NanoPack project – Biopolymer nanocomposite films for use in food packaging applications Final report

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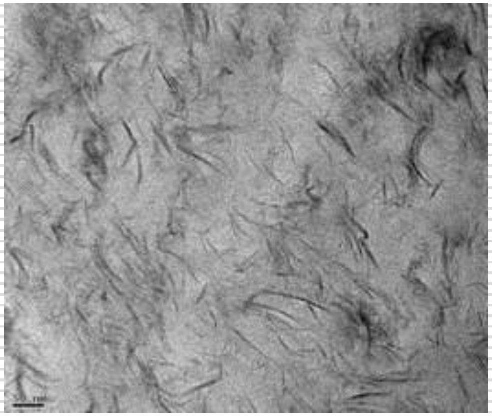

NanoPack overall goal

- Development of the technological basis for a cost-efficient production and use of biopolymer nanocomposites produced from renewable resources for use in the food packaging industry and which meet consumer requirements for functionality, sustainability and safety

NanoPack project objectives

- Development of PLA/montmorillonite clay nanocomposite films
- Development of PLA/layered double hydroxide (LDH) clay nanocomposite films
- Transfer of knowledge from laboratory to pilot scale
- Development of new analytical methods
- Knowledge dissemination

NanoPack – The project team

DTU

Risø DTU
National Laboratory for Sustainable Energy

Processing of nanocomposite films and their characterization

DTU
National Food Institute

KU-LIFE

David Plackett Vimal Katiyar

Food chemistry, toxicology and risk assessment of nanocomposites and their constituents

Jens H. Petersen, Mona-Lise B,.Erik H. Larsen, Anoop Sharma ,Bjørn Schmid

Hans Chr.B.Hansen, Jens Risbo, Christian B. Koch, Nathalie Gerds, Anna Svagan

Anionic clays

Film properties for food packaging

Carina G. Nielsen

FÆRCH PLAST

Thermoforming and tray production

DMRI
Danish Meat Research Institute

DANISH TECHNOLOGICAL INSTITUTE

Anette G. Koch Jens S. Jensen

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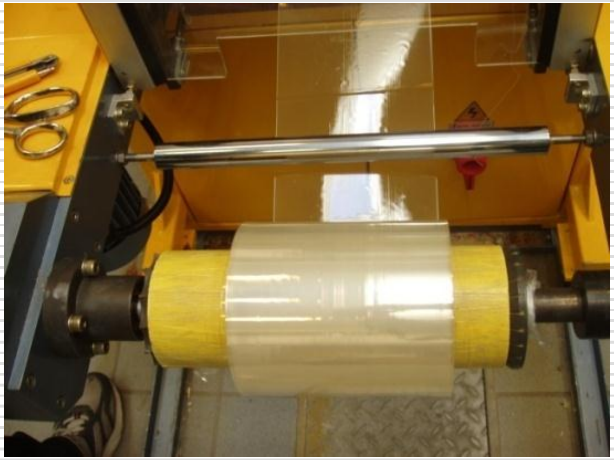
Research results

1. Materials preparation and film extrusion
2. PLA nanocomposite film characterisation
3. Nanoparticle charaterisation and migration studies
4. Toxicology
5. Meat packaging shelf life trial
6. PLA film coating technology



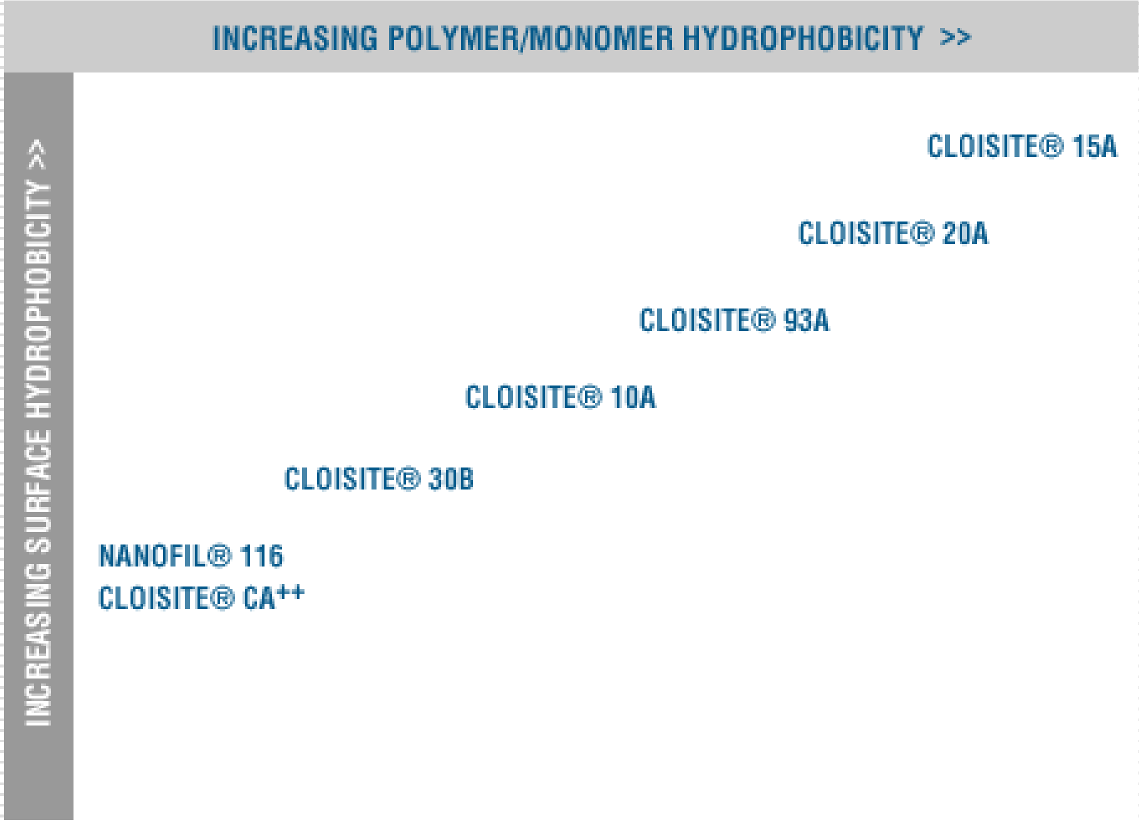
1. Materials preparation and film extrusion

Cooperation established in 2009 with Kunststof Kemi A/S, allowing access to facilities for PLA nanocomposite compounding and film extrusion

Material combinations were Ingeo™ 2003D PLA with 5% Cloisite™ 30B, chitosan-modified Cloisite Na^{+} or laurate-modified LDH clay

Kunststof Kemi twin-screw extruder (foreground) and co-kneader (background)

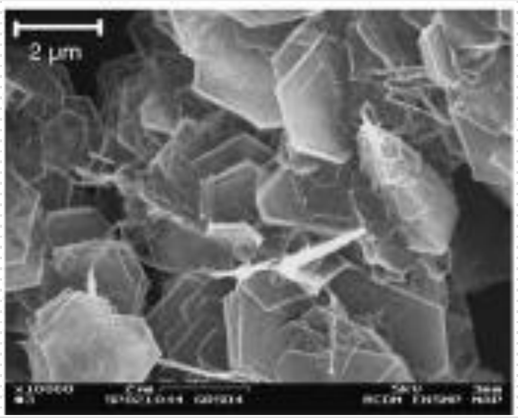
Cloisite™ clays (Southern Clay Products, USA

