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Thapaliya, Pratigya ***Barrier Properties of Starch -Based Antimicrobial Films Using Antimicrobial Nano-Particle System***

Abstract

Intercalation of antimicrobial agent into layered double hydroxides depended upon the type of antimicrobial agent, concentration of antimicrobial agents, and modification condition. Benzoate and Natamycin anion were loaded successfully into layered double hydroxide matrices and its loading was controlled by reaction condition. However, the molar concentration and the reaction time carried out for modification of benzoate did not seem to be effective for loading propionate anions. Starch films were prepared using modified LDH with Benzoate, and Natamycin (LDH-Bz and LDH-Nat).

Barrier properties in terms of water vapor, oxygen transfer and carbon dioxide were found to be improved in films made with either antimicrobials or 2% modified LDH by antimicrobials compared to the control film. However, there is no significant difference of WVTR and OTR between films with antimicrobials or modified LDH by antimicrobials. Lightness of color (L), red/green color (a) and yellow/blue color (b) of films were tested. No significant difference was found among control films, films with unmodified LDH, and films with benzoate, natamycin, and LDH-Bz. Films with LDH- Nat. were found more yellow with low “L” value, which might not be eye appealing for the use in all types of food packaging.

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My special thanks to my co-worker Disha Trivedi for her assistance during the work. I would like to express my deepest appreciation to my husband, Bijay Parajuli for his constant support, encouragement and continuous inspiration. I would also like to thank my parents and in-laws for their help and co-operation throughout my study.

Thank you,

Pratigya Thapaliya

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Chapter I: Introduction

Bio-based polymers as packaging materials is gaining great attention in industrial as well as research field. The reasons behind may be the nondegradable and nonrenewable nature of synthetic polymers used for packaging. In addition, the manufacture of plastic requires intense energy. Moreover, it also contributes to global warming by release of carbon dioxide gas as a by-product. Therefore, packaging material derived from natural source can be the alternative to this problem. The source of bio- based polymers that can be used for film making can be starch, cellulose, polysaccharides, polylactic acid (PLA) etc. However, bio-polymers do not have good barrier and mechanical properties that can compete with synthetic polymers (Krochta & Mulder-Johnston, 1997). This may be because of hydrophobic nature of bio-polymers like starch, cellulose which loose mechanical integrity as well, when expose to higher relative humidity.

This poor mechanical and barrier properties can be bio-polymers can be improved my many methods: chemical modifications, coatings, blending with biopolymers use of layered silicates (nanoclay) to produce nanocomposites (Tang, Alavi, & Herald, 2008). Nano composites are new class of materials, which has recently drawn interest among researchers. It contains polymer matrix, with nanoparticles having dimension 1-100nm (DeGruson, 2014). Layered Double hydroxides (LDH), are layered solids having outer layer positively charged and balancing anions in the inner layers (Costantino, Casciola, Massinelli, Nocchetti, & Vivani, 1997) and these LDH have potential as much as layered silicates to produce nanocomposites.

According to Evans and Duan (2006) LDH are denoted by the formula $[M^{II}_{1-x} M^{III}_{x} (OH)_2]$ x+$(A^{n-})_{x/n}$. yH_2O, where M^{II} and M^{III} are divalent and trivalent metal ion and A^{n-} is an anion, such as $CO_3^{\ 2-}$, $NO_3^{\ -}$.

On the other hand, to prevent food from spoilage by undesirable microorganism; use of antimicrobial agents directly in food or packaging material (coating for active packaging) is common. At the same time due to dilution or migration effects, there is a high probability for loss of antimicrobial activity of antimicrobials when used directly. Therefore, there is a need to control and regulate the release of antimicrobial activity according to requirement to stop growth of microorganisms in the food. As a solution to this problem, a method has been put forward which includes modification of Layered Double Hydroxides (LDH) with molecules of interest (antimicrobials) and holds active molecules into its interlayer matrices, later releases the compound in a controlled rate (DeGruson, 2014). The LDH nanoparticles in the bio nanocomposites has two functions. the inorganic part enhance the mechanical and barrier properties whereas, the organic part (antimicrobial agent) provides a functional group that incorporates a new property (antimicrobial activity) to the packaging films for food packaging application (DeGruson, 2014).

Hypothesis and Objective

The main objective of the research work, is to modify Layered Double Hydroxide with antimicrobials for food packaging application, which would improve barrier properties and color properties when used for making packaging films. The hypothesis is that, Antimicrobials can be loaded on Layered Double hydroxide and the Antimicrobial modified LDH will affect the barrier and color properties of starch based films.

Chapter II: Literature Review

Biopolymers are polymers derived from biological sources. They have potential to be broken down to organic compounds by microorganism, light, air, water etc. They are gaining attention in industrial, scientific and research areas as well. The reason behind this can be nondegradable and non- renewable nature of plastic polymers. Also, these plastic polymers add to environmental pollution, because they persist long on environment without degrading, also contributes global warming by release of carbon dioxide gas as a by-product. Therefore, use of bio-polymers as an alternative to synthetic polymers can be the best solution for this problem.

However, there is a challenge to create the large-scale production to maintain the demand and supply of bio-based polymers to replace the synthetic polymers (Rachana Shalini, 2009). Bio-based polymers can be Natural polymers like starch, cellulose, protein: produced from microorganisms like poly hydroxyalkanoates; Synthetic bio polymers like poly lactic acid (DeGruson, 2014). An overview of bio-based polymers based on their origin and method of production is shown below on *Figure 1*. Among these bio- polymers, starch is of great interest. It is because starch is commonly available and inexpensive agricultural material. Starch can be converted into thermoplastic under high temperature and pressure (Tang et al., 2008).

Starch

Starch is the principle carbohydrate found in cereals, root and tubers. They occur in very small granules consisting of linear or branched glucose chains. Starch are found in variety of shapes like round, flat, polyhedral, kidney shaped. The size of starch granules differs with its source, for example: rice starch (2-15µ), wheat (2-40 µ), corn (10-25µ), potato (20-150 µ). Starch consist of two different fractions depending on the source, they are amylose (20-30%) and amylopectin (70-80%) (Rai, 2007). Amylose is a linear chain polymer linked through α-1,4 glucoside bonds and to a lesser degree with α-1,6 linkage. Whereas, Amylopectin is highly branched molecule which is linked to the main glucose by α-1,6 linkage (Durrani & Donald, 1995). Both Amylose and amylopectin ratio regulates the crystalline property of starch. Flexibility is contributed by the linear structure of Amylose and therefore, represent amorphous fraction. Whereas, due to highly branched chain, Amylopectin contribute to the crystalline fraction (Hermansson & Svegmark, 1996).

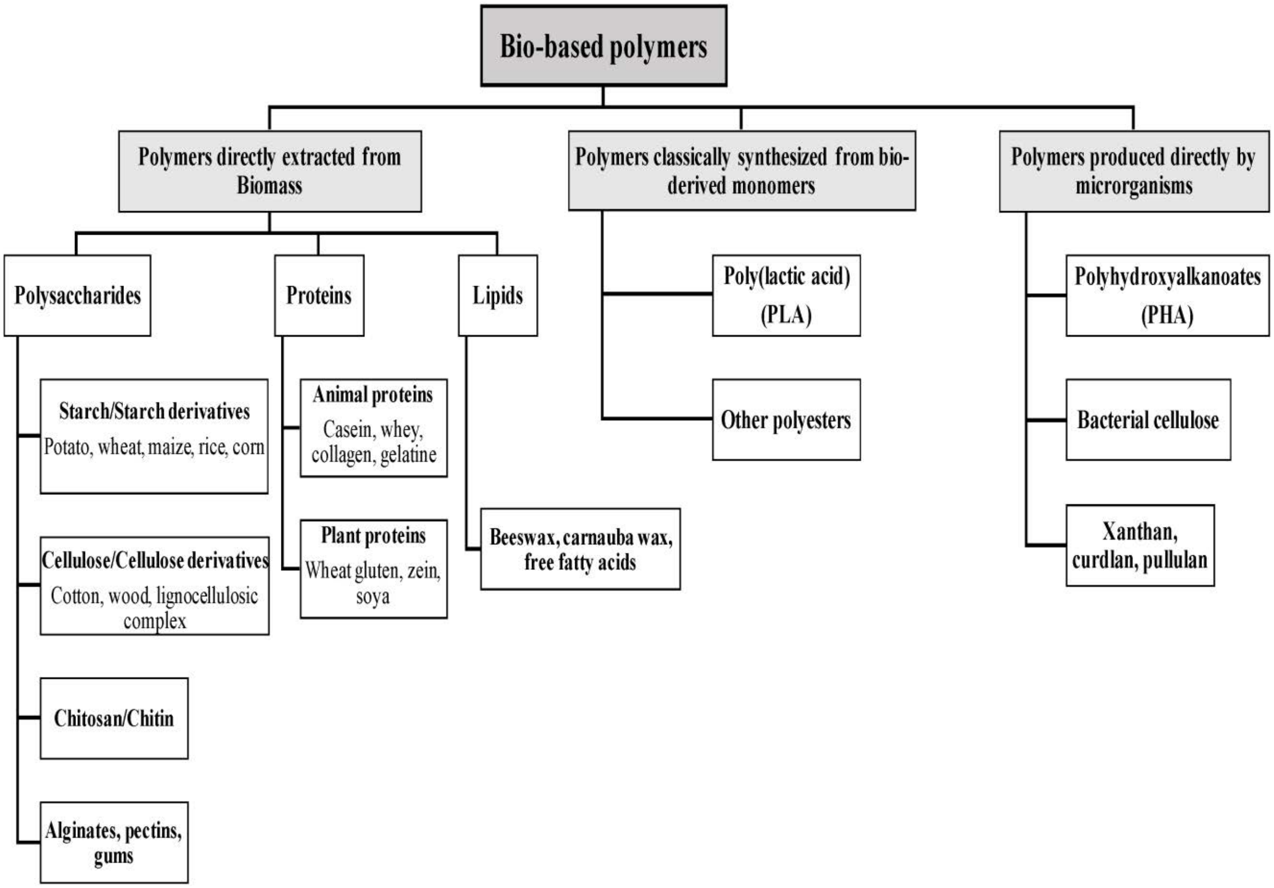

Figure 1. Categorization of bio-based polymers based on their source. (Rastogi & Samyn, 2015)

Properties. Starch is white powder with no taste and odor. They are insoluble in water and most organic solvents. Their solubility increases when the temperature is elevated. Around

60–70ºC, insoluble starch granules are broken down which results in loss of crystalline structure giving viscosity and solubilization of starch. This phenomenon is termed as starch gelatinization. During cooling after gelatinization, starch undergoes retrogradation (setback), where starch chain re-associate to give an ordered structure, resulting increase in viscosity (Bertolini, 2009). To use starch for different purpose with enhanced properties, they are modified chemically, enzymatically or physically. Starch can be categorized as: Native starch, which imparts very high viscosity whereas, modified starch uses thermal/ nonthermal treatment to obtain pregelatinized, cold water- swelling starch etc. (BeMiller & Huber, 2015).

