

BELSPO Project Report

Project: Nano-reinforced Bioplastics for Active Packagings (NanoBioPack)

Researcher: Dr.Sunil P.Lonkar

Host: Prof. Philippe Dubois

Research Place: Laboratory of Polymeric and Composite Materials (SMPC), University of Mons, Mons-7000, BELGIUM

Objectives of the research:

- **To develop nano-reinforced biodegradable polymeric materials with improved gas barrier, antimicrobial and biodegradability properties suitable for active packaging applications.**

PART-I

Multifunctional bionanocomposites of Polylactic acid and Ferulic acid intercalated layered double hydroxides for active packaging applications

Research and development of packaging materials and packaging technology have shown a clear and irreversible trend toward “green” and active packaging. Recent studies on packaging materials have emphasized the use of biobased materials in conjunction with nanotechnology. Hence, in present study we explored a concept of using bionanohybrids and their composites with biobased polymer for active packaging applications. A bioactive molecule, ferulic acid is successfully intercalated into the galleries of layered double hydroxide (LDHs) type nanoclay. The resulting bionanohybrid is used as multifunctional reinforcement to biobased polylactic acid (PLA). The intercalated biomolecules are expected to prolong the bacterial growth in PLA films, their high hydrophobic nature may facilitate the filler dispersion and simultaneously the high aspect ratio of LDH inorganic nanosheets can be reinforcing the polymer matrix. Also, the presence of long range nanoclays is expected to improve the oxygen barrier. Hence, the resulting composites are envisaged as environment friendly active packaging materials.

The objectives of this part were achieved in three different sections:

1. Design, synthesis and characterization of active multifunctional layered nanofillers for polymer reinforcement:

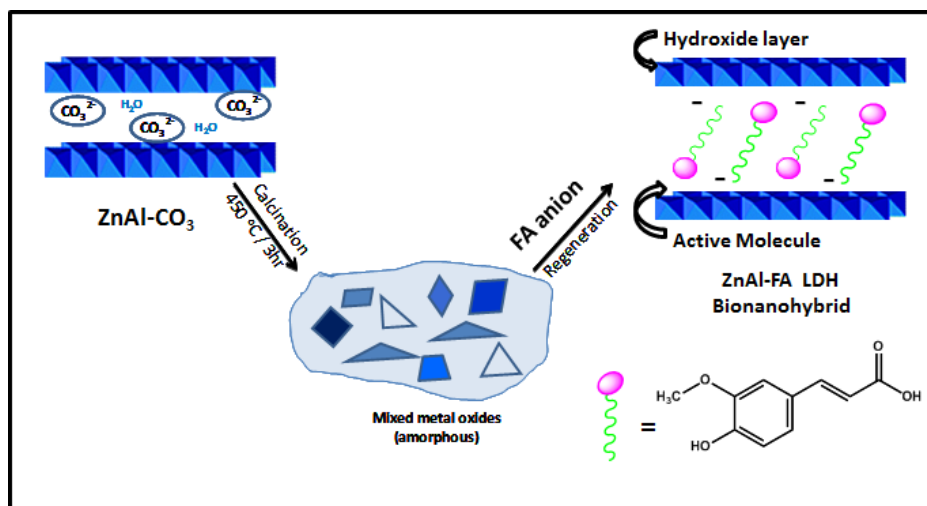
1.1 Synthesis

The selection of right nanofiller is very critical to achieve the highest possible multifunctional properties into polymers. The properties of nanofillers like dimensions, toxicity, aspect ratio, capability of host-guest interactions and active functionality etc. are of key importance to achieve a better performance in packaging applications. In present study, based on our expertise and literature survey we have selected a nanomaterial which fits into aforementioned criteria i.e

Layered double hydroxides (LDHs). This anionic nanoclay was further functionalized using Ferulic acids (FA), a naturally occurring biomolecule which possesses significant activity like potential antimicrobial, antioxidants and antifungal.

The approach was to intercalate FA active molecules into the galleries of layered double hydroxides (LDHs) in order to render LDHs more functional and to increase FA stability and longevity. The resulting bionanohybrids was assumed to be potentially active and can be used as multifunctional nanofillers for bioplastics. We believed, the finely dispersed layered inorganics can reinforce the polymer matrices and can induce barrier properties through their high aspect ratio. Moreover, intercalated FA molecules can impart antimicrobial and antioxidant properties to the polymer. So we have carried out the synthesis of such FA intercalated ZnAl-LDH bionanohybrids via regeneration approach and detailed synthetic methodology was given below:

- a. **Synthesis of ZnAl-CO₃ LDH:** The ZnAl-CO₃ LDH was synthesized by using urea hydrolysis method. An aqueous solution of Al³⁺ and Zn²⁺ with the molar fraction Al³⁺/ (Al³⁺+Zn²⁺) equal to 0.33 was prepared from hydrated AlNO₃ and ZnNO₃ in water. To this solution solid urea was added until the molar fraction [urea/ (Zn²⁺+Al³⁺)] reached 3.3. The resulting clear solution was refluxed for 36 h. The white precipitate was then filtered, washed until chloride free and dried at 60 °C till constant weight.
- b. **Preparation of ZnAl- FA LDH:** The synthesized LDH was first calcined in a muffle furnace at a temperature of 450 °C for about 3 h to convert the layered hydroxide material into its oxide form, which was named CLDH. Aqueous solution of FA anions was prepared by dissolving the calculated amount of antioxidant (for theoretical 100% anion exchange) and 0.1M NaOH. The pH of all the solution was maintained about 8 before further treatment. The calcined material (CLDH) was then dispersed in the FA solution with constant stirring, which was continued for more than 24 h at ambient temperature to ensure complete regeneration of the LDH structure. Finally, the residues were separated and washed with distilled water several times to get rid of unexchanged antioxidant molecules. The solid was then dried in oven at about 60 °C till constant weight. The schematic representation of regeneration approach is shown in **Scheme 1**.



Scheme 1: Synthesis of ZnAl-FA LDH bionanohybrids via regeneration approach

1.2 CHARACTERIZATIONS:

The successful intercalation of ferulic acid into LDH galleries was confirmed by various physico-chemical characterization techniques like FT-IR, TGA, X-ray diffraction, scanning electron microscopy, antioxidant activity and structural modelling etc.