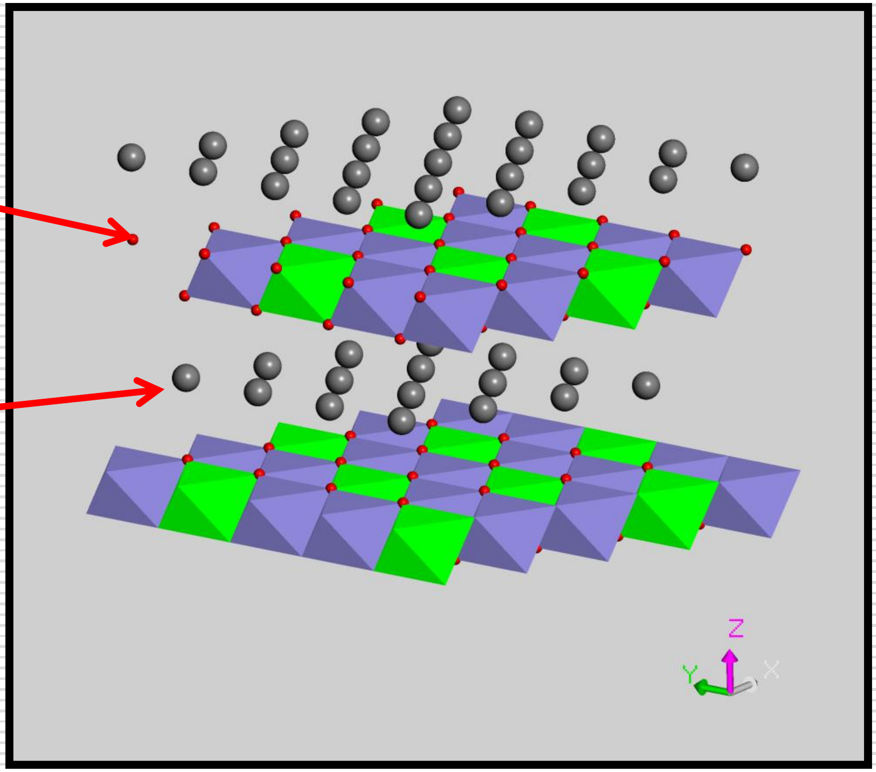


Layered double hydroxides

Positively charged mixed metal hydroxide layer

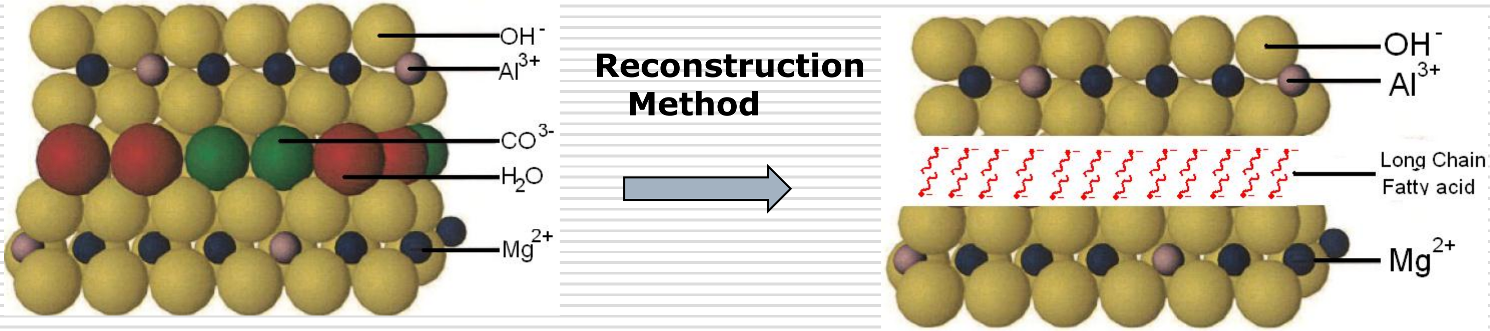
Negatively charged interlayer of anions and water



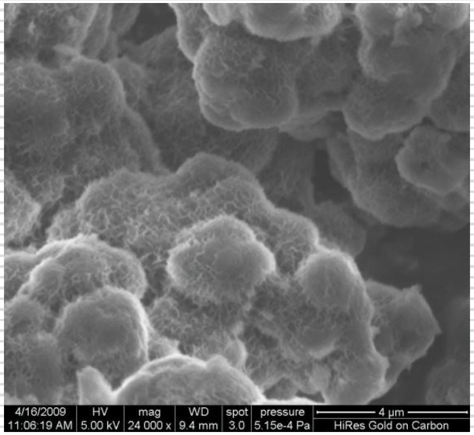


$$(Mg_{1-x}Al_x(OH)_2)^{x+}.(A^-)_x.nH_2O$$

Synthesis of long-chain carboxylic acid-modified LDH

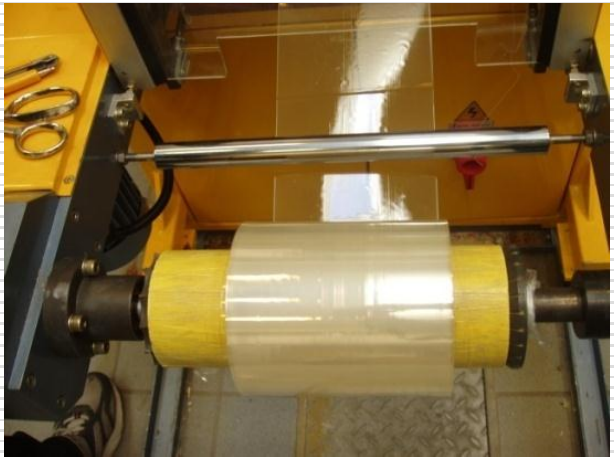


XRD shows an increase in basal spacing from 7.7 to 23.7 Å after the LDH laurate (C_{12}) synthesis from $LDHCO_3$



Film extrusion

After compounding, PLA and PLA/clay mixtures were processed using a single-screw extruder to generate films for subsequent testing and thermoforming into food packaging trays



Coherent films were obtained with all combinations with the exception of laurate-modified LDH, in which case difficulties were encountered which were consistent with PLA degrdation

2. PLA nanocomposite film characterisation

Molecular weight data

LDH-C12 introduces PLA chain scission reflected by lower molecular weights

Film raw material	Mw (Da)	Mn (Da)	Pd
PLA raw granulate	193,000	118,000	1.6
PLA pellets	165,300	99,600	1.7
PLA + 5%* Cloisite® 30B	153,900	98,300	1.6
PLA + 5% LDH-C12 (MB)	**123,400**	**60,900**	**2.0**
PLA + 5% LDH-C12 (direct mixing)	**50,700**	**25,700**	**2.0**

* Comparable data obtained for chitosan-intercalated Cloisite Na^+

PLA nanocomposite film characterisation

Relevant project publications

KATIYAR, V.; GERDS, N.; KOCH,C.B.; RISBO, J.; HANSEN, H.C.B.; PLACKETT,D. 2010. Poly-L-lactide nanocomposites via in-situ polymerization of L-lactide. *Polymer Degradation and Stability*, 95(12), 2563-2573.