Production and application. Starch is low cost, easily available material and is used in many food (57%) and nonfood applications (43%). Patil (2010) stated that starch production is estimated to be around 75 million tons worldwide. The source for starch production are namely corn, potato, cassava and wheat. However, because of high moisture content of root and tubers compared to cereals, their storage life and the extraction process of starch is quite complex. Therefore, cereals like corn and wheat are generally preferred for commercial starch production (Sriroth, Piyachomkwan, Wanlapatit, & Oates, 2000). Starch is used for several reasons in industries. In general, it can be divided into Food and Non-Food applications.

Starch is one of the main component of different food recipes and formulations, in food industries. It is generally used to increase the nutritional as well as functional properties of the developed product/processed foods. For example, starch is used as texturing, thickening, gelling agents. Starch is also used in beverage industries. Most of the sweeteners used in the beverage industries these days use sweeteners like: High Fructose Corn syrup, glucose syrups, maltodextrins, fructose etc., which are made from starch.

Bio plastics are gaining attention these days because of prolong persistent of petroleum-based plastic in the environment that adds to the environmental pollution. Bioplastics uses degradable renewable raw materials like starch, cellulose, proteins, as a major component with other minor additives like plasticizers to enhance the property of the bioplastic. Bio plastics are generally made from two processes namely: Casting and Extrusion process. Casting process uses, heating of starch with water and plasticizers (glycerol) that results to breakdown of crystalline structure and solubilization of amylose, this phenomenon is termed as swelling of starch granules. Whereas, in Extrusion process involves heating of starch granules and then mechanically processed to disrupt the internal crystalline order of the starch granules. In this method, the extruder speed, feeding rate of the feed, temperature, moisture content plays the important factor for transformation of starch for film making (Lai & Kokini, 1991).

Bio-Nanocomposites

Nano -Technology relates the study of matters with the scale size of 1 to 100 nanometer. Nano fillers in the form of nanocomposites is gaining popularity to enhance the properties (good flow, low density, surface enhancement etc.) of synthetic polymers. Generally, the nanofillers are used in very low concentration (< 5%) to get maximum enhancement in the bio polymer properties. Rhim, Park, and Ha (2013) states that amount of nanofillers used affect the mechanical properties of polymers. It is also observed that, with increase in concentration of nanofiller used, tensile strength and modulus of bio-composite material also increased along with the gas and water vapor barrier properties. However, it is claimed that improvement in barrier properties is attributed by the concentration and aspect ratio of the nano sized filler used. Nano fillers commonly used for food packaging applications are categorized as nanoparticles, nanofibrils, nanotubes etc. These nanofillers can be either organic or inorganic, natural biopolymers, antimicrobial agents, metal and metal oxides (Othman, 2014). Biopolymers also provides antimicrobial, bio-sensing, oxygen scavenging properties besides mechanical and barrier properties. Sorrentino, Gorrasi, Tortora, and Vittoria (2006), reported increase in barrier properties of polymers with the use of nanoparticles as they create the hindrance in motion in the path where they travel.

Layered Inorganic Nanoparticles

Layered double hydroxides are positively charged metal hydroxide layers, that has anions intercalated into its layers (Miyata, Kumura, Hattori, & Tanabe, 1971; Reichle, 1986). LDHs are generally expressed in the formula as $[M^{II}{}_{1-x} M^{III}{}_{x} (OH)_2]^{x+} (A^{n-})_{x/n}.yH_2O$. In the formula, M^{II} and M^{III} are represented as divalent and trivalent metal ion respectively. Likewise, A^{n-} denotes for, anions like, $CO_3{}^{2-}$, $NO_3{}^{-}$ etc. (Evans & Duan, 2006). The general structure of LDH is shown in *Figure 2.*

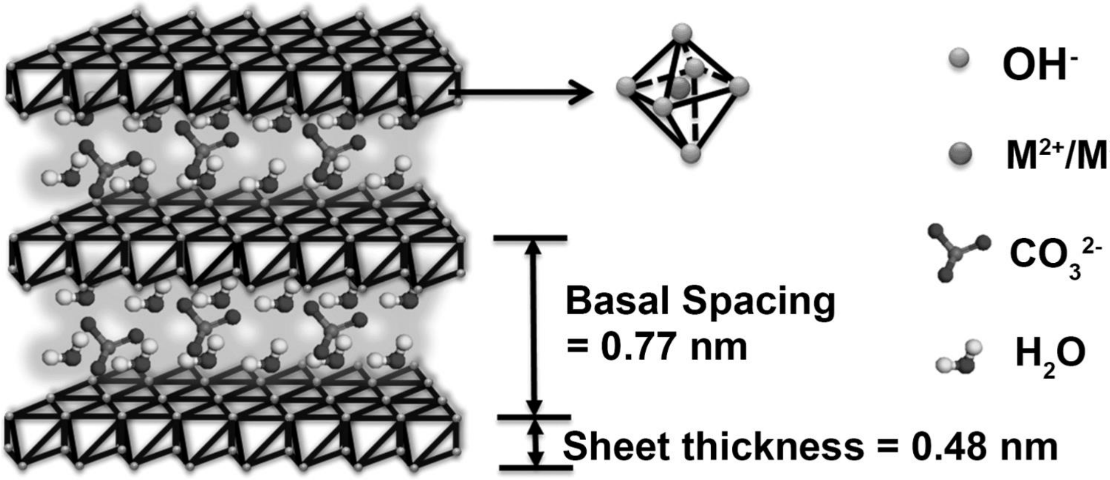

Figure 2. General structure of layered double hydroxide (LDH).

Here in LDH, M^{2+}, M^{3+} and OH^- represents the cations and metal hydroxide layers, whereas, $CO_3{}^{2-}$ is anions that gets deposited in the interlayer region. These, anions that are accumulated in the layers can be exchanged with other functional group to produce a new compound (Hwang, Han, & Choy, 2001). The structure also consists of water molecules (H_2O) in the interlayer region along with anions, which helps to bring stability in the crystalline structure. Interlayer space also called, basal space is contributed by both water and anions present in the interlayer space (DeGruson, 2014). In case of Layered Double Hydroxide, the basal space is observed around 0.77nm, which is much higher compared to brucite crystal (0.48nm) (Hwang et al., 2001).

Nano Composite Synthesis

When polymer chain diffuses between clay layers, it results to nanocomposite. It is formed either by intercalation or exfoliation. Finite expansion of multilayer clay, with ordered arrangement is observed during intercalation. Whereas, homogeneous distribution of platelets (nanometer thick) throughout the matrix is observed in exfoliation (DeGruson, 2014). Characterization of nanocomposites are commonly done by two methods, X-ray Diffraction (XRD) and Transmission Electron Microscopy (TEM). Although to study the structure of polymer nanofillers, XRD is usually used, it still has some limitations. XRD is not reliable in detecting exfoliated layers, with spacing greater than 50nm, only able to sense periodically stacked layers. TEM on the other hand can detect the nanofillers without parallel stacking but the small area of the image included during analysis makes it difficult to describe the nanocomposite morphology (Nyambo, Songtipya, Manias, Jimenez-Gasco, & Wilkie, 2008).

Bio nanocomposites for food packaging applications. There are number of bio nanocomposite that can be potentially used for food packaging application. These are; polysaccharide based bio nanocomposite (starch based, cellulose based), protein based bio nanocomposite (soy protein, corn zein, wheat-gluten, animal protein), Lipid based nanocomposite, PLA based nanocomposite, PHA based nanocomposite etc. (DeGruson, 2014).

Among them, starch-based bio nanocomposite is gaining popularity because of biodegradability, easy availability and low cost. However, they need to be modified to enhance their property as a film. Use of plasticizer alone cannot contribute to ideal food packaging application, as a result need of starch nanocomposite is must to make bio polymers capable to use for food packaging application. Different researchers have studied the problems and suggested many findings to improve the mechanical properties. For instance, kaolin as a filler (De Carvalho, Curvelo, & Agnelli, 2001) acetylated starch with MMT (Qiao, Jiang, & Sun, 2005) etc. After study, the enhancement in film properties depends upon type and concentration of nanoclay, plasticizers used. Also, the film properties did not significantly differ with starch source but varies with modification Chung and Lai (2010).

Layered double hydroxide-based antimicrobial nanocomposites. Packaging material can easily favor the growth of microorganism when used for food packaging application. In order to prevent the growth of food spoilage microorganism in the food, there is a practice of incorporating the antimicrobial agents in the food or coating in the inner layer of packaging material. However, this practice has proved to be inefficient when the food has to be opened and closed number of time during consumption. For an example cheese, which favors mold growth. In this circumstance, active packaging can be a good alternative, which controls the release of antimicrobial agent and helps to prolong the shelf life of food.

Nowadays, use of Layered Double Hydroxide (LDH) as a nanofillers is gaining interest. LDH in its original form has hydrophilic surface. When modified with organic compounds, the thus formed nanocomposites possess additional properties like antimicrobial, drug release as well the film properties like mechanical, barrier and heat stable etc. tend to increase (Costantino et al., 2009). In one of the control release study of antimicrobials, carried out by (Costantino et al., 2009), found out that when the antimicrobials were freely dispersed into the polymers, it resulted in rapid uncontrolled release of antimicrobials. On the other hand, when the LDH were modified with antimicrobials, the release of antimicrobial moiety was very slow.