1.2.1 FT-IR Spectroscopy:

The molecular structure of resulting nanohybrid materials were characterized by FT-IR spectroscopy as shown in **Fig.1a**. The parent LDH i.e ZnAl-CO₃ LDH shows characteristic peaks associated with such materials. The strong and broad absorption band in the frequency range of 3600-3200 cm⁻¹, centered around 3470 cm⁻¹ can be assigned to the stretching vibrations of the hydroxyl groups of in the brucite-like layers and water in the LDH galleries. The sharp band around 1368 cm⁻¹ corresponds to the symmetric (ν_3) stretching vibration of interlayer CO₃²⁻ groups. In the lower low-frequency region, the bands observed are associated to the lattice vibration modes and can be attributed to M-O from 850 to 600 cm⁻¹ (M = Al, Zn) in the LDH sheets. After calcination most of the stretchings have disappeared confirming the collapse of layered structure and formation of mixed metal oxides. The regeneration leads the reconstruction of layered structure with ferulic acid as an anion. In the spectrum of regenerated nanohybrid i.e ZnAl-FA LDH, most of characteristic vibration bands associated with the FA anion like -C-H, - and C=C are present besides the characteristic peaks of parent LDH. The new peaks emerged such as symmetric and asymmetric modes of carboxylate (-COO) stretching vibrations at 1584 cm⁻¹ and 1695 cm⁻¹, respectively. This collective information indicated that FA molecules were combined within LDH layers through electrostatic interactions. However, the presence of the carbonate band at 1368 cm⁻¹ indicates carbonates accompany FA anion in the intergallery of regenerated LDH.

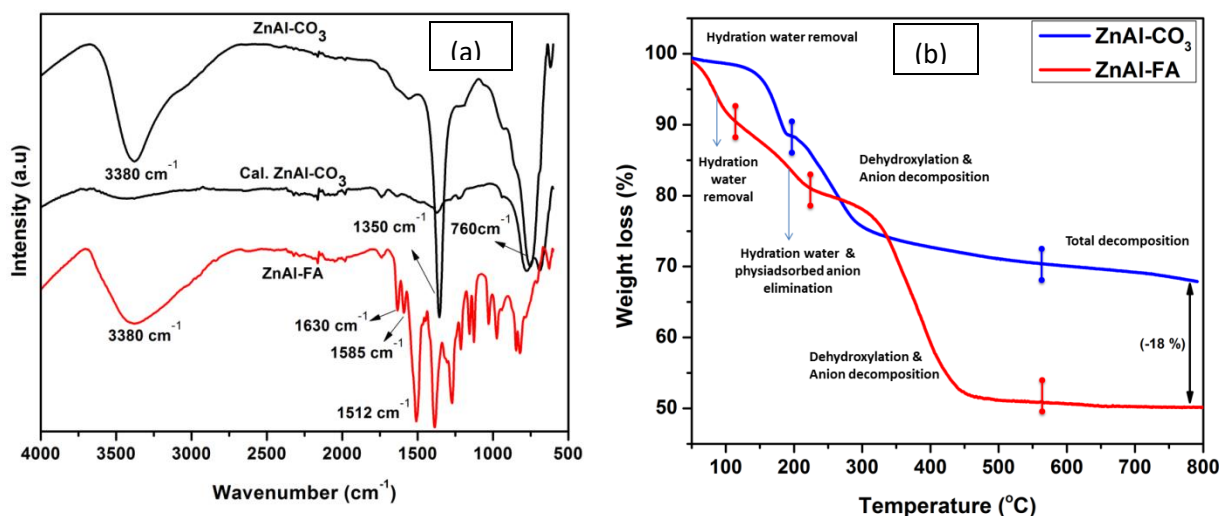


Figure 1: a) FT-IR spectroscopy of FA-LDH bionanohybrids, b) Thermogravimetric analysis of ZnAl-CO₃, Cal. ZnAl-CO₃ and ZnAl-FA LDH

1.2.2 Thermogravimetric analysis (TGA):

Figure 1b shows the thermal decomposition of pristine CO_3 and FA modified LDHs. It is evident that the TG curves displaying four thermal events, the first one is generally attributed to hydration water elimination while the second one to attributed hydration water and physisorbed anions removal. The third thermal event corresponded to the dehydroxylation of the laminae and expulsion of the anions located in the interlayer region. Finally, the fourth thermal event was assigned to the total dehydroxylation and expulsion of the remaining interlayer anions. However, the decomposition behaviour was altered by presence of FA, the CO LDH shows decomposition at 280°C and FA-LDH decomposing at 382°C which exhibit that presence of ferulic acid increase the thermal stability of LDH structure. Also, the total weight loss percentage decrease (-18%) confirms the presence of FA into LDH galleries.

1.2.3 X-ray diffraction analysis:

XRD analysis is a very important method to reveal the crystalline changes in intercalation of large size anions into layered nanostructures. XRD patterns of ZnAl-CO_3 LDH, C-LDH, and ZnAl-FA LDH are shown in Fig 2a. The ZnAl-CO_3 , a LDH precursor exhibits typical characteristics of the layered crystalline LDHs phase at (003) and (006) diffraction peaks with basal spacing value (d_{003}) of 0.65 nm. In XRD pattern of CLDH, no characteristic reflections were observed because during the calcination, LDH is converted into a total amorphous material, which is commonly referred as mixed oxide of Zn and Al. After reconstruction (ZnAl-FA LDH), the crystal structure of LDH appears to be considerably modified. The positions of the basal reflections in FA modified LDH were shifted towards higher d value indicating the expansion of the interlayer gallery distance through an accommodation of FA anions. Also, in compared to the parent ZnAl-CO_3 LDH, a slight broadening in each reflection, especially the (00 l) ones are noted. This might be related to a partial loss of crystallinity or turbostratic disorder, as is commonly observed in the intercalation of large anions into LDHs. It is noteworthy that basal spacing d_{003} is expanded by 1.52 nm after intercalation of ferulic acid anions. Hence, the XRD results clearly reveal the successful intercalation of FA anions into the LDH galleries.

