Layered Inorganic Nanoparticles (clays) – Towards a Better Understanding of Biological Interactions

D Martin, R Minchin, S Smith, ZP Xu, K Carrado, A Musumeci and G Broadhurst

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Summary

- Synthetic clays and "nanoclays": an important class of nanoparticles
- Traditional applications and markets (Mmt, hectorites and LDH)
- Emerging applications requiring a new perspective on risk governance
- Our approach to understanding biological interactions
- Some of our key findings so far
- Conclusions and outlook
Growing Importance of Synthetic and Nanoclays

- More than 9000 tonnes produced in 2007
- > $132,000,000 USD market value
  - Automotive 35%
  - Paper 26%
  - Paint 26%
  - Flame retardant 13%
  - Cosmetics and toiletries < 1%

Current Toxicological Data

- Very limited toxicological data
- Only acute (mostly in-vitro) studies performed
- No epidemiological evidence of health problems
- True chronic (nano)toxicological work has not been done
- New approach needed to address knowledge gaps


Montmorillonite (natural)

Montmorillonite (Mmt)
(-ve charge)
100-200 x ~1nm platelets
Montmorillonite “Nanoclay”

Montmorillonite (Mmt)
(-ve charge)
100-200 x ~1nm platelets

Typically 1-5% by wt nanoclay addition into “host” polymer
Clay-based Nanocomposites – Applications Today

Automotive
Sport
Packaging

Nanoclay Particles Market: Volume and Revenue Forecasts (World), 2003-2013

Note: All figures are rounded; the base year is 2006. Source: Frost & Sullivan
Clay-based Nanocomposites
Tomorrow’s Applications?

27 March 2007
Nanotechnology: the next big thing?

French food and drinks giant Danone is working with Queen’s University Belfast on a research project to develop new polymer nanocomposites, which it believes will result in stronger plastic packaging, less energy consumption and reduced waste.

On the other hand, critics point out risks. The effects of engineered nanomaterials that are released into the environment during production and disposal remain unclear. Uncontrolled incineration of nanocomposites could cause emissions of nanoparticles into the environment and nanocomposite materials could disturb plastic recycling processes.

So is nanotechnology the next big thing or just the flavour of the month?

More articles are appearing with such qualifying statements, trying to project a balanced viewpoint of the pro’s and con’s of new nanomaterials…

Robust new data measuring NP release (if any), migration and fate during complete product life cycle is needed.

It is our responsibility to work together, generate this data and communicate it!

Hectorite (synthetic)

Hectorite
(-ve charge)
25-50 x ~1nm platelets

Tetrahedral layer
Octahedral layer
Tetrahedral layer
Interlayer region

- Si
- O
- Li
- Mg
- Na
- H
Better purity, clarity and more pronounced rheological effects
Trade names “Laponite” (Rockwood) and “Lucentite” (Kobo)

Cosmetics
Toothpaste
Antiseptics
Shower gels
Catalyst supports
Paint additives

Synthetic Hectorites – Applications Today

Synthetic Hectorites – Tomorrow’s Applications?

Biomedical polyurethane nanocomposites, cochlear implants, etc

Table 1 – Key property values for silicone versus ElastEon and UQ nanocomposites

<table>
<thead>
<tr>
<th>Material</th>
<th>Shore A Hardness</th>
<th>Ultimate Tensile Stress (MPa)</th>
<th>Stress @ 200% Strain (MPa)</th>
<th>% Elongation at Break</th>
</tr>
</thead>
<tbody>
<tr>
<td>NuSil MED-4860</td>
<td>60</td>
<td>9</td>
<td>4</td>
<td>525</td>
</tr>
<tr>
<td>ElastEon 700A</td>
<td>70</td>
<td>25</td>
<td>13</td>
<td>400</td>
</tr>
<tr>
<td>UQ TPU Nanocomposite</td>
<td>80</td>
<td>60</td>
<td>6</td>
<td>1200</td>
</tr>
</tbody>
</table>


Patent No. WO 2006/024068 A1, The University of Queensland, “Polymer Composite” (Filed May 2005)
Release kinetics of synthetic clays from PU composites in 37 °C MilliQ water before and after stretching (200% strain) – effect of nanofiller size

- indication that nanoclays do come out (Mg in solution via ICP-OES)
- 75nm nanoclay had best dispersion → lower release rate from host polymer
- equates to a maximum of ~2-4 particles/1000 coming out into solution in 7 days
Layered Double Hydroxide (LDH) (+ve charge) 20-200 x ~10nm platelets

Antacids
Adsorbents
Flame retardants

Hybrid delivery agent
- vitamins, drugs, DNA strands, organic acids
(NB. LDH is very cheap to make)

“Interaction of Engineered Nanoparticles with Biological Systems”