Chapter III: Modification and Characterization of Layered Double Hydroxide Nanoparticles with Different Antimicrobial Agents

Layered double Hydroxide are a family of layered solid with general chemical formula [MII 1−xM III x(OH)2] x+(An−) x/n.yH2O, where MII is a divalent metal ion, for example Mg $^{2+}$, Ca $^{2+}$, Zn $^{2+}$, etc., MIII is a trivalent metal ion, for example Al $^{3+}$, Cr $^{3+}$, Fe $^{3+}$ etc. and An− is an anion, such as $CO_3{}^{2-}$, Cl^-, $NO_3{}^-$ etc. (Evans & Duan, 2006). LDH are layered solids with positive charged layers and anions in interlayers (Costantino et al., 1997).

LDH are used in organic synthesis because of their catalytic activities (Tichit & Coq, 2003). LDH and its modified form are used in the various field such as photochemistry and photo physics (Ogawa & Kuroda, 1995), gene therapy, drug storage and release (Del Hoyo, 2007) LDH are gaining popularity these days as Nano fillers, used for the synthesis of polymer nanocomposite. The surface of the LDH are hydrophobic in nature, therefore the modification by organic molecules is generally required to make it miscible with polymers. The nano composite using modified LDH helps to enhance mechanical and barrier properties (Nyambo et al., 2008).

The objective of the study here is to prepare nanocomposite for “active packaging”. For the purpose, modification of LDH by antimicrobial is done using one step biosynthesis method. Three antimicrobial agents used in foods, sodium benzoate, sodium propionate and Natamycin were taken as modifiers. The structure and chemical composition of modified LDH are analyzed using X-ray Diffraction, Fourier transform infrared spectroscopy (FTIR) and thermogravimetric analysis (TGA).

Materials

Unmodified nanoparticles LDH-CO_3 of Sigma-Aldrich, Inc (St. Louis, MO, USA) with the formula ($CH_{16}Al_2Mg_6O_{19}.4H_2O$) were used. Sodium benzoate and sodium propionate from

(Alfa Aesar), Natamax with 50% Natamycin/ Lactose from DANISCO and DelvoCid (50% Natamycin/ sodium chloride (DSM, The Netherlands) were also used during modification.

Nano particle modification. Initially LDH-NO3 was prepared. In order to obtain the nitrate form (LDH-NO3), Sodium Nitrate solution (0.1 M, 350 ml) was added into 3 -neck flasks. Metal nitrate salt solution (0.2 M Mg2+, and 0.1 M Al 3+ prepared in DI water, 300ml) added dropwise into nitrate solution using additional funnel under continuous stirring and maintaining the reaction temperature at 50°C. During the synthesis, the pH value maintained at 10±0.2 by adding suitable amount of 1M NaOH solutions. Nitrogen was purged into the flask all the time. The slurry then continuously stirred at the same temperature for 30 min and allowed to age in a heater at 60°C for 18 hr. Centrifugation of the solution for 5 min with 4000rpm to separate the solid particles and solution. Washing the solid particles for several times with CO2 free water and then filtered the solution using 0.22µm Millipore filtration. Drying the white powder at 50°C in the vacuum oven till the weight was constant.

LDH-Benzoate was modified under three conditions. They were named as LDH-Bz1, LDH-Bz2 and LDH-Bz3. For the preparation of LDH-Bz1, Sodium benzoate was taken and its aqueous solution of 0.1M was prepared using De-ionized water. During titration pH was maintained at 10±0.2, ageing and drying temperature 60°C and 50°C respectively. Likewise, LDH- Bz2 was prepared by using 0.1M aqueous solution of Sodium benzoate, pH maintained at 10±0.2 throughout the titration, ageing and drying temperature 60°C and 70°C respectively. LDH- Bz3 was also obtained from 0.1 M aqueous solution of Sodium benzoate, pH maintained at 10±0.2, but different ageing and drying temperature (75°C and 70°C respectively) compared to LDH- Bz1 and LDH-Bz2.

Propionate modification was done under two conditions, they were named as LDH-P1 and LDH-P2. LDH-P1 and LDH-P2 were prepared with same molar concentration (0.1 M sodium propionate) and titration pH 10±0.2, but different ageing and drying temperature (60°C and 50°C for LDH-P1 and 75°C and 70°C for LDH-P2).

Likewise, Natamycin modification was done under three conditions. LDH- Nat. 1 was prepared by using, Natamycin with 50% Lactose and its aqueous solution of 0.1M was prepared using 1:1 ratio of ethanol: water and the pH of the solution was adjusted to 5 with 0.1M HCl. During titration pH was maintained at 10±0.2, ageing and drying temperature 60°C and 50°C respectively. LDH- Nat. 2 was also prepared with 50% Lactose and 0.05M molar concentration using same ratio of ethanol: water and the pH of the solution was also adjusted to 5 and titration pH maintained at 10±0.2. However, they had different ageing and drying temperature 75°C and 70°C respectively. LDH- Nat. 3 was prepared using Natamycin with 50% salt, molar concentration of aqueous solution adjusted to 0.05M, using, 2:1 ratio of methanol: water. During titration pH was maintained at 9, ageing and drying temperature 60°C and 50°C respectively.

X-ray diffraction. X-ray diffraction patterns of nanoparticles were taken with an X-ray diffractometer (Bruker, D8 Advance) and operating 40 kV and 40 mA and a scanning rate of 1 °/min.

Fourier transform infrared spectroscopy. Smart Miracle spectrometer, equipped with a diamond crystal was used. Spectra of the nanoparticles were obtained at room temperature over the wave number range of 4000–400 cm−1, with an accumulation of 32 scans and a resolution of 6 cm−1.

Thermogravimetric analysis. Thermal Gravimetric analysis of the nanoparticles were carried out in a TGA Q series- (Q50-1194). Samples were heated from 20 °C to 800 °C, under a flowing nitrogen atmosphere.

Result and Discussion

Result of three different tests namely x-ray diffraction, fourier transform infrared spectroscopy and thermogravimetric analysis will be discussed below.

X- ray diffraction. X-ray Diffraction test was carried out for different modified LDH samples along with unmodified LDH and LDH- NO3. Unmodified LDH (LDH-CO3) is the most common anionic group, at the same time because of higher affinity of carbonates for LDH, it is quite difficult for anion exchange (DeGruson, 2014).

Table 1 shows the first basal distance of anion trapped in matrices of LDH. The basal spacing is calculated with the help of Bragg's Law. In Unmodified LDH, the first level reflection at 2θ = 11.52°, that resembled to an interlayer distance of 0.76nm. Likewise, for LDH-NO_3, interlayer distance was found to be 0.866nm with 2θ = 10.2. This increase in interlayer distance from 0.76nm to 0.866 in LDH-NO_3 might be due to bigger anion size of nitrate compared to carbonate (DeGruson, 2014). Similar kind of result were observed by Marino and Mascolo (1982) and DeGruson (2014) with 0.84 nm and 0.87 interlayer distance for LDH-NO_3 respectively.

Costantino et al. (2009) stated that with the information of interlayer distance and space available for the guest anion, it is possible to calculate the intercalation of guest spices within that space. The intercalation was done next with three different compounds benzoate, propionate and natamycin under different conditions. *Figure 3* illustrates the XRD patterns LDH modified by sodium benzoate. The peak position of the lowest 2θ angle of 3.8° for two LDH-Bz (2 and 3) is found to be same. That relates to interlayer distance of 2.3nm. For LDH-Bz1, the peak shifted to 2θ angle of 3.9° with distance 2.66nm. The next peak was observed at 2θ = 6.6° for LDH-Bz 2 and LDH-Bz-3 with distance 1.3nm. Again, for LDH-Bz1, 2θ angle of 6.8° was found with distance 1.29nm. Similar result was obtained by DeGruson (2014) with lowest 2θ angle of 3.92°, corresponding 2.25 nm for LDH- benzoates.

Table 1

Calculation of First Basal Distance of Anion Trapped in Matrices of LDH

Sample	Wavelength (nm)	Angle (2Θ)	Distance (nm)
Unmodified LDH	0.154	11.52	0.76
LDH-NO_3	0.154	10.20	0.86
LDH-Bz1	0.154	3.91	2.26
LDH-Bz2	0.154	3.80	2.30
LDH-Bz3	0.154	3.80	2.30
LDH- P1	0.154	10.72	0.82
LDH- P2	0.154	9.80	0.90
LDH-Nat.1	0.154	3.10	2.84
LDH-Nat. 2	0.154	3.10	2.84
LDH-Nat.3	0.154	10.60	0.84

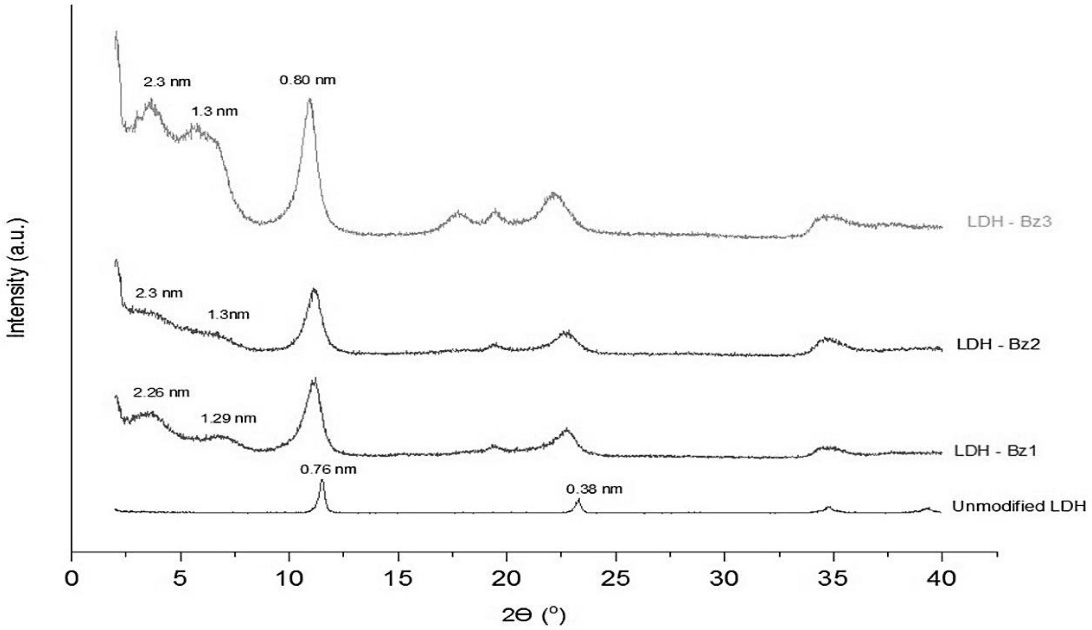

Figure 3. XRD patterns of LDH modified by sodium benzoate with different modification temperatures (LDH- Bz1: 60°C ageing, 50°C drying: LDH Bz2 - 60°C ageing, 70°C drying: LDH Bz3 - 75°C ageing, 70°C drying) and unmodified LDH.