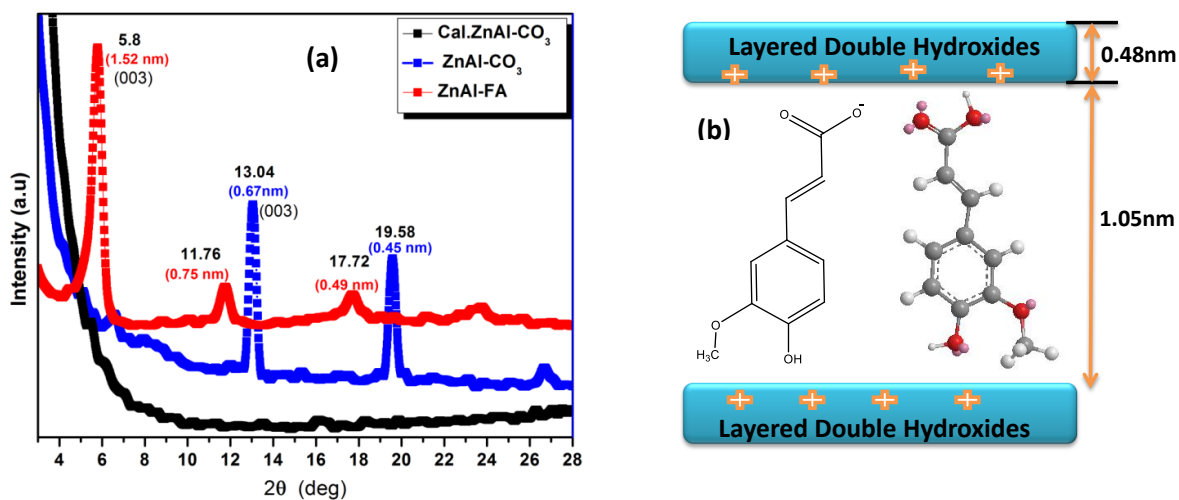


Figure 2: a) Powder X-ray diffraction pattern for ZnAl-CO_3 , Cal. ZnAl-CO_3 and ZnAl-FA LDH, b) Figure 5: Possible arrangement of FA anions in the interlayer galleries of the LDH

1.2.4 Structural Modeling:

In order to understand the arrangement of intercalated FA anion into LDH galleries, a structural modelling experiment was carried out. The intercalation compound formed by regeneration of LDH with FA was assumed to adhere the space-filling postulate. The energy minimization procedures were carried out after the molecular model of FA was drawn in ChemDraw Ultra 12, to give the most stable configuration with minimum energy (5.33 kcal/mol) as shown in Fig2b. Then, the length of the FA anion in its longest direction was calculated using ChemDraw Ultra 12; the value obtained was 0.95 nm. The XRD data indicate that the basal spacing of ZnAl-FA LDH is 1.52 nm. Subtracting the thickness of the LDH layer (0.48 nm), the gallery height of LDH is 1.05nm. This suggests that the FA anions are arranged in a monolayer into hydroxide layers. The excessive space (0.1 nm) is accounted for the length of the hydrogen bonds established between the anions and the LDH hydroxyl groups. According to the above analysis, a possible arrangement of the FA anions in the interlayer space.

1.2.5 SEM analysis

The morphology of resulting bionanohybrids was studied by scanning electron microscopy and displayed in Fig.3. It can be seen that the parent ZnAl-CO₃ LDH exhibit a regular hexagonal platelet structure which is characteristic for LDH type particles. The regularity of the LDH particles confirmed the fine crystallinity as evidenced by X-ray measurement. The thickness of these platelets is around few hundred nanometers and the lateral dimension is in the range of 5 micron. After organo-modification with ferulic acid (ZnAl-FA LDH), the morphological features seem quite similar that to parent LDH but better constructed as individual hexagonal platelets. However, the shape of the particles seems slightly changed in comparison to the unmodified LDH. This was probably due to the hydrophobic interaction of the LDH surface-adsorbed FA particles. The size and interlayer arrangements of antioxidant anions may be a potential factor that influences stacking and growth of the metal hydroxide layers during the regeneration process. Overall, it can be said that such methods not only regenerates the metal hydroxide sheets, but also the plate-like geometry of the primary particles. Conclusively, the nanohybrid structure of ZnAl- FA LDH was confirmed by SEM analysis.

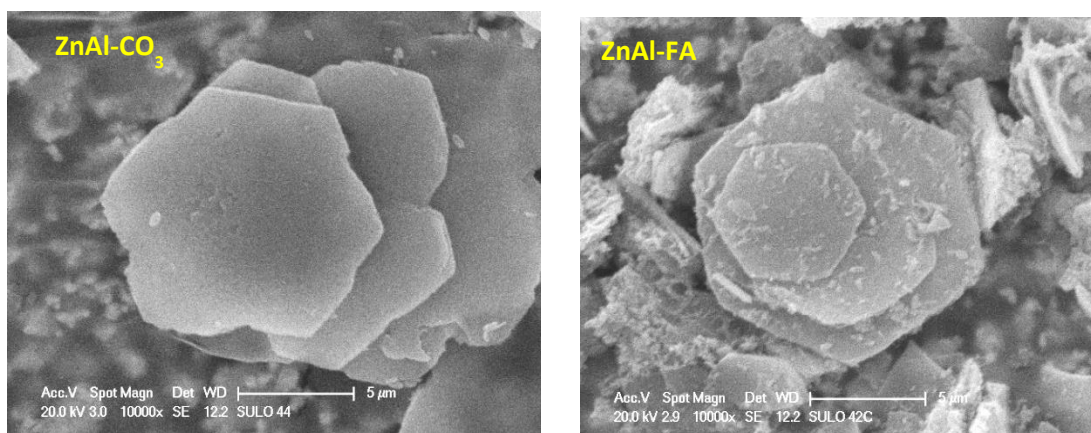


Figure 3: SEM micrograph for pristine LDH (ZnAl-CO₃ LDH) and functionalized LDHs (ZnAl-FA LDH)

1.2.6 Antioxidant activity of LDH/FA nano hybrids

The Ferulic acid is considered as one of the most active natural antioxidant. It is therefore, important to investigate whether the FA retain their chemical integrity, in terms of antioxidant activity, upon intercalation in the LDH galleries. In order to assess the radical scavenging activity, a DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is often used. It can be seen in Fig. 4(i) that the absorption maximum of a stable DPPH radical in ethanol was measured at 517 nm in UV-Vis spectrum. In presence of ZnAl-FA LDH Fig. 4(ii), the decrease in absorbance of DPPH radical is observed mostly because of the reaction between antioxidant molecules and radical, progresses, which results in the scavenging of the radical by hydrogen donation from FA. Moreover, another important evidence is colour change, a deep violet colour of DPPH radical is gradually changes yellow when the radical is scavenged by potential antioxidant. Here too it is visually noticeable that the colour has been changed from purple to yellow.