KATIYAR, V.; GERDS, N.; KOCH,C.B.; RISBO, J.; HANSEN, H.C.B.; PLACKETT,D. 2011. Melt processing of poly(L-lactic acid) in the presence of organomodified anionic and cationic clays. *Journal of Applied Polymer Science*, 122 (1), 112-125.

SCHMIDT, B.; KATIYAR, V.; PLACKETT, D.; LARSEN, E.H.; KOCH, C.B.; PETERSEN, J.H. 2011. Migration of nanosized layered double hydroxide platelets from polylactide nanocomposite films. *Food Additives and Contaminants: Part A,* 28 (7), 956-966.

PLA nanocomposite film characterisation

Film type	**Oxygen permeability (OP)***
PLA (from dry granulate)	13.6
PLA (from processed pellets)	13.4
PLA-Cloisite™ 30B (direct mixing)	5.17
PLA-Cloisite™ 30B (from masterbatch)	7.22

* Units= $cm^3.mm.m^{-2}.day.bar$ at 23^oC/50%RH

~60% OP reduction when Cloisite™ 30B was directly mixed into PLA

PLA nanocomposite film characterisation

Film type	Water vapour permeability (WVP)*
PLA (from dry granulate)	6.5
PLA (from processed pellets)	5.8
PLA-Cloisite™ 30B (direct mixing)	3.8
PLA-Cloisite™ 30B (from masterbatch)	4.2

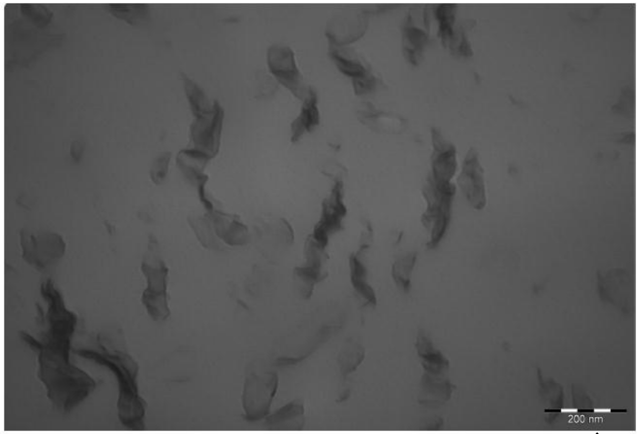
* Units= gm.mm.m^{-2}.day.bar at 38^{o}C/90% RH

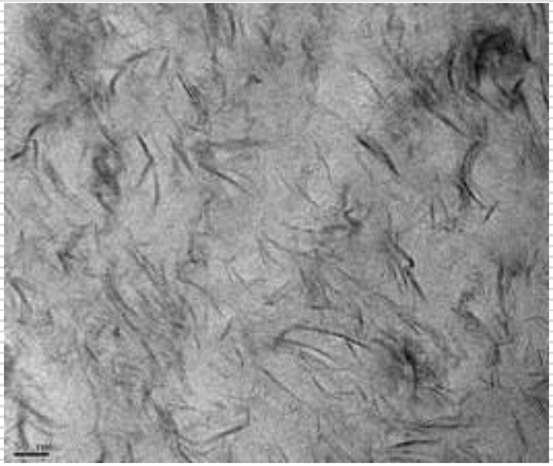
~40% WVP reduction when Cloisite™ 30B was directly mixed into PLA

Other PLA film properties

Thermal: DSC tests indicate films are largely amorphous. Addition of Cloisite™ clays causes a slight reduction in Tg and Tc but has very little effect on Tm

Mechanical: Addition of Cloisite™ clays had little significant effect on PLA tensile properties even though Cloisite 30B, for example, was well dispersed (see TEM below)





200 nm scale bar

50 nm scale bar

Summary of PLA film properties

1. Significant reduction in oxygen and water vapour permeability in PLA/Cloisite™ 30B films

2. No significant changes in thermal or mechanical properties

3. TEM suggests some nano-scale dispersion of Cloisite ™ 30B

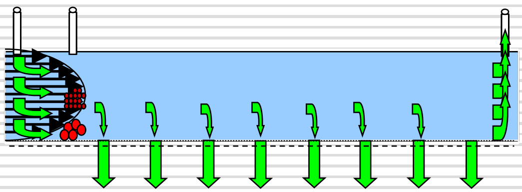
3. Nanoparticle characterisation and migration studies

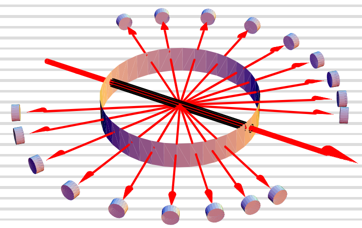
1. A multi-instrument technique was established for nanoparticle detection, size determination and chemical analysis

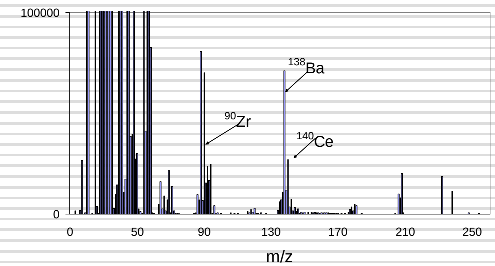
2. This technique (AFFF-MALS-ICP-MS) was validated and used to assess migration of clay particles from PLA films under conditions simulating exposure to foodstuff

3. The results indicated that clay nanoparticle migration did not occur when PLA/Cloisite™ 30B films were tested but did occur in PLA/LDH-C12 films in which PLA had been significantly degraded

Nanoparticle characterisation platform at DTU Food







Asymmetric flow field flow fractionation

Small NPs elute first

Optical detection (multi-angle and dynamic light scattering, UV and fluorescence)

Size determination (root mean square, hydrodynamic and geometric radius)

Inductively coupled plasma mass spectrometry (ICP-MS)