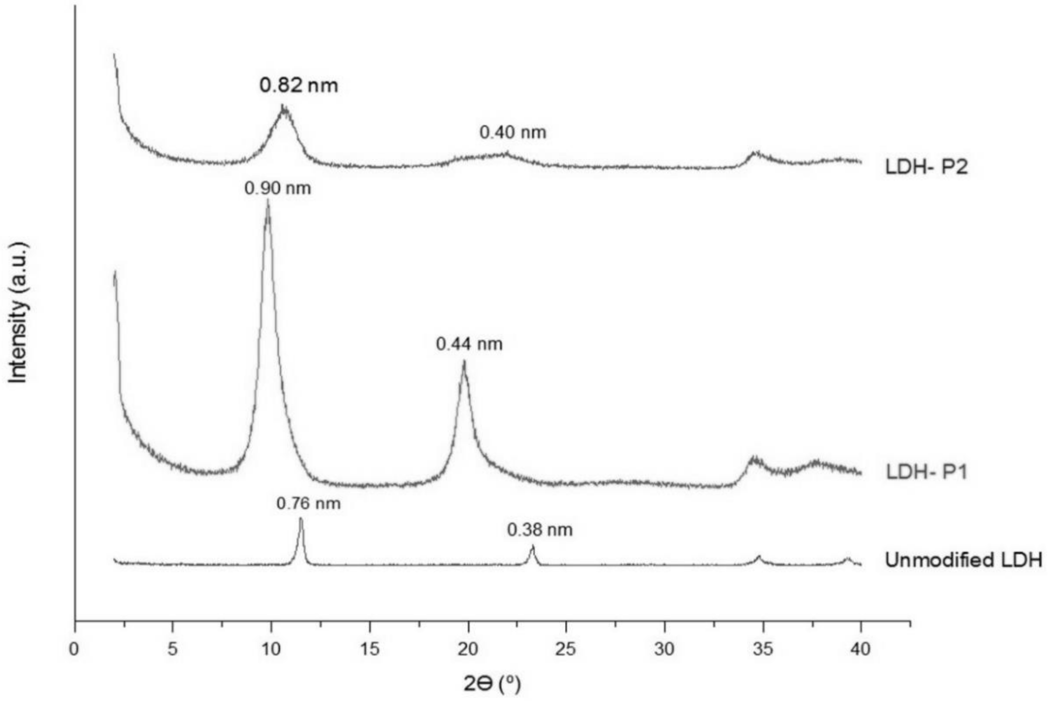

Figure 4. XRD patterns of LDH modified by sodium propionate with different modification temperatures (LDH- P 1: 60°C ageing, 50°C drying: LDH P 2 - 60°C ageing, 70°C drying) and unmodified LDH.

Figure 4 illustrates the XRD patterns LDH modified by sodium propionate. The peak position of the lowest 2θ angle of 9.8° and 10.72° was observed and corresponds to 0.90nm and 0.82 nm for LDH-P1 and LDH P2 respectively. The next peak was observed at 2θ = 19.78° for LDH-P1 and 22° for LDH-P2 with distance 0.4nm and 0.44nm. The interlayer distance calculated for both the batches of propionate were lesser or equal than the inter layer distance of LDH-NO_3 (0.866nm for the first peak and 0.439 second peak). Therefore, it can be predicted that the propionate anions were not exchanged and loaded inside the LDH matrices.

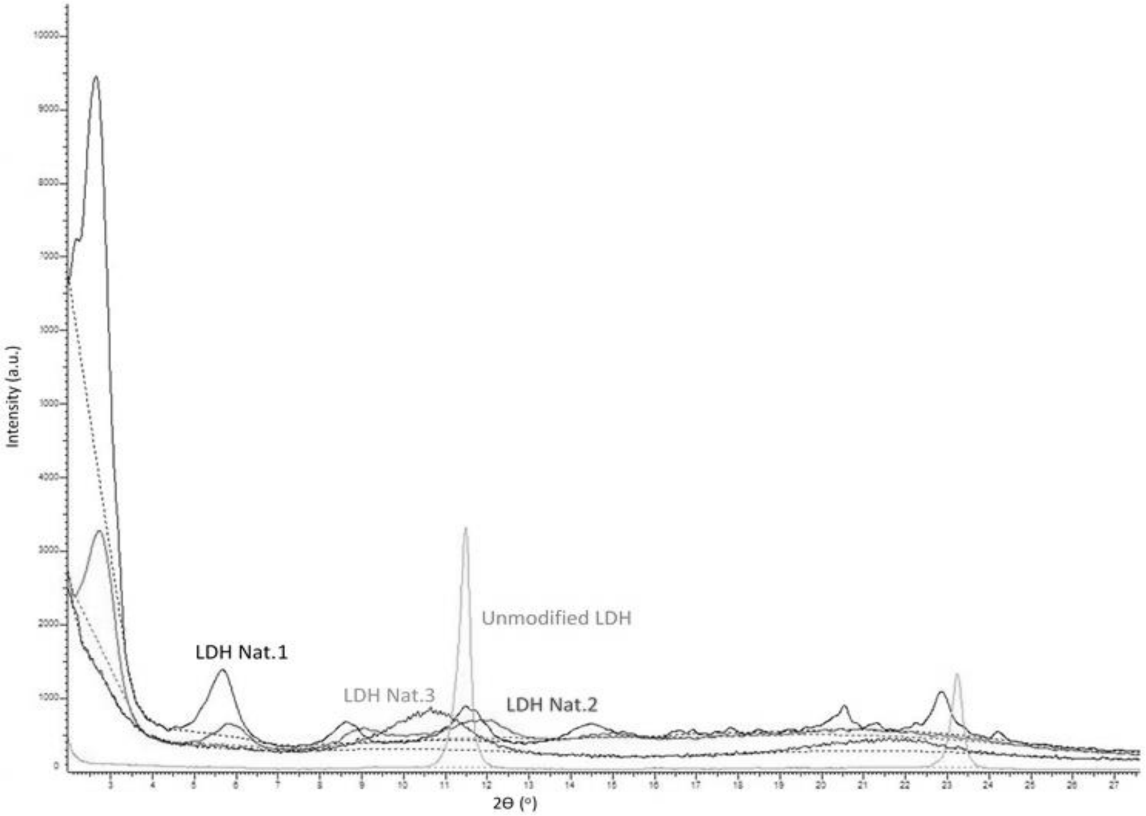

Figure 5. XRD patterns of LDH modified by Natamycin with different concentration and modification temperatures (LDH- Nat 1: 0.1 M, 60°C ageing, 50°C drying: LDH Nat 2: 0.05M, 60°C ageing, 70°C drying: Natamycin 3: 0.05M , 75°C ageing, 70°C drying) and unmodified LDH.

Figure 5, illustrates the XRD patterns LDH modified by Natamycin. The peak position of the lowest 2θ angle of 3.1° was observed for two batches of LDH-Nat. (1 and 2) and it corresponds to 2.84nm. For LDH-Nat.3, the peak shifted to 2θ angle of 10.6 ° with distance 0.84nm. For LDH-Nat 1 and LDH- Nat 2, the next peak was seen at 2θ = 5.7° and 5.8° with distance 1.54nm and 1.52nm respectively. The second peak for LDH- Nat 3 was found to be around 2θ = 21°, with distance 0.42nm.

The interlayer distance calculated for LDH- Nat 1 and LDH- Nat 2 were greater than LDH-NO_3 (0.866nm for the first peak and 0.439 second peak). But, LDH- Nat 3 had low space distance with respected to LDH-NO_3. Therefore, it can be concluded that Natamycin was successfully loaded during modification in the matrices of LDH for the first and second batch, at the same time the loading of Natamycin for the third batch was not successful.

Fourier Transform Infrared Spectroscopy. FTIR spectra of unmodified LDH and modified nitrate form (LDH-NO3) is shown in Figure 6. It displays broad band in wave length range of 3000 – 3800 cm-1 which might be due to OH- stretching vibrations in the layers (DeGruson, 2014). Kloprogge (2005) states that water HOH bending mode is generally observed in the range of 1580 cm-1 and 1655 cm-1 for hydrotalcite. *Figure 6*, Illustrates that water HOH bending for unmodified LDH and LDH- NO3 is 1573.6 cm-1 and 1650.7 cm-1 respectively. The next strong single band for both unmodified LDH and modified nitrate form (LDH-NO3) was observed around 1373 cm-1 and 1357.6 cm-1 and this may be due to asymmetric stretching vibration of CO3 2- and NO3 respectively (DeGruson, 2014).