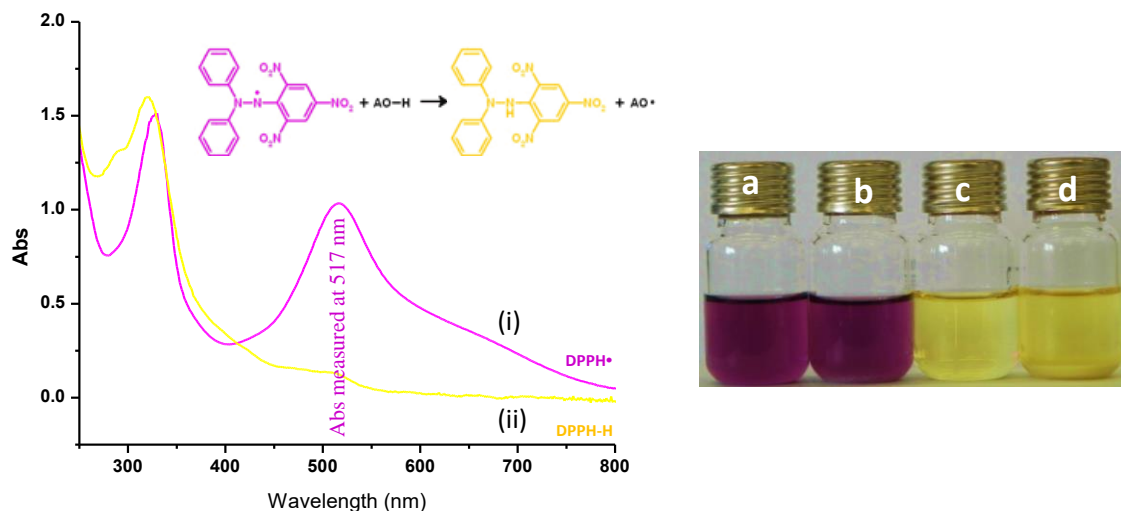


Figure 4: Change in UV-absorption-i) DPPH radical ii) FA-LDH in DPPH solution and color change before and after radical scavenging a) DPPH b) ZnAl-CO₃ in DPPH c) FA in DPPH d) ZnAl-FA in DPPH

2. FABRICATION OF PLA/ZnAl-FA LDH NANOCOMPOSITES:

The nanocomposites of bio resourced and degradable polylactic acid (PLA) and ZnAl-FA LDH were prepared by twin melt extrusion (DSM microcompounder 15cc, Fig.5). The processing parameters used are: Temperature-190 °C, RPM-100, Time- 6 min under nitrogen flow. The abbreviations and composition has been given in Table1.

| Abbreviation | PLA (Wt%) | ZnAl-FA (wt%) | ZnAl-CO ₃ (wt%) |
|--------------|-----------|---------------|----------------------------|
| PAL-0 | 100 | 0 | 0 |
| PAL-1 | 99 | 1 | 0 |
| PAL-2 | 97 | 3 | 0 |
| PAL-3 | 95 | 5 | 0 |
| PAL-4 | 99 | 0 | 1 |
| PAL-5 | 95 | 0 | 5 |



Figure5: DMS micro-compounder used for melt extrusion of PLA/LDH nanocomposites

2.1 CHARACTERIZATIONS:

The practical applications of resulting bionanocomposites are subject their structure-property relationships. Hence, the resulting bionanocomposites were thoroughly characterized in order to assess their structure and properties and ability to use as active packaging materials. The properties like gas barrier, biodegradation, thermo-mechanical were assessed in terms of confirming the intended applications.

2.1.1 Oxygen barrier properties:

The resulting bionanocomposites are featured as active packaging materials so the assessment of their gas barrier properties *esp.* Oxygen is of prime importance. Hence we measured the oxygen permeability of resulting bionanocomposites using LabThink permeability meter at 100 kPa differential pressure. It can be seen from **Fig.6a** that O_2 permeability of resulting hybrids was significantly altered by addition of Ferulic acid modified LDHs into PLA. An incorporation 5wt-% decreases the permeability of PLA by 61% in compared PLA and 59% in compared to LDH- CO_3 filled PLA. The decrease in O_2 permeability for FA-LDHs was observed due creation of highly tortuous path for gas molecules by well dispersed LDHs nanosheets. Whereas, unmodified CO_3 -LDHs may not have well dispersed because of highly hydrophilic nature causing agglomeration into PLA matrix.

2.1.2 DHPP radical assay:

In order to assess the radical scavenging ability of FA/LDH in PLA matrix, a DHPP radical assay method was used. The sample of neat PLA, PLA/FA-LDH and PLA/ CO_3 -LDH was dissolved in $CHCl_3$

to the same 0.01 methanolic solution of DHPP was added. It can be seen from **Fig.6b** that after an incubation of 1hr the sample containing FA-LDH turned violet to yellow, confirming the living radical scavenging ability of FA-LDH even after intercalating into PLA in contrast to neat PLA and CO₃-LDH filled PLA.

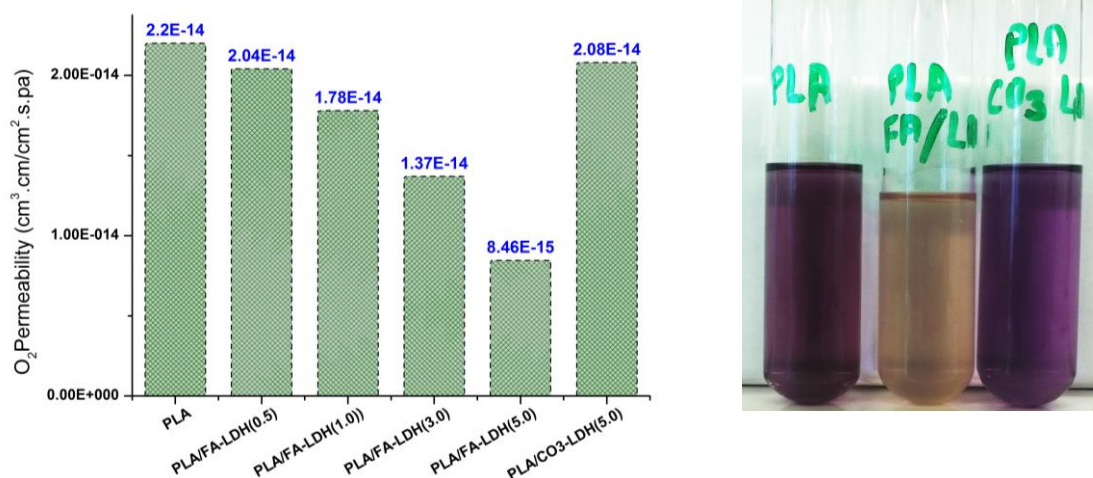


Fig.6: a) Oxygen permeability of PLA/LDH bionanocomposites, b) The DHPP assay test for PAL-0, PAL-1 and PAL-4 bionanocomposites

2.1.3 Hydrolytic Degradation:

The polymer used in presents study is PLA which is considered to be as biodegradable under composting conditions. So it was interesting to assess the effect of these nanoclays both unmodified and ferulic modified LDHs towards rate of degradability of PLA.

In present study we have carried out the biodegradation of PLA/FA-LDHs under hydrolytic conditions for 3 months in buffer solutions of K₂HPO₄ and Na₂HPO₄ at pH=7.6 and 37 °C. The resulting degradation is monitored by measuring the changes in crystallinity, weight loss and opacity.