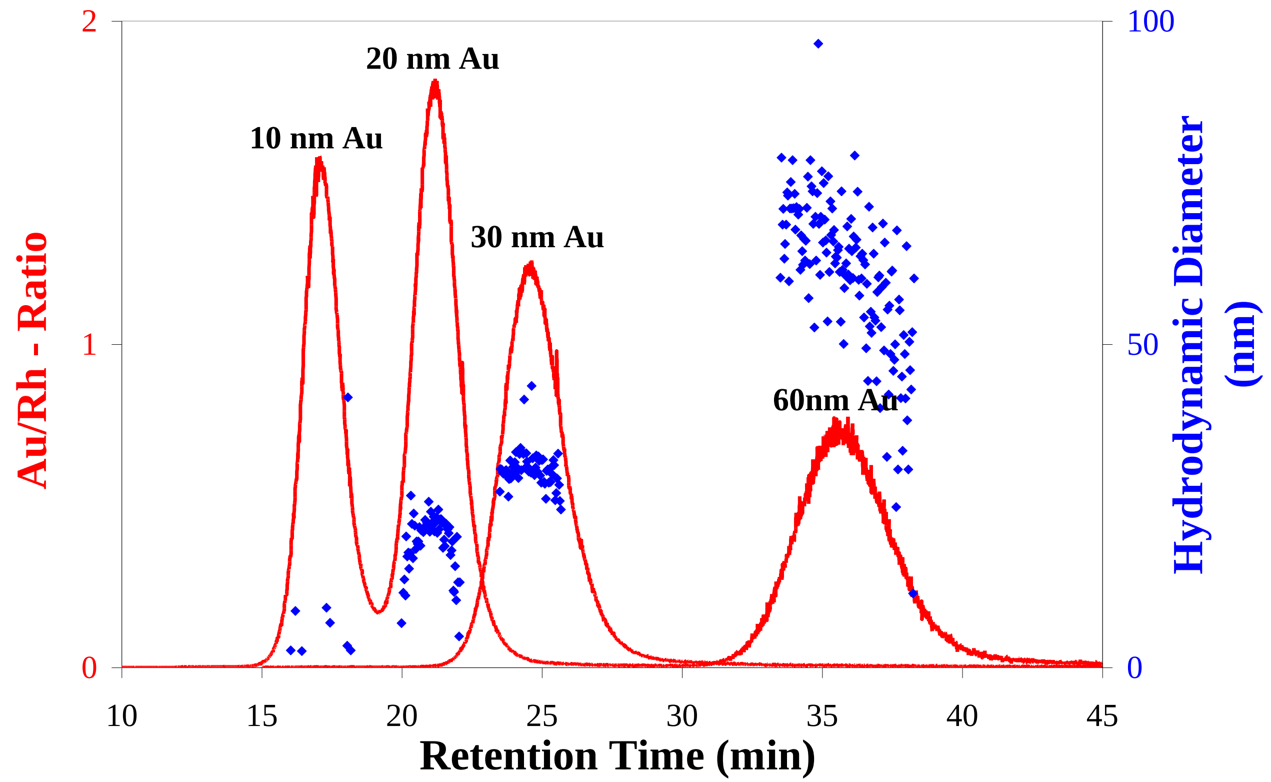
Elemental detection

Platform validation using gold nanoparticles

Separation of 3 gold sizes plus an overlay of a 30 nm Au NP single run

Red solid line: Au signal from ICP-MS

Blue Dots: Calculated hydrodynamic sizes by dynamic light scattering



References: Schmidt, B. et al. 2009. *Food Additives and Contaminants*: Part A, 26 (12), 1619-1627; Schmidt , B. et al. 2011. Analytical Chemistry, 83 (7), 2461-2468.

Migration experiments on PLA/nanoclay films

Migration study results

Total migration and acid digestion of migrates followed by ICP-MS analysis

Sample	Clay Load	Total Migration	Clay Det. ICP-MS
PLA	-	1.7±0.6 mg/dm^2	No
PLA/30B	5.0 %	6.7±0.5 mg/dm^2	No
30B Spike	1.9 mg	2.1±0.5 mg/dm^2	Yes
PLA	-	4.2±0.8 mg/dm^2	No
PLA/15A	5 %	11.5±1.9 mg/dm^2	No
15A Spike	2.8 mg	3.2±1.0 mg	Yes
PLA/20A	5 %	5.4±0.3 mg/dm^2	No
20A Spike	3.3 mg	3.0±1.3 mg	Yes
PLA-PF	-	2.5±0.6 mg/dm^2	No
PLA-PF3*	1.8 %	8.3±0.8 mg/dm^2	Yes
PLA-PF1*	1.8 %	9.6±1.9 mg/dm^2	Yes
PLA-PF2*	5.5 %	31.9±7.4 mg/dm^2	Yes
LDH Spike*	5.0 mg	5.2±0.2 mg	Yes

Notes on migration study results

1. Other Cloisite™ clays (15A and 20A) were included in this study and, as with Cloisite™ 30B, no clay migration could be chemically detected using ICP-MS

2. Total migration determined gravimetrically at levels below the permissible 10 mg /dm^2 was ascribed to PLA oligomers migrating from film samples

3. Film samples containing PLA-LDHC12 (PLA-PF series) showed chemical evidence for clay migration from the ICP-MS results

4. The validity of the method was apparent from the spiked reference sample results

4. Toxicology

- Toxicology studies were focused on Cloisite™ 30B nanoclay and its assessment by both *in-vitro* and *in-vivo* methods

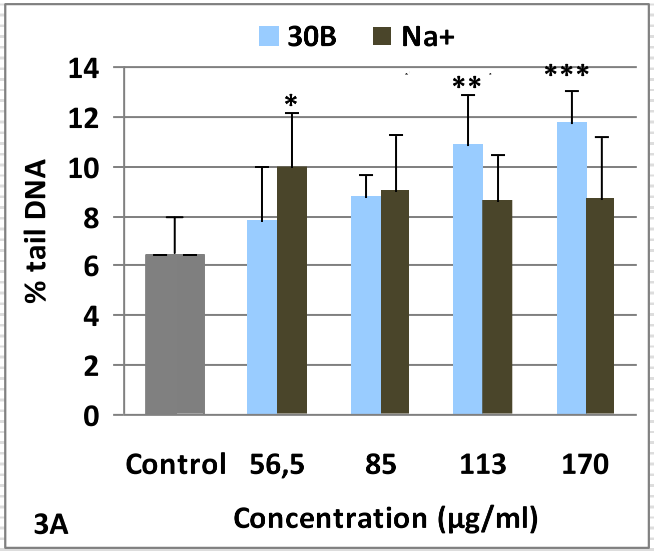
- A decision to proceed with these tests was made because although not fully meeting the targeted film barrier properties, this nanoclay was deemed to be the best choice at this stage in the project

In-vitro genotoxicity tests

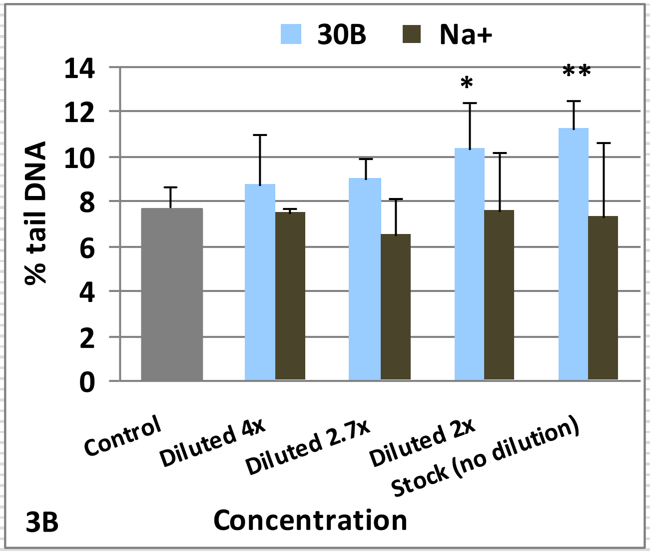
Suspensions of Cloisite® 30B and Cloisite® Na+ were not mutagenic in a *Salmonella*/microsome assay at the test conditions used