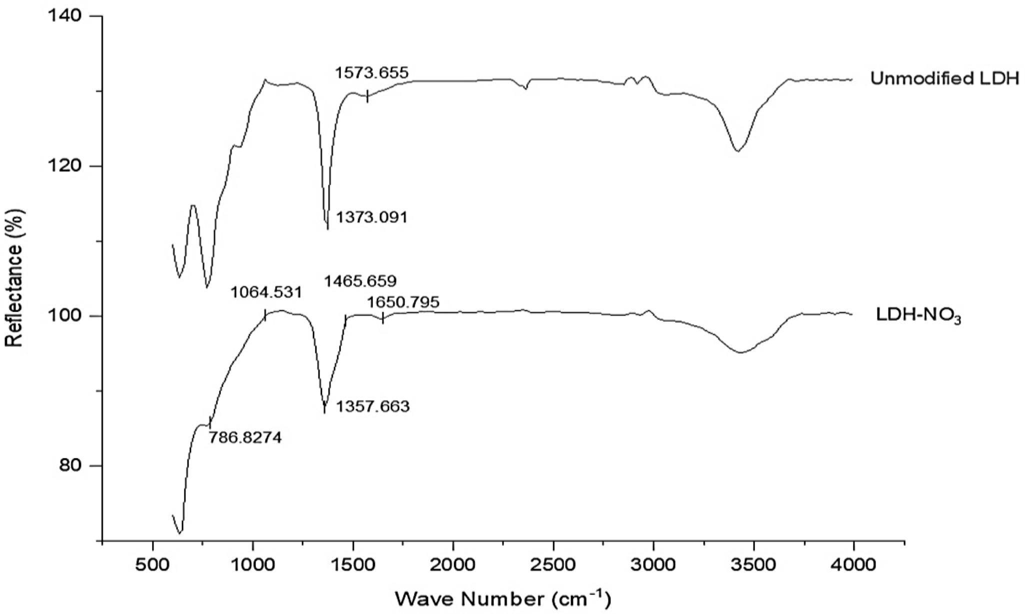

Figure 6. FTIR spectra of unmodified LDH and modified sodium nitrate (LDH- NO_3).

FTIR spectra of sodium benzoate standard and LDH- Bz are shown in *figure 7*. Bands at 1540 cm^{-1} and 1400 cm^{-1} in sodium benzoate standard signify asymmetric and symmetric stretching vibration of C-O bonds of COO- groups according to different literatures (Costantino et al., 2009). The peak at 1590 cm^{-1} signify to the in- plane skeletal vibration for aromatic ring. This peak did not change after modification into LDH layers. After the benzoate was intercalated, asymmetric stretching did not change whereas, symmetric vibration were observed to shift to somewhat lower wavenumbers: 1370 cm^{-1} for LDH - Bz1, 1390 cm^{-1} for LDH-Bz2 and LDH-Bz3 . This phenomenon as described by (Kloprogge, 2005) is due to loss of freedom and lowering of symmetry because of interaction with the surrounding interlayer water molecule.

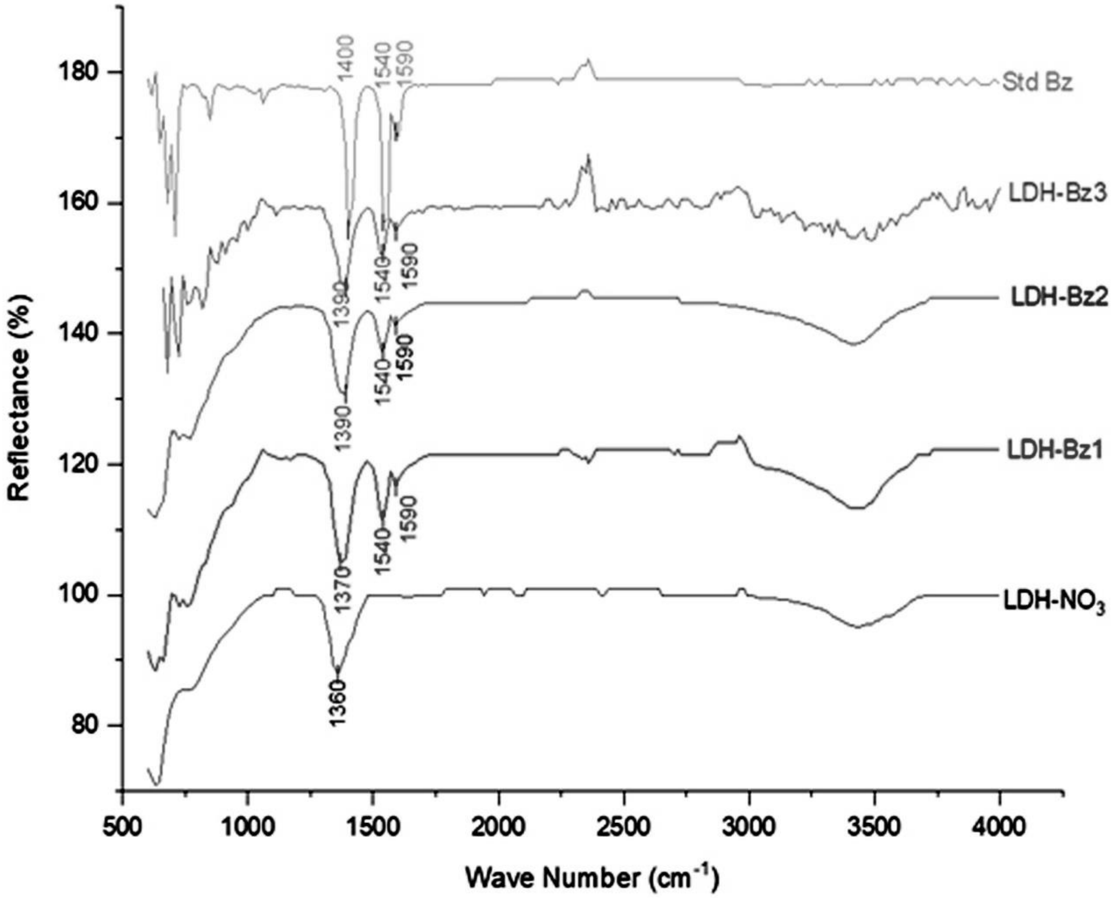

Figure 7. FTIR spectra of LDH modified by sodium benzoate with different modification temperatures (LDH- Bz1: 60°C ageing, 50°C drying: LDH Bz2 - 60°C ageing, 70°C drying: LDH Bz3 - 75°C ageing, 70°C drying), LDH in nitrate form: LDH- NO_3, and sodium benzoate.

FTIR spectra of sodium propionate standard and LDH- P are shown in *Figure 8*. For propionate, the CO_2 asymmetric vibration was observed around 1570 cm^{-1} also band 1420 cm^{-1} for symmetric CO_2 stretching mode. Kakihana and Nagumo (1987), detected similar result for CO_2 asymmetric and symmetric vibration in the band 1563.2 cm^{-1} and 1428.6 cm^{-1} respectively for sodium propionate during study on isotopic shift. Also, CH_3 deformation modes and CH_2 bending mode was found to be in the band range 1400- 1470 cm^{-1}, which agrees to Kakihana and Nagumo (1987).

After propionate was intercalated in the LDH layers, the distinct sharp bands of asymmetric and symmetric CO_2 stretching mode were not visible in both modified batches of propionate. However, distinct sharp band was observed around 1360 cm^{-1} for LDH- NO_3, LDH-P1 and LDH- P2, which represent asymmetric stretching vibration of NO_3. Therefore, propionate was not successfully intercalated during modification of LDH.

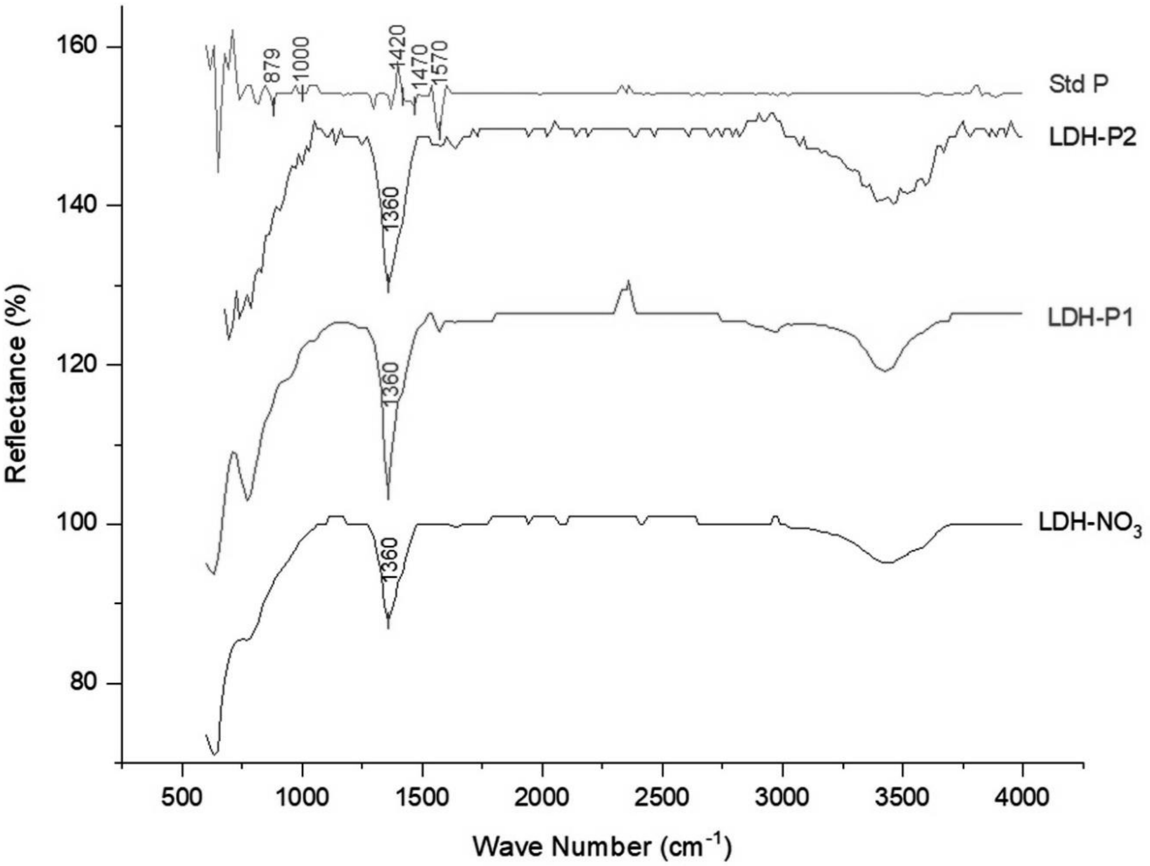

Figure 8. FTIR spectra of LDH modified by sodium propionate with different modification temperatures (LDH- P 1 : 60°C ageing, 50°C drying : LDH P 2 - 60°C ageing, 70°C drying), LDH in nitrate form: LDH- NO_3, and standard sodium propionate (Std.P).