2.1.3.1 Crystallinity:

The change in crystallinity is an important measure to follow hydrolytic degradation in polymers. The crystalline structures of PLA and PLA/LDH bionanocomposites both before and after hydrolytic degradation were followed by DSC studies. It is observed that the all samples undergo hydrolytic degradation but the rate is much higher in case of LDH filled PLA. It can be seen from **Fig.7** that with the addition of 1 wt% of nano charge, the sample with FA-LDH showed higher increase in crystallinity (8.8%) in compared with neat PLA (3.4%) and PLA-CO₃/LDH (5.0%), respectively. These results reveal that the nanohybrids of FA-LDH causing faster hydrolytic degradation and this may be due high affinity of FA with aqueous medium or the highly dispersed LDH sheets catalyzing the hydrolysis of PLA leaving behind more crystalline domains.

Also, in DSC scan spectra (**Fig.8**) the T_{cc} peaks of all samples differ after the degradation, the peak with FA-LDH appears to be more downside showing faster crystallization. Also, in cooling scan,

only PLA/FA-LDH shows small peak revealing highly crystalline domains. Hence, overall results confirm that FA-LDH is causing PLA to degrade faster under hydrolytic degradation conditions.

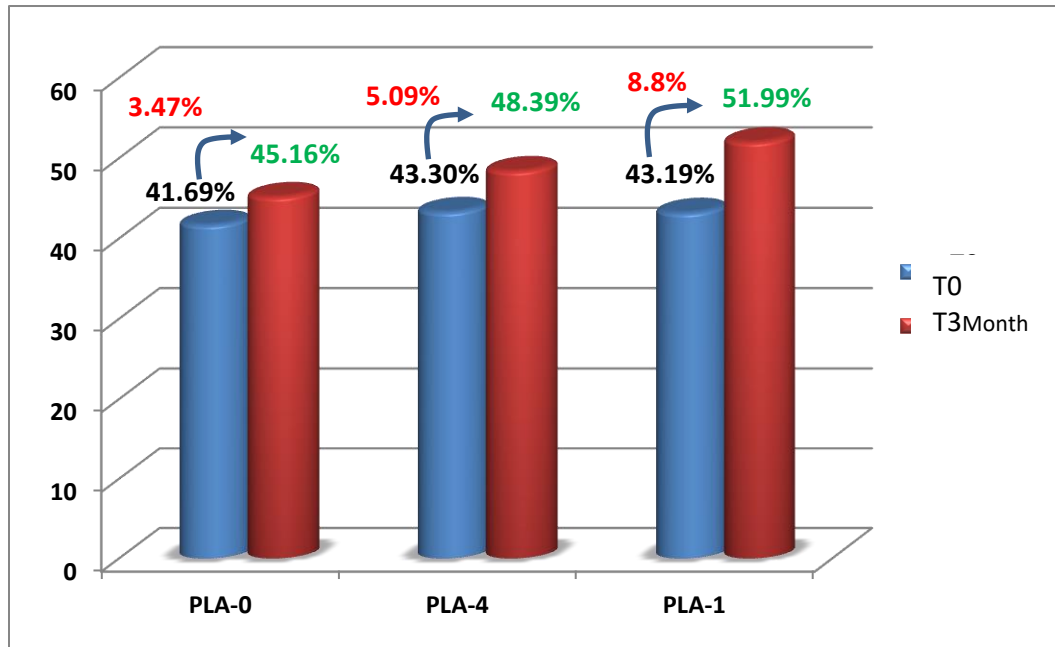


Figure7: Crystallinity changes after hydrolytic degradation

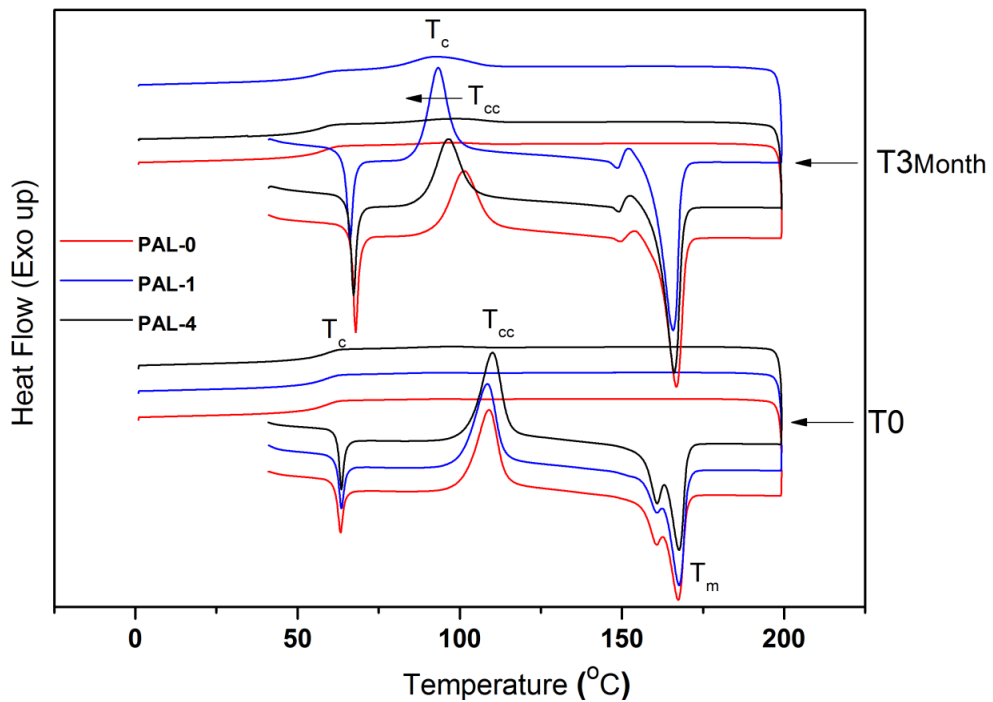


Figure8: DSC scan spectra of PLA/LDH bionanocomposites both before and after hydrolytic degradation

2.1.3.2 Opacity and weight loss:

From Fig.9 it can be seen that the opacity and weight of LDH filled PLA has been affected upon hydrolytic exposure. Moreover, the opacity intensity seems (Fig.9a) higher in case of FA-LDH which underlines the significant effect of FA-LDH on crystalline structure of PLA after 3 month of degradation. The amorphous part seems degraded first leaving behind the highly crystalline domains. Such crystalline changes were also evidenced by fore-mentioned DSC measurements. Moreover, the difference in weight loss (Fig.9b) also confirms the faster degradation rates in PLA/FA-LDH composites.

