6. Enhanced permeation and retention (EPR)

Well exploited by modern nanomedicines
Nanotechnology is built on microscopy

Microscopists understand nanostructures well, so they are best equipped for exploring nano-medicinal systems (and more).

- Our Microscopy and Nanotechnology led us to the applications of nanotechnology for health care
- IIT B is well equipped with tools, techniques and expertise in nanotechnology, bioengineering & biotechnology.
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Some examples …
1. Micro-devices for cardiac use (minimally invasive surgery)
2. Nanocomposites for dental and orthopedic use (bone grafts)
3. Ocular drug delivery with nanoparticles
4. Green QDs for diagnostics
5. Nano particles in traditional medicine
6. Nano particles in alternative medicine
7. Nanostructured hollow fibers for superior kidney dialysis
8. Stem cell bioreactors and scaffolds
Nanotechnology for Health Care
Work in lab of Prof. Jayesh Bellare \( jb@iitb.ac.in \) and colleagues

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Dr. Bharat Dalvi, Nanavati
Dr. Vivek Soni, MGM, Thane
Dr. Santosh Honavar, LVPEI, Dr. Shome
Vaidya Ajit Joshi, Pune

... vox populi ...

Dr. Varsha Degwekar
How we got to Nanotech for Health Care at IITB

Our work in Nanotechnology + collaboration with Medical Doctors =

Led to our first research in medicines:
Our work in Nanotechnology + collaboration with Medical Doctors =

Led to our first research in medicines:

First: Modern Medicine (Allopathic) [Doctor driven]
**Example: Ophthalmic drug delivery with nanoparticles**
(with R. Banerjee; now with D Shome, S Honavar)

- Physical and chemical barriers to effective drug delivery to eye
- Pharmacokinetic limitations of conventional drug delivery systems in ophthalmology
- Requirement for new drug delivery forms for sustained release
TEM photograph of aspirin loaded albumin nanoparticle:
Diameter of the particles 21nm
Drug loaded nanoparticles for the eye

- Developed aspirin loaded protein nanoparticles of uniform 40-60 nm size, which were stable for 3 months

- Achieved sustained release of drug over 72 hours

- Effective even with one-third of the therapeutic dose

- Will be advantageous for use in diabetic retinopathy where aspirin is used orally

*(Das et al. BIMAO 2003)*

*Patent pending*
Newest: First in Human trials: Nano-carboplatin for retinal cancer

• Multicentric trials for first in human started at L V Prasad Eye Hospital (Dr. Santosh Honavar) Apollo Hospitals (Dr. Debraj Shome)
• Nanoparticulate form reached significant therapeutic levels near the retina
Surfactant Nanoparticles for alveolar drug delivery: cures neonatal respiratory distress

- Developed surfactant nanoparticles of 100-200 nm size having low polydispersity; Will be non-invasive and have better alveolar reach than intratracheal instillation
- Can be used for Inhalation therapy: more homogenous pulmonary distribution than instillation

*(Banerjee R, Editorial, Chest, 2004)*
How we got to Homeopathy at IITB

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How we got to Homeopathy at IITB

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Led to: Ayurvedic and Homeopathic medicines
Controversial subjects?

Traditional and Alternative systems of medicine are controversial:

Are Ayurveda and Homeopathy B.S. (Bad Science)?
Traditional and Alternative systems of medicine are controversial: Is Ayurveda and Homeopathy B.S. (Bad Science)?

- They are not studied in context of modern science, nor validated by modern standards
- This suppresses open discussion … but …
- Could new science emerge?
How we got to Homeopathy at IITB

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Then: Ayurvedic bhasmas [Vaidya driven]
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We did not make the medicines: we studied standard medicines.
Nanoparticles in Traditional medicinal systems: *Bhasma*

*Bhasmas* are unique form of medicinal preparation used in *Ayurveda / Siddha* system for thousands of years.

- They are powders and made by grinding, heating, quenching a variety of base materials.

- **Examples:**
  - *Tamra Bhasma*  Cu
  - *Mouktika Bhasma*  Pearl
  - *Suvarna Bhasma*  Au
  - Others containing Zn, Pb, As, mixtures
Nanoparticles in Traditional medicinal systems: Bhasma

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- Examples:
  - Tamra Bhasma Cu
  - Mouktika Bhasma Pearl
  - Suvarna Bhasma Au
  - Others containing Zn, Pb, As, mixtures

Toxic!
Objective: Bring into framework of modern medicine with use of modern techniques of science

1. Physical Characterization (crystalline phases, Size analysis, Shape analysis).

2. Elemental composition analysis for major and minor elements.

3. Biological assay for activity testing of the medicine.
   • We use samples from authentic source
     a. Sample made by Vaidya of several generations
     b. Sample from reputed commercial manufacturer

Method Used for Analysis:
 a) Size and Shape: Dynamic Light Scattering, TEM
 b) Crystalline Phase: XRD
 c) Elemental Analysis: ICP, EDAX, XPS
Physicochemical Result Summary

Dynamic Light Scattering of Jasada Bhasma: 30 nm particles

Electron Microscopy of Jasada Bhasma

Major finding-1: Jasada Bhasma has nanoparticles!