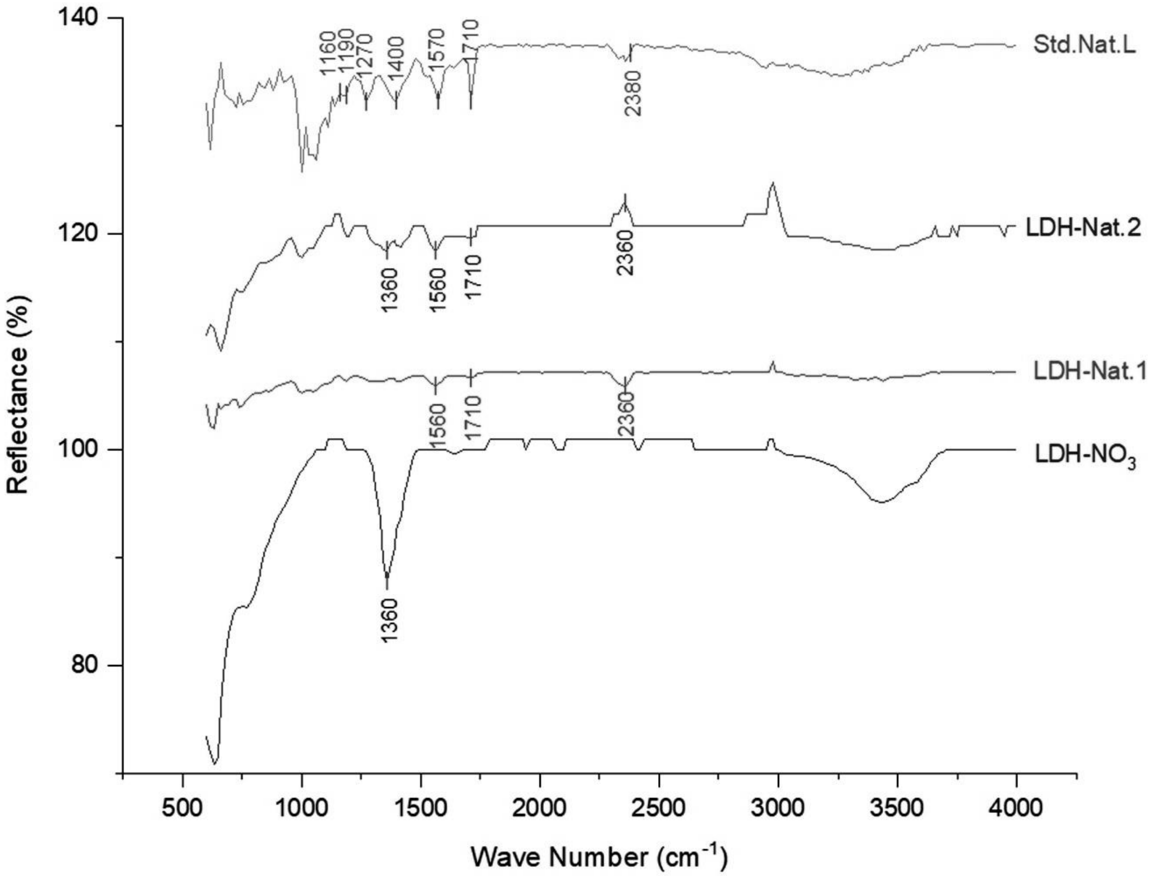

Figure 9. FTIR spectra of LDH modified by natamycin (50% Lactose) with different concentration and modification temperatures (LDH- Nat 1 : 0.1 M, 60°C ageing, 50°C drying : LDH Nat 2: 0.05M, 60°C ageing, 70°C drying), LDH in nitrate form: LDH- NO_3, and Natamycin (50% Lactose) as standard (Std. Nat-L).

FTIR spectra of Natamycin- Lactose 50%, LDH- Nat1, LDH-Nat 2 & Natamycin -salt 50%, LDH- Nat 3 are shown in *Figure 9* and *10* respectively. In both the Natamycin standard (Lactose and salt) typical carboxyl peak structure was observed around band 3000 cm-1. The peak at 1710 reflected the conjugated ester group whereas, 1570 cm-1 assigned to primary amine present in Natamycin chemical structure. The result was supported by Atta, Selim, and Zayed

(2012). They also supported that peaks in the range 1294-1116 cm^{-1} reflects existence of different C- O group.

After intercalation of Natamycin in LDH layers, various results were observed. LDH- Nat 1 showed no LDH properties at all. The modified particles looked powdery than crystalline after modification. The sharp and distinct peak at 1360 cm^{-1} neither is present, which represent asymmetric stretching vibration of NO_3. This may be because use of high molar concentration (0.1 M) during modification. Peak in 1360 cm^{-1} seemed more visible in LDH- Nat.2 and LDH-Nat. 3. Also, in these two batches, the presences of peak at 1710 $cm^{-1}$and 1570 cm^{-1} confirms the successful intercalation of Natamycin during modification.

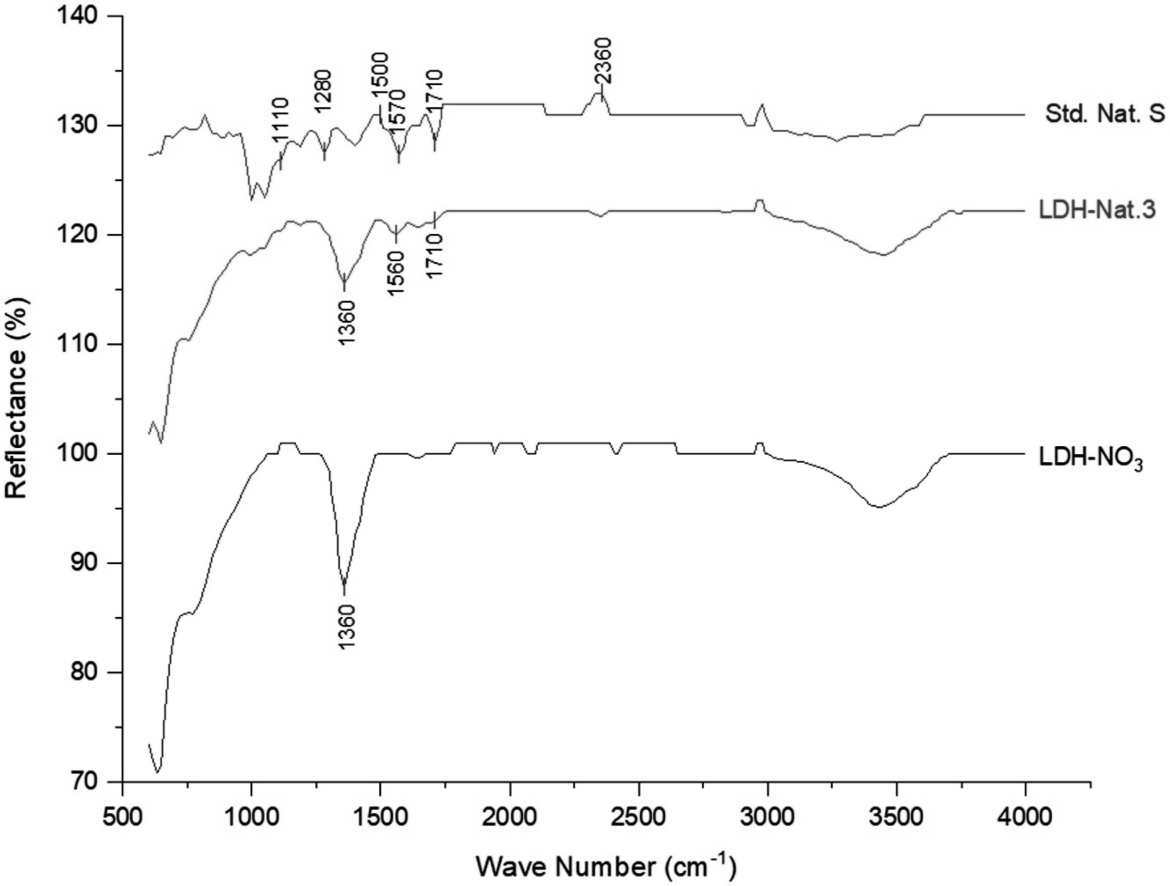

Figure 10. FTIR spectra of LDH modified by natamycin (50% salt) with 0.05 M concentration and modification temperatures (75°C ageing, 70°C drying), LDH in nitrate form: LDH- NO_3, and Natamycin (50% Lactose) as standard (Std. Nat-L).

Thermogravimetric analysis. TGA was used to determine, the composition of nanoparticles containing different anions. All nanoparticles showed similar type of initial weight loss from 7 to 19% up to 200◦C. This weight loss can be reported as loss of adsorbed water followed by the interlayer. For LDH- NO3, the second weight loss was between 200 to 500◦C. Similar loss were observed by DeGruson (2014), which can relate to the loss of water derived from de-hydroxylation of the inorganic layers and of the interlayer anions, carbonate released as CO2 and nitrate as NO.

For three LDH-Bz, similar pattern of weight loss was found. The second step between 200 °C and 400 °C was assigned to de-hydroxylation of the layers and decarboxylation of benzoate (Costantino et al., 2009). The third step between 400 °C and 800 °C was assigned to removal of the benzene from benzoate and carbonate released as CO_2. Likewise, LDH-P and LDH Nat. also followed similar behavior as LDH-Bz. The percentage of residue varied in the nanoparticles dependence on the interlayer anions. The composition was calculated using the formula $[Mg_2\,Al(OH)_6].\ Bz_X + (NO_3)_Y + H_2O)_Z$, with maintaining Mg :Al ratio 2:1 during modification . MgO and $Mg\,Al_2\,O_4$ were recognized as the residues (Costantino et al., 2009). Table 2

Composition of Nanoparticles Containing Various Anions below reports the composition for all nanoparticles. It can be seen that, ageing and drying temperature plays significant roles in loading the anions in the LDH.