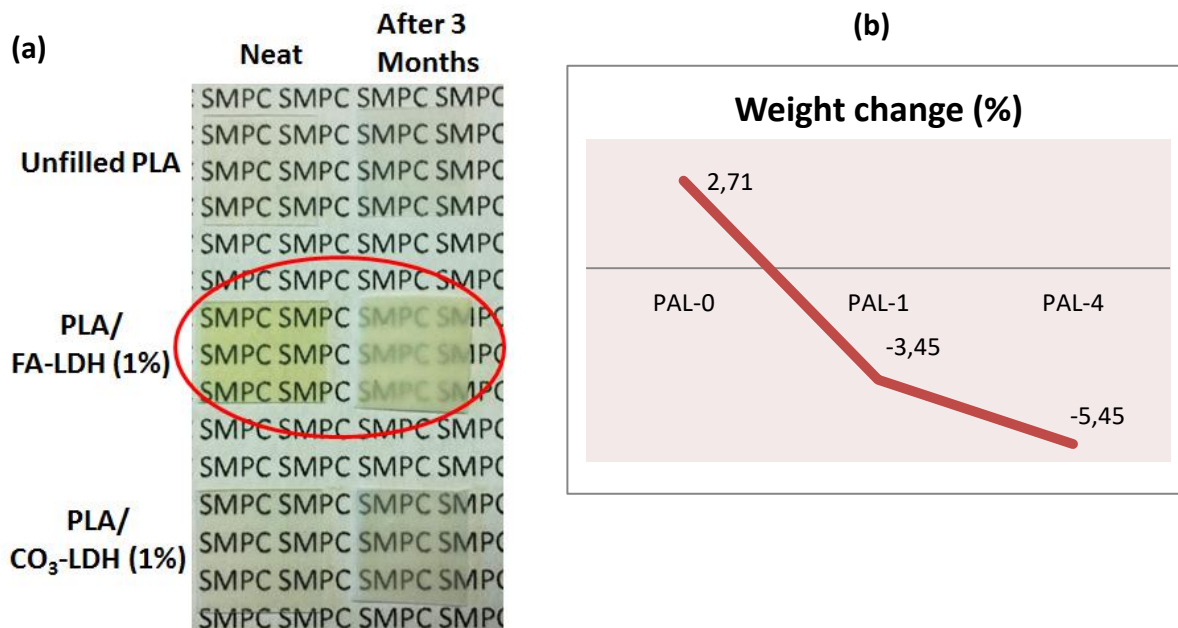


Figure9. a) The colour change in PLA/LDH bionanocomposites before and after degradation and b) The weight change in PLA/LDH bionanocomposites before and after degradation

2.1.4 Antimicrobial test

In order to validate resulting materials for active packaging, assessment of their antimicrobial properties are critical. Hence we performed the antimicrobial tests by using liquid incubation method. For liquid incubation tests, 200 mg small pieces of each film sample of PLA and composites were placed in test tubes each containing 50 mL liquid medium Nutrient Broth (NB) (approx. 1×10^6 cells). To each test tube 100 μ L of DMSO was also added to facilitate the release of intercalated Ferulic acid (FA) into the medium. All the test tubes were sealed and shaken at 100 rpm for 24 hrs. At each sampling time, aliquots containing 1 mL of incubated sample were serially diluted with double distilled water and absorbance has been taken at 600 nm. The measured absorption value at 600 nm is significant for Turbidity Method which is well established. The increase in the absorbance shows the growth of cells whereas decrease in the absorbance or turbidity shows decrease in the number of the cells. Cell numbers = Absorbance \times 10,00000.00. (Average of three measurements is reported in each case with standard error of approx. 5 %). From Fig.10 it can be seen that the sample containing FA-LDH showed significant inhibition

towards bacterial cell growth, in compared to both unfilled and unmodified LDHs. This underlines that the incorporation of FA-LDH into PLA makes composite more antimicrobial.