DNA damage by the Comet assay

Unfiltered suspensions



Filtered suspensions

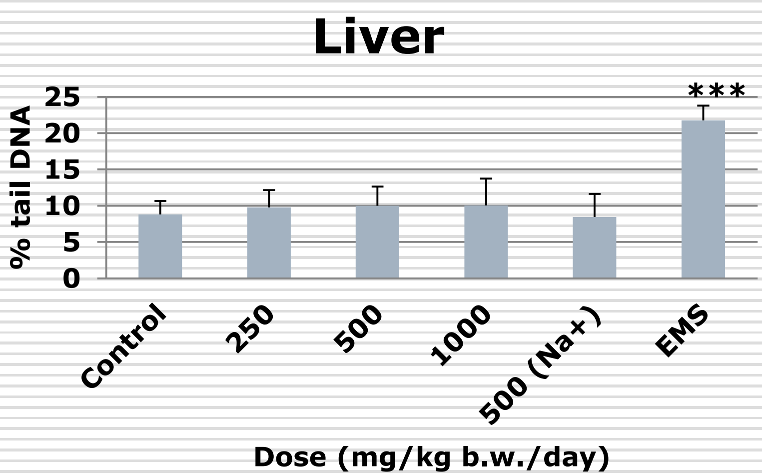


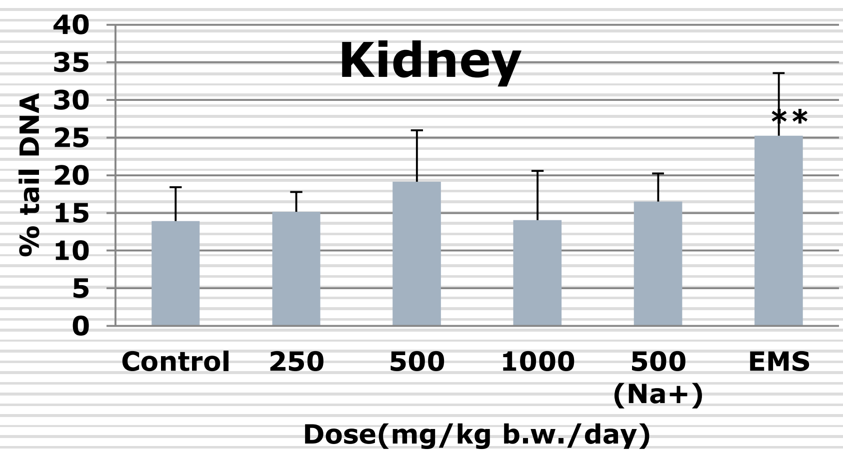
Cloisite Na^{+} was not genotoxic. Cloisite 30B in filtered* and unfiltered suspensions was genotoxic in the Comet assay (i.e., causes DNA damage)

Reference: Sharma et al. 2010. Genotoxicity of unmodified and organo-modified montmorillonite. Mut. Res. Mutagen. Environ. 700, 18-25

In-vivo genotoxicity tests

DNA damage by the Comet assay

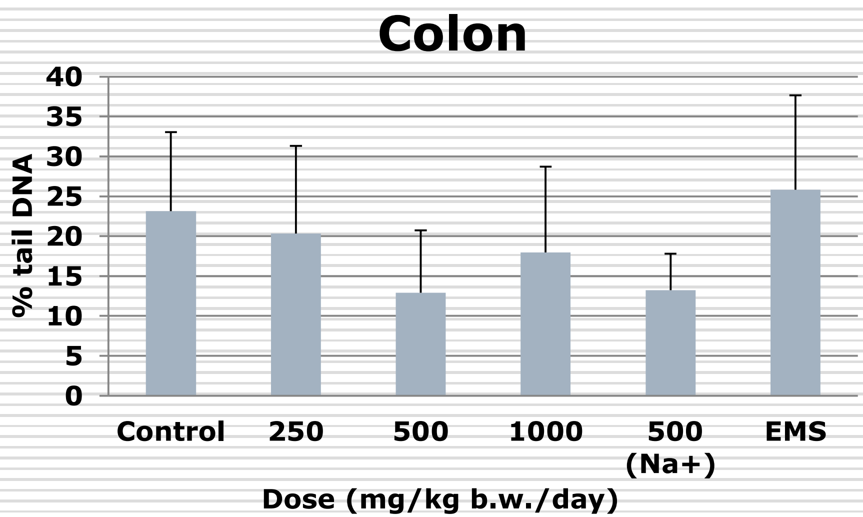




Cloisite™ 30B and Cloisite™ Na+ did not induce DNA damage in the *in vivo* Comet assay study. Cloisite™ 30B and Cloisite™ Na+ did not induce inflammatory or immuno responses in blood samples from rats

In-vivo genotoxicity tests

Similar results were obtained when testing colon cells from test rats using the Comet assay method



Notes regarding the genotoxicity studies

1. Although Cloisite™ 30B was genotixic as a result of *in-vitro* testing, this finding could not be confirmed by *in-vivo* tests
2. However, it should be noted that:

a) OECD guidelines require more strains to be tested

b) Further *in-vitro* tests should include liver and kidney cells and not only colon cells

c) Further work should determine whether the Cloisite™ 30B organomodifier causes DNA damage *in vivo*

d) Ideally, mutation frequency assays would be undertaken as they are more robust than the Comet assay

e) Although encouraging, the results obtained do not give the complete ”green light” to Cloisite™ 30B

5. Meat packaging shelf life trial on fresh and processed meat products

- Discoloration of cooked cured meat products
  - NitrosoMGb (pink)
  - MetMGb (greyish/brown)
- Discoloration of fresh meat
  - Meat color
    - MetMGB (brown)
    - OxyMGB (red)
    - DeoxyMGB (purple)
- Oxidation of fat and protein
  - Taste/smell (rancidity)
- Microbial growth
  - Pathogens
  - Spoilage bacteria/fungi

Meat packaging shelf life details

- **Products:**
  - Pork chop and sliced saveloy

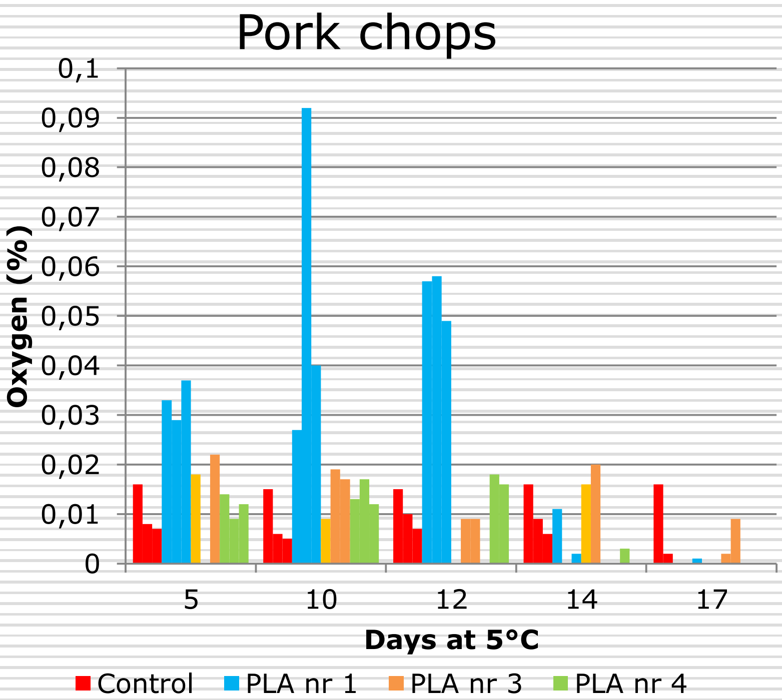
- **Packaging:**
  - Control (low OTR)
  - PLA
  - PLA (5% Cloisite 30B)
  - PLA (5% Cloisite/chitosan)
  - Modified atmosphere: 30% CO_2/70% N_2