<table>
<thead>
<tr>
<th>Current Team</th>
<th>Skills Set</th>
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<tbody>
<tr>
<td>Dr Darren Martin</td>
<td>(AIBN, Materials Scientist)</td>
</tr>
<tr>
<td>Prof Rod Minchin</td>
<td>(UQ, Pharmacologist/ Toxicologist)</td>
</tr>
<tr>
<td>Dr Suzanne Smith</td>
<td>(ANSTO, Expert in tailored radio tracer probe chemistry)</td>
</tr>
<tr>
<td>Dr Gordon Xu</td>
<td>(AIBN, Materials Chemist, LDH specialist)</td>
</tr>
<tr>
<td>Dr Katie Carrado</td>
<td>(Argonne Nat Labs, USA, Inorganic Chemist, synthetic hectorite and synchrotron specialist)</td>
</tr>
<tr>
<td>Dr Lawrie Gahan</td>
<td>(UQ Chemistry, cage ligand expert)</td>
</tr>
<tr>
<td>Mr Anthony Musumeci</td>
<td>(AIBN PhD student, radiolabelling materials chemistry)</td>
</tr>
<tr>
<td>Ms Gysell Broadhurst</td>
<td>(UQ Pharmacology PhD student, development of nanotox biological assays)</td>
</tr>
<tr>
<td>Ms Kayleen Campbell</td>
<td>Postdoc</td>
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</tbody>
</table>
Methodology

Effective use of radio tracer probes and study of kinetics, distribution and persistence in vivo (e.g., rats, fish)
Interactions with bio-molecules (plasma proteins, etc)
Interactions with cells (adherent vs suspension cell lines)
Interactions with other organisms (e.g., algae)

LDH
(+ve charge)
40-200+ x ~10nm platelets

HECT
(-ve charge)
25-200+ x ~1nm platelets

DWCNT
(neutral)
Variable length X 2nm

Octahedral layer
Interlayer anions
The Challenge?
Functional NPs for Toxicology Work Must Have:

Ability to track & image at ultra-low dosage in vivo (radiochemistry best?)

Good control of size and/or charge to study these effects on biodistribution/bioaccumulation and toxicokinetics

An understanding of NP stability in various *in-vitro*/*in-vivo* environments (pH, ionic strength etc)

An understanding of NP-biomolecule interactions

Biological (fluorescent?) probes for *in-vitro* cell studies

*Equivalent structure, props and behaviour* of new functional NPs with respect to original NPs of interest
Results – LDH Materials Chemistry/ Radiolabelling
Have done the most NP synthesis with LDH so far

Determined max “cold” Co and Ga allowable as isomorphous substitution into LDH lattice, without changing NP structure and properties

Successfully performed direct hydrothermal substitution of $^{57}$Co and $^{67}$Ga isotopes into lattice

Studied LDH chemical stability at various pH

Can now control LDH platelet dia/ thickness distribution very nicely (~40nm dia and up, 10-18nm crystallite thickness)

Explored the insertion of Tb into structure in an attempt to obtain fluorescence for biological work
This forms “pre-LDH”, then employ hydrothermal to produce well-ordered brucite-type structure

Xu, ZP; Stevenson, G; Lu, CQ; Lu, GQ (Max); Bartlett, PF; Gray, PP: “Stable suspension of layered double hydroxide nanoparticles in aqueous solution”. J. Am. Chem. Soc. 128, 36-37, 2006
RESULTS: LDH synthesis & characterisation

Monodisperse hexagonal LDH platelets (~65 nm)

Impurity free

Dynamic Light Scattering

Transmission Electron Microscopy

X-ray Diffraction
**Aim:** Incorporate $^{57}\text{Co}$ and $^{67}\text{Ga}$ into LDH structure to mimic behaviour and properties of $\text{Mg}^{2+}$ and $\text{Al}^{3+}$, respectively. LDH stability studies over a range of pH’s and tracking of the radioisotopic species using instant thin layer chromatography.

<table>
<thead>
<tr>
<th>Element</th>
<th>Ionic Radius / Å</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg</td>
<td>0.72</td>
</tr>
<tr>
<td>Co</td>
<td>0.745</td>
</tr>
<tr>
<td>Al</td>
<td>0.535</td>
</tr>
<tr>
<td>Ga</td>
<td>0.62</td>
</tr>
</tbody>
</table>

**Half life:**

$^{57}\text{Co} = 273$ d  \hspace{1cm} $^{67}\text{Ga} = 3.26$ d
Effect of “cold” Cobalt on LDH Nanoparticles

Particle Size

Minimal changes in measured solution particle size distribution up to a loading of 0.5% Cobalt

Some evidence of aggregation at 1% Cobalt loading

Zeta potential consistently implies a stable suspension has been formed

Zeta Potential

<table>
<thead>
<tr>
<th>Sample</th>
<th>Pure LDH</th>
<th>0.1% Co-LDH</th>
<th>0.25% Co-LDH</th>
<th>0.5% Co-LDH</th>
<th>1% Co-LDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zeta Potential (mV)</td>
<td>44.4</td>
<td>43.7</td>
<td>40.1</td>
<td>42.7</td>
<td>42.4</td>
</tr>
</tbody>
</table>
LDH Stability at Various pH – Co57 Leaching Studies