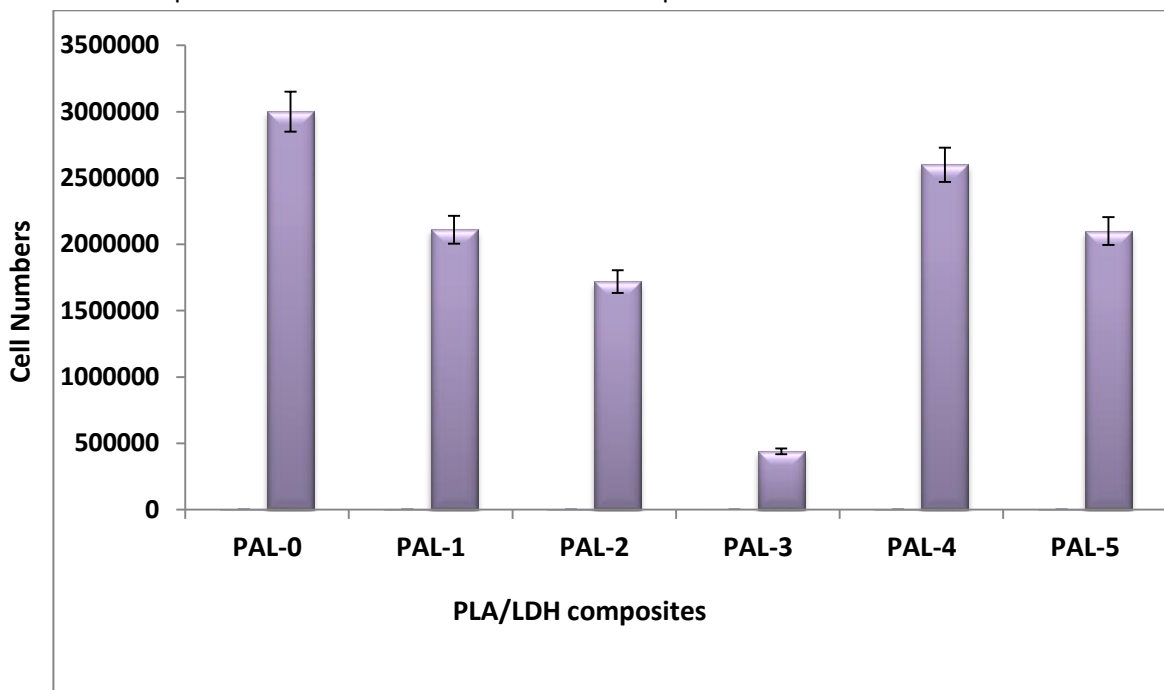


Figure 10. Inhibition towards cell growth by PLA/LDH composites

2.1.5 Thermal properties:

The thermal stability of resulting bionanocomposites was assessed by TGA analysis. **Fig. 11** illustrates the TGA curves in nitrogen atmosphere for neat PLA and its nanocomposites with 5 wt-% loadings of LDHs. Surprisingly, it is observed that the degradation of nanocomposites occurs at lower temperature as compared to the neat PLA and the onset thermal degradation temperature ($T_{0.1}$) decreases gradually by 42 °C (for ZnAl-CO₃ LDH) and by 22 °C (for ZnAl-FA LDH) in compared unfilled PLA. However, when 50% weight loss was selected as the point of comparison, the thermal decomposition temperatures ($T_{0.5}$) for neat PLA and its LDH nanocomposites shows temperature difference of 32 °C and 9 °C, respectively. For the first sight it seems that the residual water which is known to escape from LDH galleries around 200 °C might have cause the hydrolytic degradation of PLA during processing. The effect seems less pronounced in case of ZnAl-FA which may be attributed to the high hydrophobic nature of modifier, antioxidant effect of FA and highly dispersed LDH in compared to unmodified LDH. However, further investigations are required to confirm this early degradation of PLA in presence of layered double hydroxides.

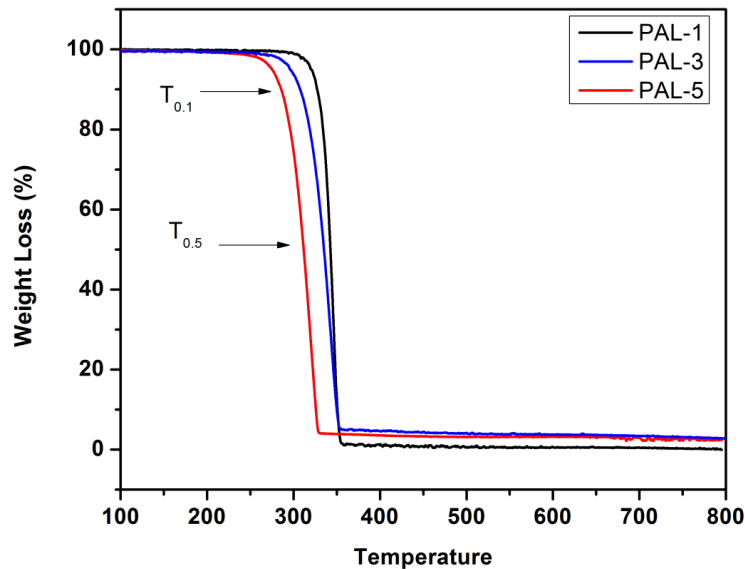


Figure11. TGA analysis of PLA and PLA/LDH nanocomposites

➤ Conclusions

New multifunctional and active layered nanofiller were effectively synthesized based on layered double hydroxides and bioactive Ferulic acid using regeneration approach. The host-guest structure of resulting FA-LDH nanohybrids were confirmed by various physico-chemical characterizations. Moreover, resulting bionanohybrids exhibits sustained antioxidant activity. Subsequently, the resulting layered bionanohybrids were successfully employed as active and reinforcing filler for bio-resourced polylactic acid. The bionanocomposites of PLA/LDH with varying compositions were fabricated using melt extrusion technique. The resulting composites showed significant increase in oxygen barrier properties and sustained antioxidant activity. The hydrolytic degradation studies also confirmed that the FA-LDHs have pro-degrading effect on PLA films. Also, the FA-LDH composites effectively control the bacterial growth and possess good antimicrobial properties. The thermal properties on resulting composites were slightly reduced but high enough for packaging applications. Hence, the resulting PLA/FA-LDH bionanocomposites can be a highly potential material to obtain green and active packaging materials.

PART-II

Synthesis and characterization of functional graphene and their polymer nanocomposites

Recently emerged one atom thick wonder material 'Graphene' possesses significant properties like high surface area which hold potential for gas barrier and antimicrobial. Their incorporation in polymeric matrices could be beneficial to make active packaging materials. However, their poor dispersibility into organic and polymeric material can limit their applications. To overcome, we have developed a new approach based on supramolecular chemistry to obtain highly dispersible graphene. Moreover, their nanocomposites with biodegradable poly-caprolactone were synthesized using in-situ polymerization.

1. Synthesis of imidazolium functionalized Graphene and their nanocomposites with polycaprolactone:

The aqueous mixture of graphene oxide and imidazolium ionic liquid was in-situ reduced by hydrazine hydrate to obtain imidazolium functionalized graphene. After, a solvent free polymerization of ϵ -caprolactone was carried out by dispersing varying content functionalized graphene (0.1, 0.5, 1.0 and 2.0 wt%). The polymerization was carried out at 70 deg. for 20h using tin-octate as a catalyst.

2. Characterizations:

The resulting nanocomposites were characterized by different physico-chemical techniques to reveal successful functionalization and nanocomposite formation.

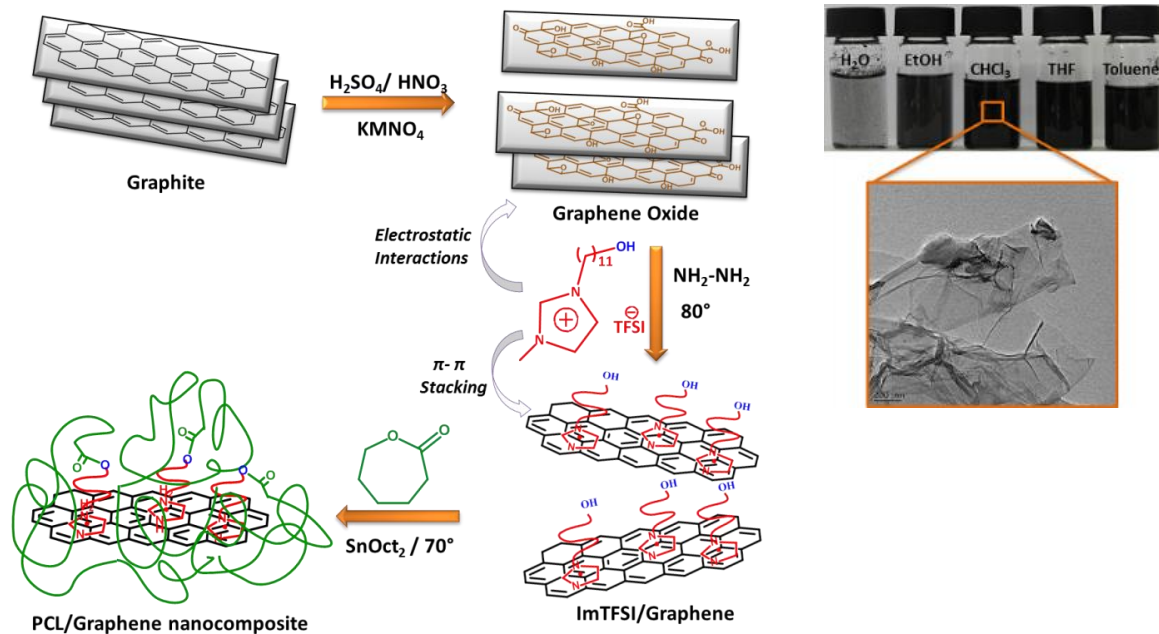


Fig.1 a) Schematic representation of functionalization of graphene and fabrication PCL nanocomposites b) Dispersion of functional graphene into different solvents