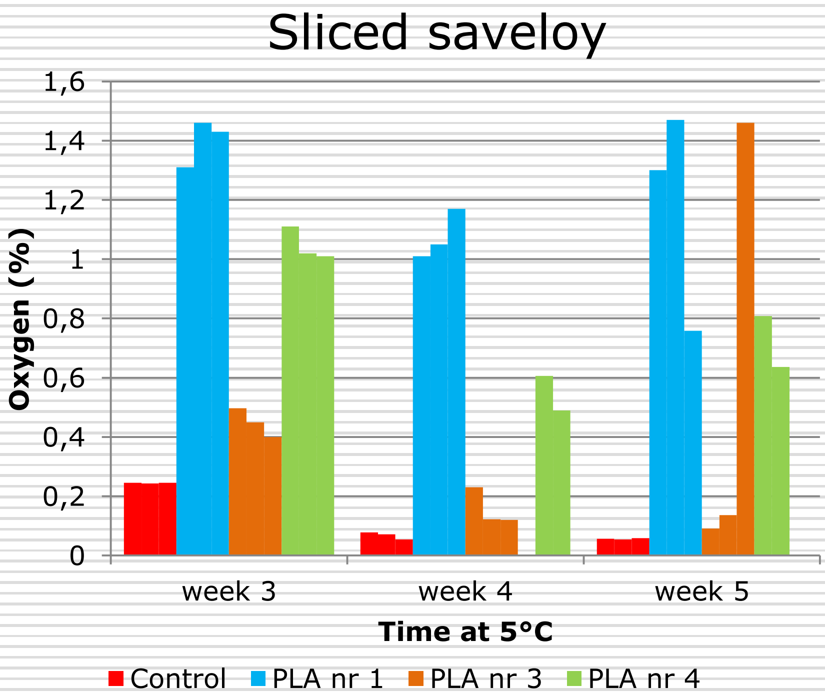
- **Storage:** 5°C for 2 weeks (pork chop) or 5 weeks (saveloy)

- **Light:** 7 am – 7 pm; appr 1200 lux (pork chop every day; saveloy 1 week)

Oxygen in the packages during storage

- The O_2 barrier was improved in PLA with added nanoclay
- No rancidity was observed in pork loin and saveloy samples





Overall results of the meat packaging shelf life trial

Color maintenance of fresh meat and processed meat is affected by oxygen

Residual oxygen, headspace, OTR, light (brown, loss of redness)

PLA with nanoclay had and improved O_2 barrier but effects were small in practice

Oxidation is affected by packaging but also by anti-oxidative ingredients

Rancidity = oxidation of proteins and fat

No rancidity observed in any packages of pork chop or sliced saveloy.

Microbial growth is affected by oxygen residual content but preservatives and temperature have a greater impact

The improved barrier did not affect the microbiological shelf life of pork chop and sliced saveloy stored at 5°C.

Unintended migration

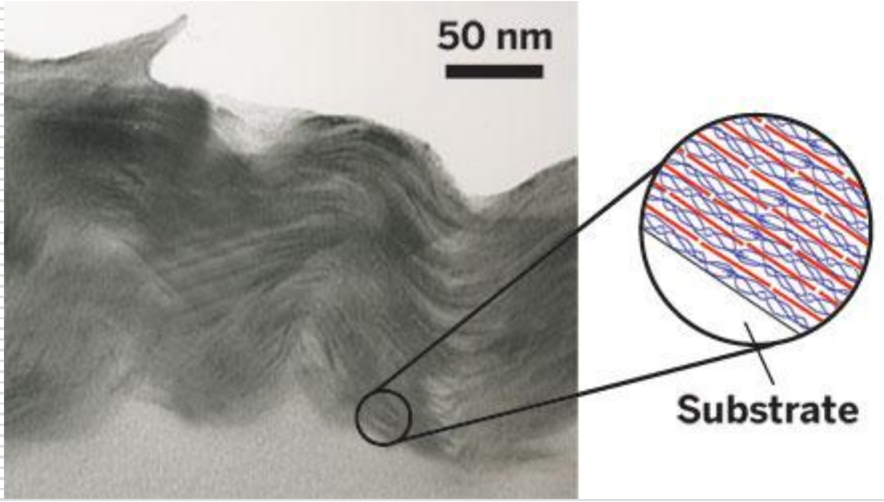
PLA with the chitosan-modified Cloisite ™ resulted in a smell of tartare sauce/curry in packages containing saveloy, pork chop and pure water.

Summary: Use packaging material with low OTR but ensure the other parameters also are optimized

6. Film coating technology using layer-by-layer (LbL) technique

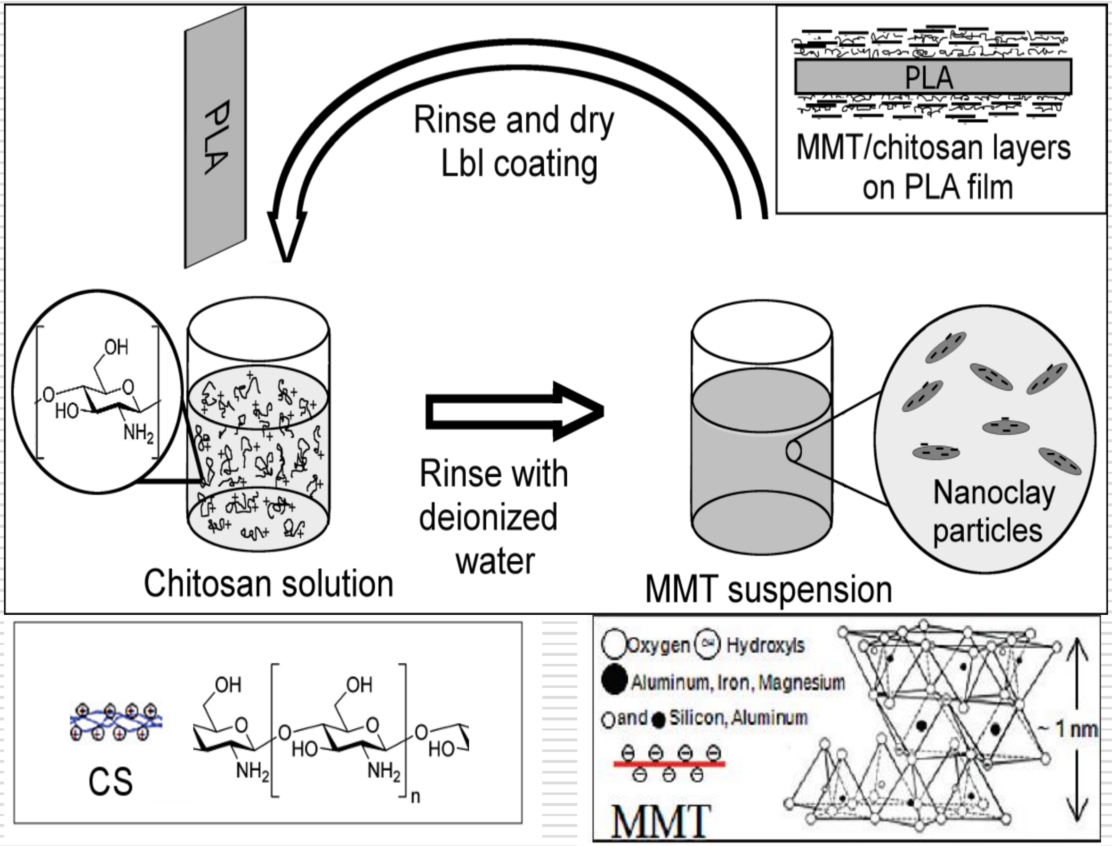
Example from the literature

LbL electrostatic assembly using alternating application of NaMMT and polyethyleneimine with thickness adjustable by pH control