Elemental Analysis of Jasada Bhasma by XRD, ICP, EDAX, XPS: new technique development gives complete analysis and new insight.

Major finding-2: Jasada Bhasma is Oxygen Deficient Material
How to test Biological effects of Bhasma?

We used model organism: Yeast Cell

Growth Medium Composition

<table>
<thead>
<tr>
<th>Medium YPG</th>
<th>Medium YPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol- 4%</td>
<td>Glucose- 4%</td>
</tr>
<tr>
<td>Yeast Extract- 2%</td>
<td>Yeast Extract- 2%</td>
</tr>
<tr>
<td>Peptone- 1%</td>
<td>Peptone- 1%</td>
</tr>
</tbody>
</table>
Effect on biomass production of Yeast cells

With Jasada Bhasma fed on glycerol

Yeast Bioassay [Batch-13] [Jasada Bhasma], Dose-300ppm, Date-26.03.05, C-4% Glycerol

O.D.@600nm vs Time-Hr

- Control
- Jasada Bhasma
Effect on biomass production of Yeast cells

With Jasada Bhasma fed on glycerol

Yeast Bioassay[Batch-13] [Jasada Bhasma], Dose-300ppm, Date-26.03.05, C-4% Glycerol

Preserves cells longer into late growth phase
Change carbon substrate to glucose

Yeast Bioassay[Batch-14][Jasada Bhasma], Dose 300ppm, Date 18.04.05 C-4% Glucose
Yeast Bioassay [Batch-14] [Jasada Bhasma], Dose 300ppm, Date 18.04.05 C-4% Glucose

Change carbon substrate to glucose

Opposite: kills cells quicker in late growth phase
Yeast Bioassay [Batch-14] [Jasada Bhasma], Dose 300ppm, Date-18.04.05 C-4% Glucose

Food type affects drug action!

Change carbon substrate to glucose
Morphology Study of Yeast Cells by TEM

Batch-18 (4% Glycerol)
Summary of work done with *Jasad bhasma*

**DLS:** showing Nano sizes

**SEM and TEM** showing nanoparticles

<table>
<thead>
<tr>
<th>XPS &amp; EDAX</th>
<th>ZnO</th>
<th><em>Jasad bhasma</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn2p1/2 (eV)</td>
<td>1045.1</td>
<td>1045.0</td>
</tr>
<tr>
<td>Zn2p3/2 (eV)</td>
<td>1022.0</td>
<td>1021.9</td>
</tr>
<tr>
<td>O/Zn (EDAX)</td>
<td>0.98</td>
<td>0.603</td>
</tr>
<tr>
<td>O/Zn (XPS)</td>
<td>0.977</td>
<td>0.591</td>
</tr>
</tbody>
</table>

**Conclusions: *Jasad Bhasma***
- Has nanoparticles
- Is highly oxygen deficient ZnO
- Has biological effect ➞ protection of chemical entities from ROS

Yeast cell (Control): damaged internal Organelles, with thin cell wall, and No nucleopores

Yeast cell (*Bhasma* treated): With better preserved internal Organelles, thick cell wall and nucleopore (marked with an arrow)

- Reduced DNA fragmentation
- Higher intracellular proteins
- Higher content of biomolecules

*Note: The graph shows Raman Peak Area (Ar. Unit) vs. Wavenumber (cm⁻¹). The peaks are labeled for quartz treated A549 cells and *Jasad Bhasma* treated A549 cells.*
Conclusions of Ayurvedic Bhasma study

- There are nano-particles 5-30nm in *Bhasma*
- There is non-stoichiometric ZnO (50% O deficient)
- Jasada Bhasma promotes / inhibits growth of Saccharomyces (substrate mediated)
- Many open questions remain: do non-stoichiometric nano-particles affect toxicology?

Exciting science, much more to be done!
How we got to Homeopathy at IITB

Our work in Nanotechnology + collaboration with Medical Doctors =

Led to our research in medicines:

First: Allopathic [Doctor driven]

Then: Ayurvedic bhasmas [Vaidya driven]

Now: Homeopathic [Patient driven]
What we have done in Homeopathy:
Physico-chemical Characterization of certain Homeopathic Medicines
(to understand super-Avogadro dilution)
Motivation:
Homeopathy vs. modern scientists: What's the problem?