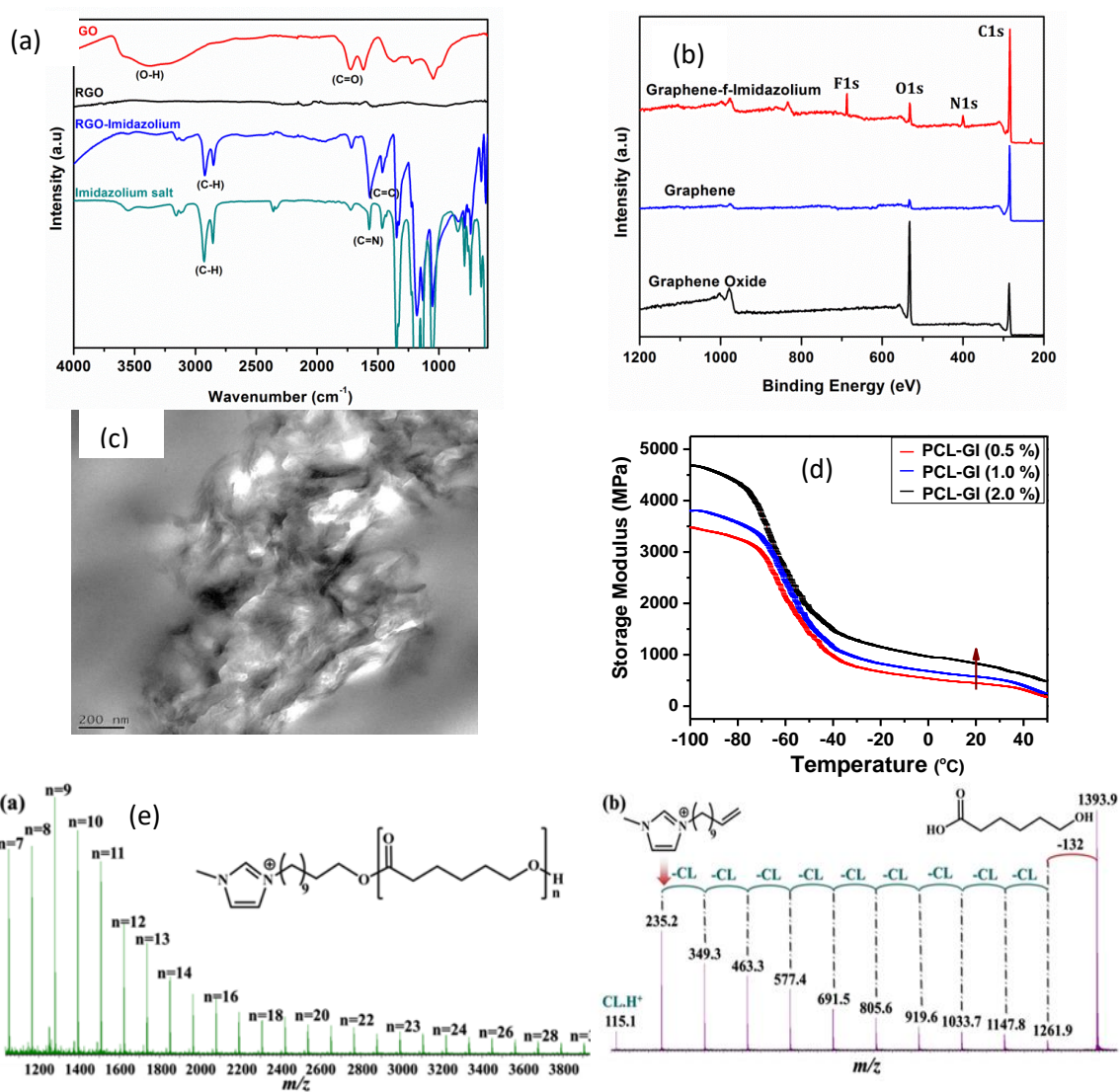


Fig.2 Characterization of functional graphene a) FT-IR, b) XPS and their nanocomposites c) TEM, d) DMA analysis and e) MALDI-ToF spectroscopy

Conclusions:

- Successfully demonstrated a supramolecular approach to produce stable, organo-dispersible graphene nanosheets via non-covalent functionalization.
- Functionalized Im-OH/TFSI/RGO hybrid was successfully employed as an active initiator for ROP to fabricate graphene/PCL nanocomposites.
- The resulting graphene reinforced polymer shows improved mechanical performance.

❖ Achievements:

➤ Journal Publications:

1. Lonkar, SP., Bobenrieth A., Raquez J-M., De Winter J., Dubois Ph. (2012), A supramolecular approach toward organo-dispersible graphene and their straightforward polymer nanocomposites. *Journal of Materials Chemistry*. 22, 18124-18126.
2. Lonkar, SP., Raquez J-M, Dubois Ph. (2013) Bionanohybrids of LDH/Ferulic acid and their multifunctional reinforcement to the biobased polylactic acid (In preparation).
3. Lonkar, SP., Bobenrieth A., Oden J., Raquez J-M., Dubois Ph. (2013), Structure-Property investigation in PLA/Graphene nanocomposites (In preparation).
4. Lonkar, SP., Raquez J-M, Dubois Ph. (2013), Microwave assisted graphene wrapped LDHs nanostructures for supercapacitor applications (In preparation).

➤ Conference Publications:

- 1 **One paper presented:** Lonkar, SP., Bobenrieth A., Raquez J-M., Dubois Ph., Graphene Based Bionanocomposites: Green Materials for Advanced Applications. Proceedings of International Conference on Advances in Polymeric Materials & Nanotechnology (*PolyTech-2012*), Dec. 15 – 17, 2012 , Pune, INDIA (Oral Presentation).
- 2 **One paper presented:** Lonkar, SP., Bobenrieth A., Raquez J-M., Dubois Ph. Imidazolium assisted synthesis of Graphene /PCL nanocomposites via ring opening polymerization. Proceedings of Belgium Polymer Group Meeting (*BPG-2012*), Blankenberge, BELGIUM, May 2012. (Poster Presentation)