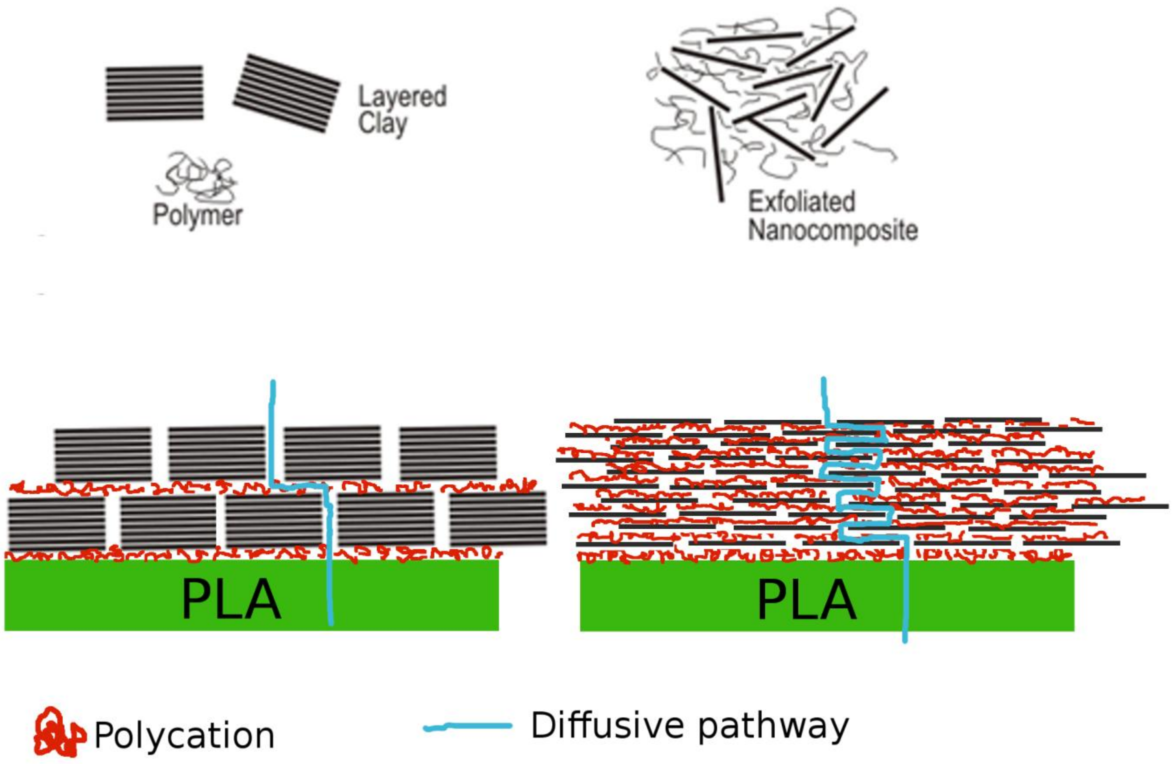


Reference: Priolo et al. 2010 Transparent clay-polymer nano brick wall assemblies with tailorable oxygen barrier. *Applied Materials and Interfaces*, 2, 312-320.

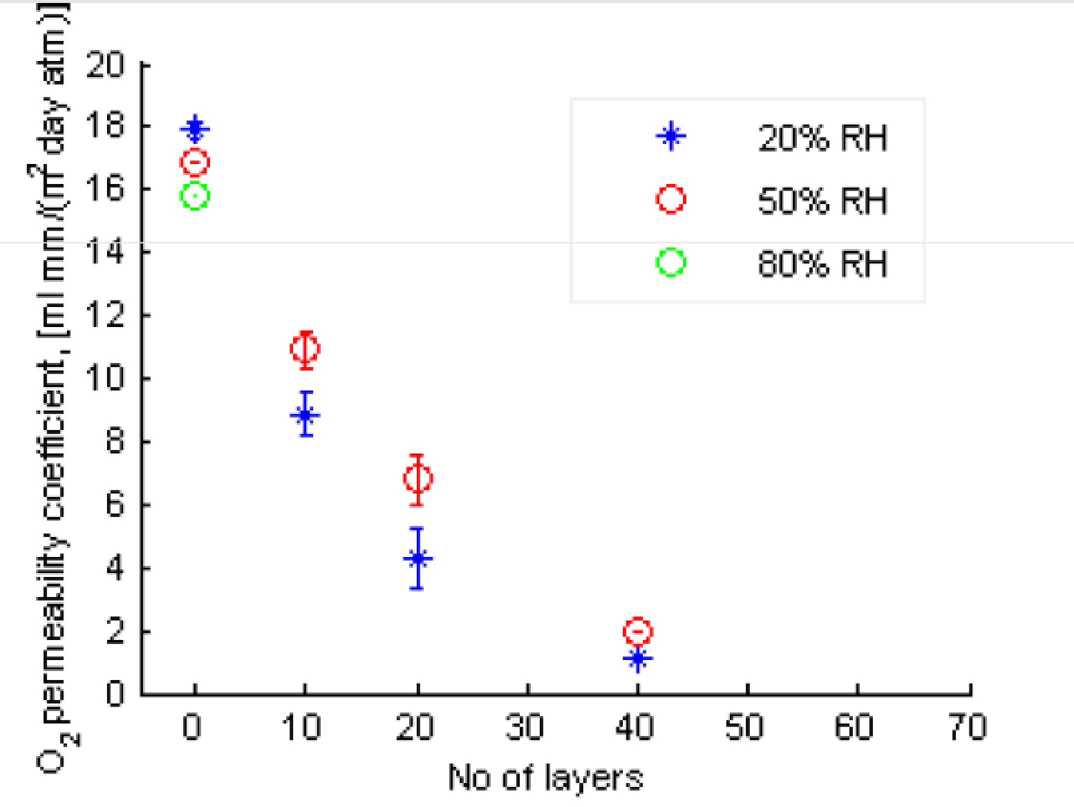
LbL approach adopted in the NanoPack project