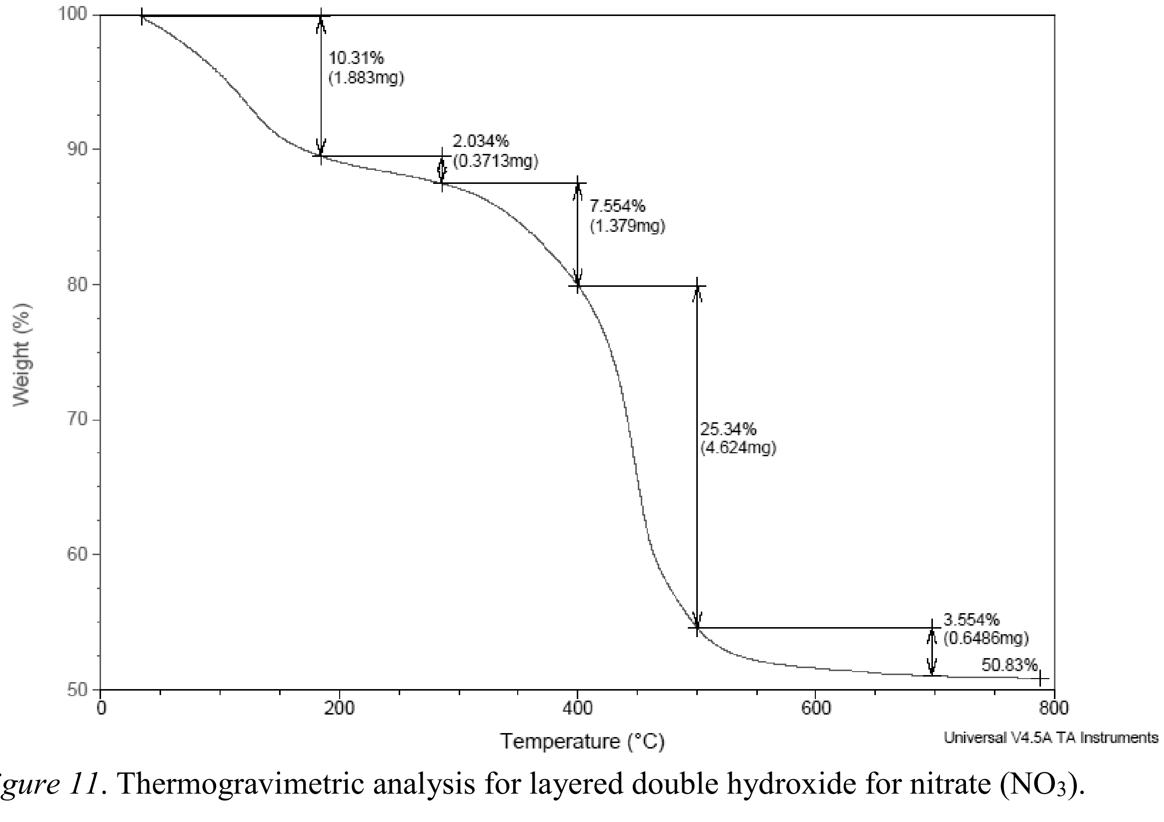

Figure 11. Thermogravimetric analysis for layered double hydroxide for nitrate (NO_3).

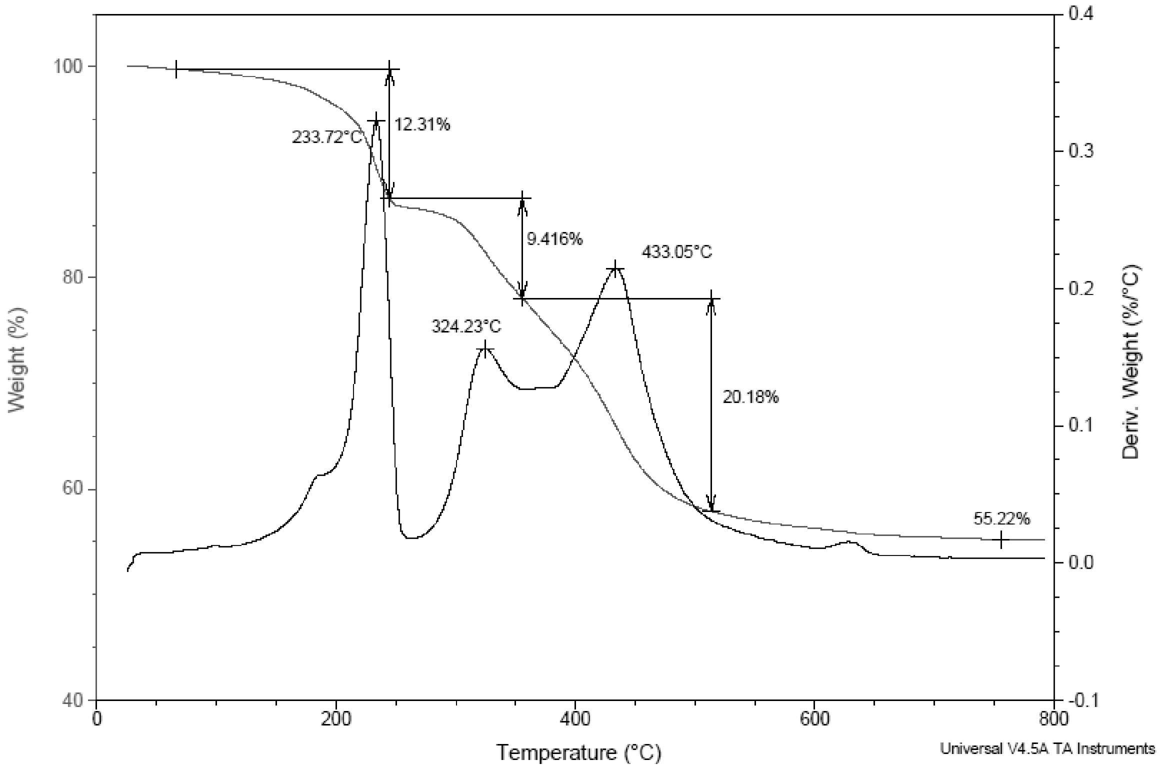

Figure 12. Thermogravimetric analysis for unmodified layered double hydroxide.

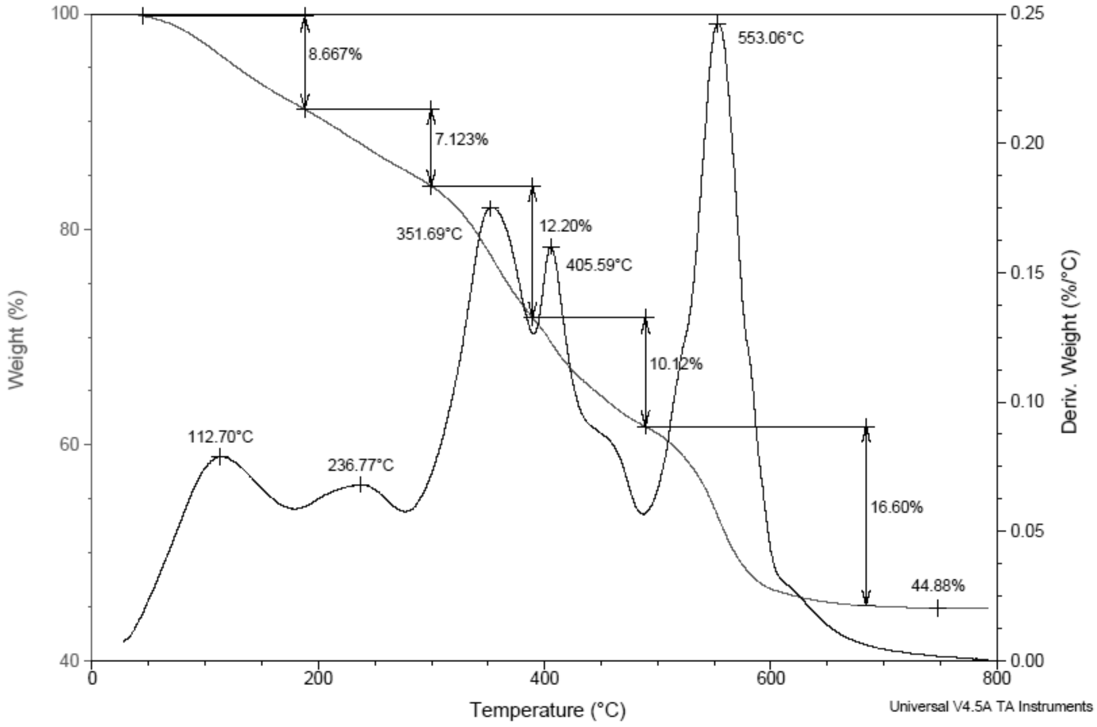

Figure 13. Thermogravimetric analysis for modified layered double hydroxide with sodium benzoate (LDH-Bz1).

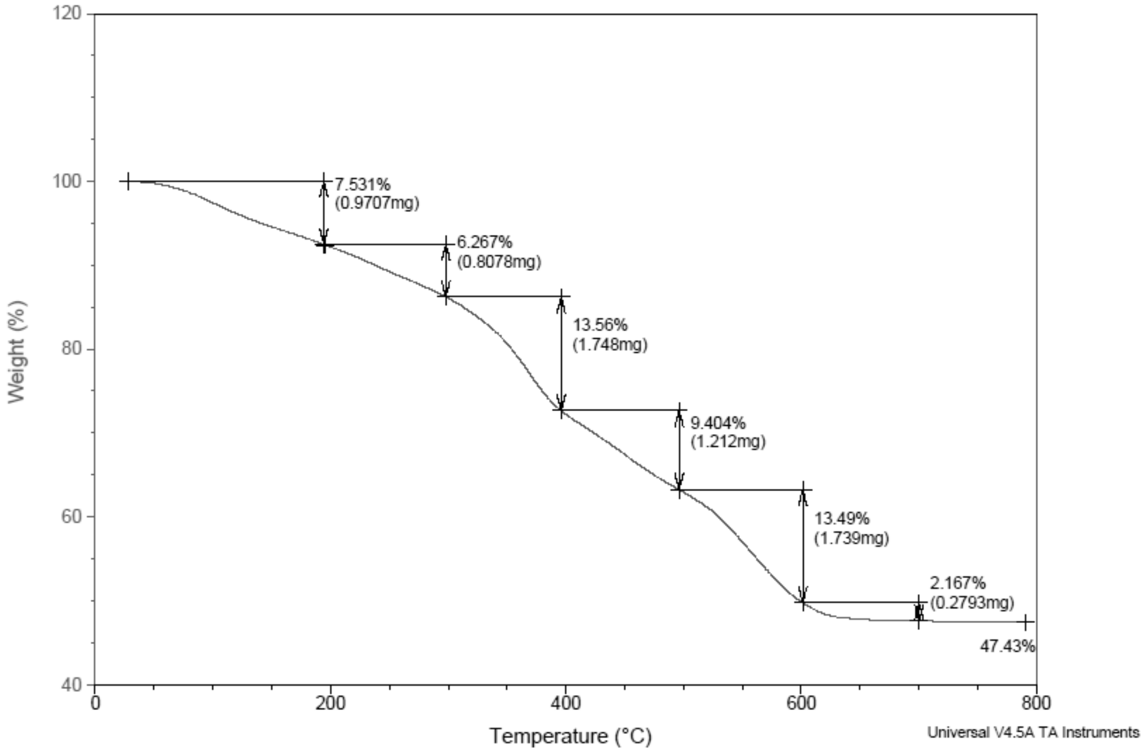

Figure 14. Thermogravimetric analysis for modified layered double hydroxide with sodium benzoate (LDH-Bz2).