• Shows $^{57}$Co activity leaching via TLC separation technique at ANSTO
• NB only $2 \times 10^{-12}$ Moles of $^{57}$Co are required to achieve this activity signal
LDH Dissolution at Various pH

XRD and TEM confirm that $^{57}$Co leach rate over acidic pH’s corresponds with dissolution of LDH structure.
Particle Size Control, Hydrothermal Time or Choice of Counter-ion

0.5% Co LDH
Scale bar 200nm
Use Combination of Hydrothermal Time and Centrifugation to Control LDH Particle Size
LDH size Fractionation

Varying synthesis conditions + High speed centrifugation → Clay nanoparticles of tailorable dimensions

TEM images of fractionated LDH particles, (bottom right) DLS of fractionated LDH’s
**Fluorescent Labelling of LDH**

**Aim:** Develop a non-invasive fluorescent label for LDH nanoparticles

Terbium (Tb) is a rare earth element, from the lanthanide series. Tb$^{3+}$ is brilliantly fluorescent with a strong green emission line (543 nm)

<table>
<thead>
<tr>
<th>Element</th>
<th>Ionic Radius / Å</th>
</tr>
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<tr>
<td>Mg</td>
<td>0.72</td>
</tr>
<tr>
<td>Eu</td>
<td>0.947</td>
</tr>
<tr>
<td>Gd</td>
<td>0.938</td>
</tr>
<tr>
<td>Tb</td>
<td>0.923</td>
</tr>
<tr>
<td>Al</td>
<td>0.535</td>
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</tbody>
</table>
X-ray diffraction (XRD) revealed:

- Presence of a single crystalline LDH phase
- Increase in LDH interlayer spacing → Tb successfully incorporated!

Scherrer Equation:

\[
\text{Crystallite Size} = \frac{K \times \lambda}{\text{FWHM} \times \cos(q)}
\]

<table>
<thead>
<tr>
<th>Sample</th>
<th>d-spacing (Å)</th>
<th>Crystallite thickness (nm)</th>
<th>Aspect ratio (W/H)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO$_3$-LDH</td>
<td>7.60</td>
<td>18</td>
<td>4</td>
</tr>
<tr>
<td>0.1% Tb-CO$_3$-LDH</td>
<td>7.63</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>5% Tb-CO$_3$-LDH</td>
<td>7.79</td>
<td>11</td>
<td>17</td>
</tr>
</tbody>
</table>
Fluorescent Labelling of LDH

Fluorescence intensity too low for biological applications?

\[ \lambda_{\text{exc}} = 238\text{nm} \]
Progress Summary - HECT

Have done the most in-vitro biological work with HECT so far

Working with a number of commercial products (Laponite™, Lucentite™, etc)

Determined robust fluorescent label

Have begun studying the interactions with various cell types (adherent, non-adherent, etc)

Studies on biomolecule interactions (eg plasma proteins) underway

Hydrothermal synthetic work will commence after LDH work is completed
4 day incubation of differentiated THP-1 cells with Lucentite-SWN labeled with YOYO-1
(2pM of YOYO-1 per ug of hectorite, 8 ug of hectorite per 200k cells)

Fluorescent, white light and overlay images, Strong evidence of internalisation of HECT by macrophages

Rod Minchin and Zell Broadhurst, UQ Pharmacology
Zetasizer data for YOYO-1 labelled HECT and non-labelled HECT
(same particle size in solution)
Direct TEM Image – HECT Internalisation
(osmium tetroxide staining employed)
Where will they go *in-vivo*? Radiolabelling will help answer this question....
Synthetic and nano clays are a growing $132M business for which new approaches to risk governance are required in order to safely and responsibly transfer new technologies.

- Have synthesised LDH libraries of differing spatial dimensions.
- LDH dissolution characterised at a range of biologically relevant pH’s – confirmed by XRD and TEM.
- Radioisotopic labeling is an effective non-invasive way to label LDH nanoparticles (dual isotopic labelling possible).
- Demonstrated HECT internalisation by macrophage cells.
- This represents a sensible and thorough approach towards a more complete understanding of biological interactions of these NPs.
Acknowledgements

- Mr Anthony Musumeci
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- Dr Kayleen Campbell and Dr Lili Tan
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- Fellow ARC Centre of Excellence for Functional Nanomaterials researchers
- UQ Centre for Microscopy and microanalysis staff

Scholarships / Funding:
- University of Queensland Joint Research Scholarship
- AIBN – top up scholarship
- AINSE – post graduate research award
- AIBN Challenge Project funding
- Queensland state government – facilitation fund
- ARC Centre of Excellence for Functional Nanomaterials – project funding