Principle of LbL approach used in the NanoPack project



Oxygen permeability of LbL-coated PLA films

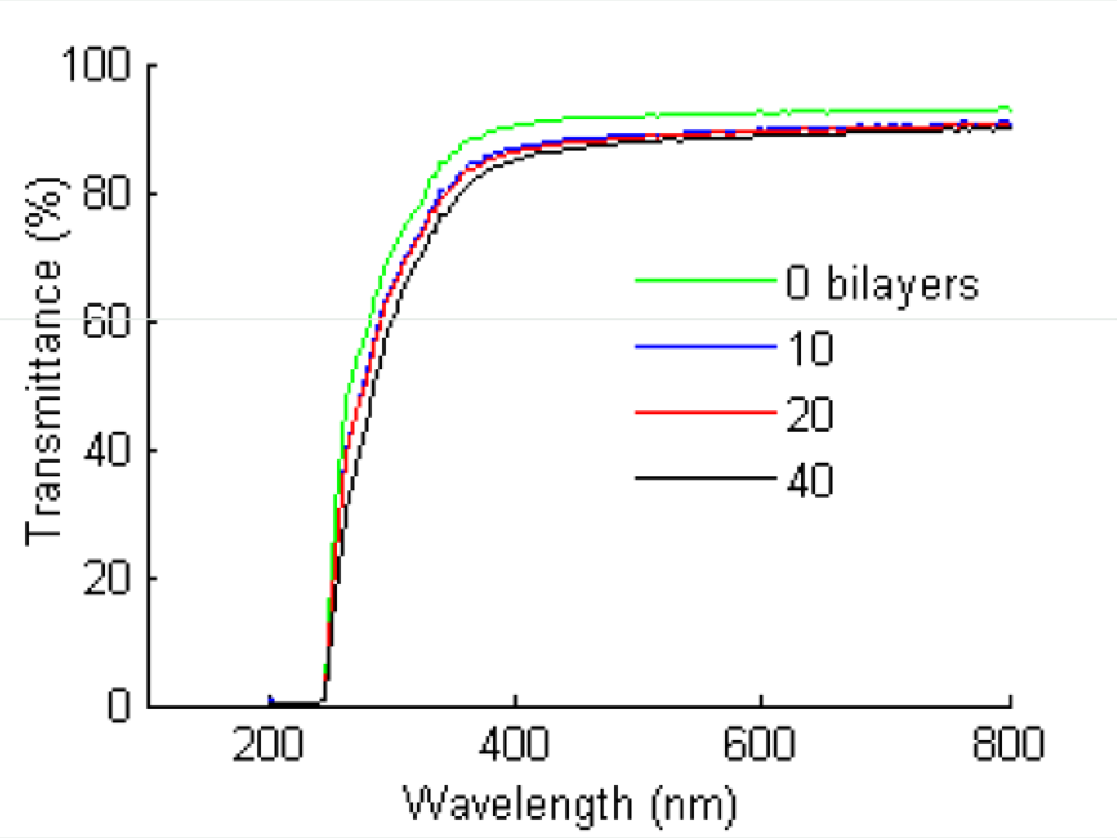


Measurements performed at 23°C

Transparency of LbL-coated 400 µm PLA films

Remaining challenges:

1. Thermoforming
2. Water vapour barrier



Research results summary

- Oxygen and water vapour permeability of PLA films could be reduced by ~60% through addition of Cloisite™ 30B nanoclay
- A multi-instrument technique was established and applied to clay migration studies, which showed no evidence for migration of Cloisite™ 30B when films were exposed to a food simulant
- *In-vitro* and *in-vivo* studies indicated that Cloisite™ 30B is not genotoxic
- A meat packaging shelf life trial showed that best overall results were obtained when using reference PET trays
- Research on use of an organomodified LDH incorporated in PLA revealed significant melt processing problems due to degradation of PLA
- In contrast to melt extrusion of PLA/nanoclay mixtures, the use of a layer-by-layer coating method allowed the target reduction in oxygen permeability to be achieved

Commercial-societal results

Although the project did not lead to new patentable or licensable technology, it did identify four areas of new knowledge which may be of benefit to business and society in the future:

1. **Business**: Layered double hydroxides (LDHs) could still be useful PLA-enhancing additives but further research is needed to enhance melt-processing stability.
2. **Business**: The LbL method looks promising as a commercially viable process providing at least two key processing questions can be addressed.
3. **Society**: The project has demonstrated an effective method for nanoparticle characterisation and confirmed its suitability for food contact migration studies.
4. **Society**: Nanoparticle toxicity has become a more important topic over the years of the project and results of the project have contributed significantly to this field.

Educational aspects

PhD 1: Bjørn Schmidt (DTU) Development of a quantitative method for characterising nanoparticles: Validation and application. Defence due early 2012.

PhD 2: Nathalie Gerds (KU) Synthesis and characterisation of organomodified layered double hydroxides for use as nanofillers in polylactide films. Defence due early 2012.

Post Doc 1: Vimal Katiyar (Risø DTU). Synthesis, characterisation and processing of PLA/nanoclay combinations. Current employment: Assistant Professor, IIT Guwahati, India.

Post Doc 2: Anna Svagan (KU/Risø DTU). Characterisation of PLA/nanoclay films and LbL coating method development. Starting Danish Science Council-funded post doc employment at KU in January 2012.

Assessment of cooperation

Internal: Six-monthly project review meetings worked well and were held at all the partner locations. When needed, sub-project meetings were also held. A project teamsite was effectively used to communicate all project information.

Inter-partner cooperation also worked well and the involvement of Færch Plast A/S in the project was a particularly positive aspect. Possible improvements could have included more discussions at the work package level and more interaction with the advisory board.

National/International: Assistance from Kunststof Kemi A/S with processing trials in 2009-2010 was pivotal to the success of the project. Cooperation with CEN DTU and KIT, Karlsruhe was very positive and was of considerable benefit in regards to materials investigations using microscopy. Frank Friedrich from KIT visited Risø DTU for three months in 2009 as part of this cooperation.

Information dissemination - publications

Published

1. Schmidt et al. 2009. *Food Addit Contam,* 6, 1619-1627
2. Sharma et al. 2010. *Mutation Research/Gen Tox Env Mut*, 700, 18-25
3. Katiyar et al. 2010. *Polym Degr Stab*, 95, 2563-2573
4. Schmidt et al. 2011. *Analyt Chem*, 83, 2461-2468
5. Katiyar et al. 2011. *J Appl Polym Sci*, 122, 112-125
6. Schmidt et al. 2011. *Food Addit Contam, 28,* 956-966

In progress

1. Svagan et al. 2012. *Biomacromolecules*, returned and under revision
2. Gerds et al. 2012. *Polym Degr Stab*, to be submitted December 2011
3. Sharma et al. 2012. To be drafted
4. Petersen et al. To be drafted
5. Koch et al. To be drafted
6. Katiyar et al. First draft prepared
7. Gerds et al. Submitted to *Appl Clay Sci* and presently under revision

Information dissemination – project conferences

1. March 2009: Mid-way seminar held at Risø DTU with ~40 in attendance

2. October 2011: One-day conference held in Copenhagen with support from *Polymer Teknisk Selskab* and ~45 in attendance plus international invited speakers

Overall assessment

Project accounting: Project accounting was undertaken with assistance and key input from Helen Vagner Jørgensen at Risø DTU. Helen regularly attended the six-monthly project meetings and acted as the liaison with the partners on all financial issues

Project impact: The main impact of the project will be in terms of new knowledge in key areas concerning PLA/nanoclay composites and groundwork for new developments/projects in bio-packaging materials