1. May be evidence-based at patient-doctor level.

2. Not evidence-based at molecular science level (as per modern scientists) because of dilution beyond Avogadro's number at 12C ($10^{24}$ dilution).


Many scientists may believe that homeopathy only provides a placebo effect.
Manufacturing methods of Homeopathic medicines

1. Start with metal in foil or granule form.

2. Tituration: grinding with lactose

3. First dilution: add solid to solvent, mix by succussion

4. Successive Dilution: 1 part of previously prepared dilution (prepared by succussion) is taken out and added to 99 parts fresh solvent

5. Repeat 6 times (6C) or 30 times (30C) etc.
Background: Reluctance to accept Homeopathy by modern scientists

1. Homeopathic high potencies are ultra-high dilutions.

2. Dilutions are far beyond Avogadro’s number: e.g. 30C is $10^{60}$ times and 200C is $10^{400}$ times dilution.

3. Presence of physical entities cannot be visualized (so far).

4. Liquid memory theories not validated experimentally.

5. Experimental results in animals and other biological models - Non-reproducible and inconclusive.

6. Not easily amenable to double blind clinical trials.

Some scientists may believe that homeopathy only provides a placebo effect.
Our work is published, peer-reviewed:

**ORIGINAL PAPER**

**Extreme homeopathic dilutions retain starting materials: A nanoparticulate perspective**

Prashant Satish Chikramane¹, Akkihebal K Suresh¹,², Jayesh Ramesh Bellare¹,²,* and Shantaram Govind Kane¹,*

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Homeopathy is controversial because medicines in high potencies such as 30c and 200c involve huge dilution factors ($10^{60}$ and $10^{400}$ respectively) which are many orders of magnitude greater than Avogadro’s number, so that theoretically there should be no measurable remnants of the starting materials. No hypothesis which predicts the retention of properties of starting materials has been proposed nor has any physical entity been shown to exist in these high potency medicines. Using market samples of metal-
Our work got Editorial attention:

GUEST EDITORIAL

Do serial dilutions really dilute?

The article by Chikramane et al. ‘Extreme Homeopathic Dilutions Retain Starting Materials: A Nanoparticulate Perspective’, in this issue reports the fascinating observation that high potency homeopathic remedies made from

Principle of Similars and, beyond a certain threshold, how much the potency matters.

References


One might expect a different outcome if the starting material were an organic compound as much of the chemistry described here would have very different implications. In addition, there are several other difficulties in determining


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Published in mainstream journal!

What we have done:

Physico-chemical Characterization of certain Homeopathic Medicines

(We showed by electron microscopy that nanoparticles remain despite dilution)
## Homeopathic medicines analyzed in our studies

<table>
<thead>
<tr>
<th>Medicines Analyzed</th>
<th>Potencies used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zincum metallicum</td>
<td>6C, 30C, 200C</td>
</tr>
<tr>
<td>Cuprum metallicum</td>
<td>6C, 30C, 200C</td>
</tr>
<tr>
<td>Stannum metallicum</td>
<td>6C, 30C, 200C</td>
</tr>
<tr>
<td>Aurum metallicum</td>
<td>30C, 200C</td>
</tr>
<tr>
<td>Argentum metallicum</td>
<td>6C, 30C, 200C</td>
</tr>
<tr>
<td>Platinum metallicum</td>
<td>6C, 30C, 200C</td>
</tr>
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</table>

Source: WSI and SBL, from their authorized dealers
Main tool: Transmission Electron Microscope (TEM)
TEM provides Images & Electron Diffraction (ED)

Bright Field Image – Scale Bar 200nm

Dark Field Image – Scale Bar 200nm

ED or electron diffraction
Determination of presence and identity of nanoparticles – TEM & ED

**Zincum met 30C (SBL)**

Size of aggregate:
- Length: 1950nm / Width: 1540nm
Determination of presence and identity of nanoparticles – TEM & ED

*Zincum met 200C (SBL)*

Bright Field Image – Scale Bar 200nm  
Dark Field Image – Scale Bar 200nm

Size of aggregate:

Length: ~700nm / Width: ~500nm
Determination of presence and identity of nanoparticles – TEM & ED

**Zincum met 30C (WSI)**

**a** Bright Field Image – Scale Bar 200nm  **b** Dark Field Image – Scale Bar 200nm

**Size of aggregate:**

**Length:** ~490nm / **Width:** ~200nm
Determination of presence and identity of nanoparticles – TEM & ED

**Zincum met 200C (WSI)**

Bright Field Image – Scale Bar 200nm

Dark Field Image – Scale Bar 200nm

Size of aggregate:

Length: ~1300nm / Width: ~950nm
Crystallite size determination of *Zincum met* (SBL and WSI) by dark-field TEM (corresponding to the crystallite sizes determined in the dark-field images represented for *Zincum met*)
Evidence so far:

So far, we have shown that:

1. Particles of the starting material remain despite super-Avogadro dilution.

2. They contain nanoparticles and nano-crystallites.

So …

Can we quantitatively measure them for concentration?
Inductively Coupled Plasma-Atomic Emission Spectroscopy – Introduction

- Solution is drawn by peristaltic pump
- Turned into fine aerosol by nebulizer
- Aerosol introduced into a plasma which excites atomic species in the aerosol
Inductively Coupled Plasma-Atomic Emission Spectroscopy – Introduction (contd.)…

Principle of ICP-AES

1. Electrons of an atom absorb energy and jump to higher energy levels
2. When they return to normal states, they emit characteristic photons of energy
3. By isolating these photon wavelengths, we can determine the types and concentrations of the elements present.

http://www.mri.psu.edu/facilities/MCL/events/presentations/ICP-AES
# Inductively Coupled Plasma-Atomic Emission Spectroscopy – Emission Wavelengths

<table>
<thead>
<tr>
<th>Metal analyzed</th>
<th>Emission wavelength (nm)</th>
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<tbody>
<tr>
<td>Zinc</td>
<td>213.856</td>
</tr>
<tr>
<td>Copper</td>
<td>324.754</td>
</tr>
<tr>
<td>Tin</td>
<td>283.999</td>
</tr>
<tr>
<td>Gold</td>
<td>242.795</td>
</tr>
<tr>
<td>Silver</td>
<td>328.068</td>
</tr>
<tr>
<td>Platinum</td>
<td>265.945</td>
</tr>
</tbody>
</table>
**Concentration measurement with ICP-AES**

**Concentrations of Starting Materials in Homeopathic medicines**

**Part ‘A’** – Estimated by Röder *et al* – Solid symbols: expected concentrations, open: estimated concentrations, circles: Au³⁺, star: Fe³⁺, left triangle: Hg²⁺, right triangle: Zn²⁺

**Part ‘B’** – Estimated by ICP-AES in our work – Squares: Zinc concentrations, open: *Zincum met* (SBL), solid: *Zincum met* (WSI), open triangles: gold concentrations in *Aurum met* (SBL) samples. The dotted line at 20pg/ml indicates the LOD of the instrument.
How does this happen?: Explanation: Segregation of particles and carry-through

- Nanoparticles form due to shear during initial trituration and succussion at each dilution step
- Succussion process generates bubbles
- Nanoparticles cling to these bubbles and rise to the surface of the solvent forming a monolayer at concentrations below 100 ppb (4C)
- The monolayer on top is retained during each dilution step in both Hahnemannian and Korsakovian methods
- Hence, dilution beyond 4C is apparent and not real
Froth flotation of nanoparticles

Langmuir 2012 http://dx.doi.org/10.1021/la303477s
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Interaction of AuNPs and Zinc particles with Lactose during trituration – FT-IR analysis

Change in vibrational frequencies of methylene C-H bonds

- C-H at 2901 cm⁻¹ (aliphatic methyne [R₂CH⁻]) constant in all cases
- C-H stretching of methylene (RCH₂⁻) at 2930 cm⁻¹ in AuNP and 2933 cm⁻¹ in zinc. H-Bonding interactions

OH stretching freq. at of sec. alcohol at 3382 cm⁻¹ constant. Stretching freq. of primary OH [RCH₂OH] changed from 3350 cm⁻¹ in free lactose to 3345 cm⁻¹ in AuNP and 3343 cm⁻¹ in zinc. H-Bonding interactions
TEM analysis of lactose-AuNP triturated mixture

Formation of nanoclusters of lactose embedded metal nanoparticles

A – Bright-field TEM image of nanocluster
B – SAED pattern of polycrystalline region consistent with Gold (JCPDS) in nanocluster
C – SAED pattern of amorphous region (Lactose)
High-speed video snapshots – Succussion (Bottom layer)
High-speed video snapshots – Succussion (Top layer)
Summary and Conclusions

1. Nanotechnology has an important future in drug delivery

2. Manufacturing and regulatory issues remain

3. A study of alternative and traditional medicinal systems is important.

4. TEM helped establish presence of nanoparticles in traditional and alternative medicines, Ayurveda and Homeopathy.

5. Some medicinal materials have non-stoichiometric chemistry, which may be new candidates for modern medicines

6. The process of manufacture needs to be understood: it explains results of action

7. Much further study is needed. It may lead to exciting new science & drugs.
Nanotechnology for healthcare

Collaboration across disciplines enabled us to do things we couldn’t do before, or didn’t even know we wanted to do.
Thank You!

Acknowledge support from:
DST, DBT, IITB-IRCC, Alumni

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