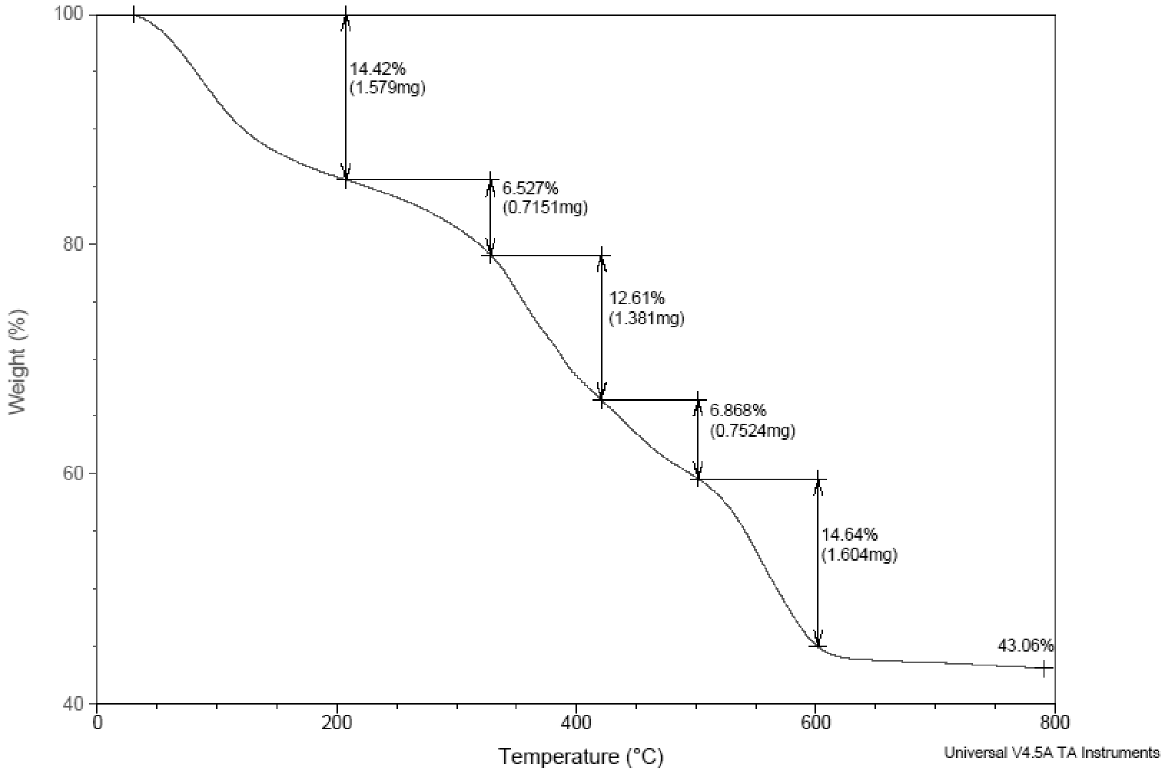

Figure 15. Thermogravimetric analysis for modified layered double hydroxide with sodium benzoate (LDH-Bz3).

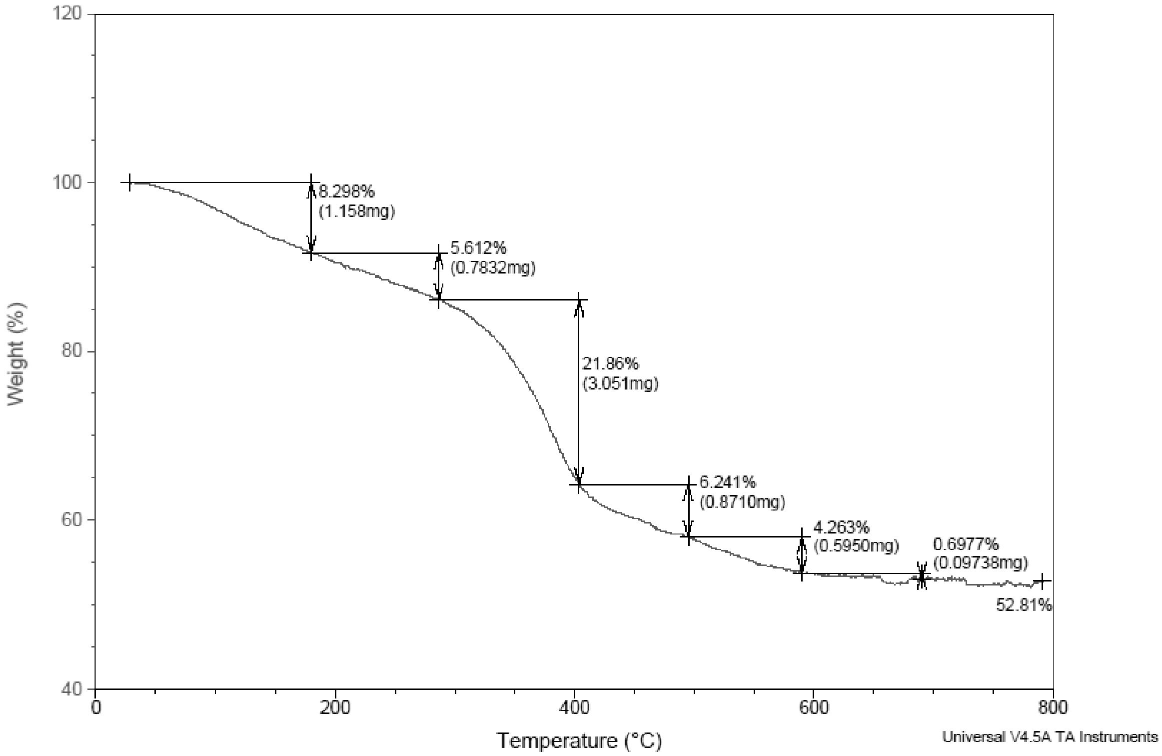

Figure 16. Thermogravimetric analysis for modified layered double hydroxide with sodium propionate (LDH-P1).

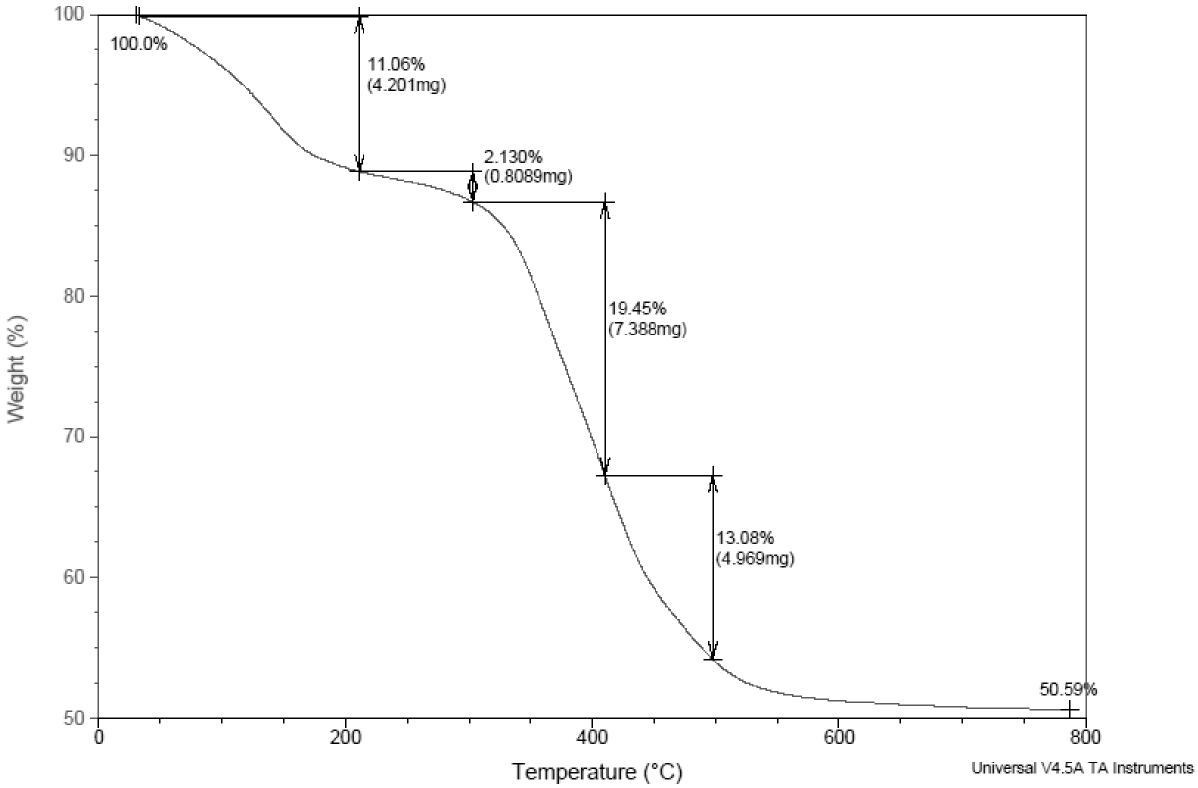

Figure 17. Thermogravimetric analysis for modified layered double hydroxide with sodium propionate (LDH-P2).

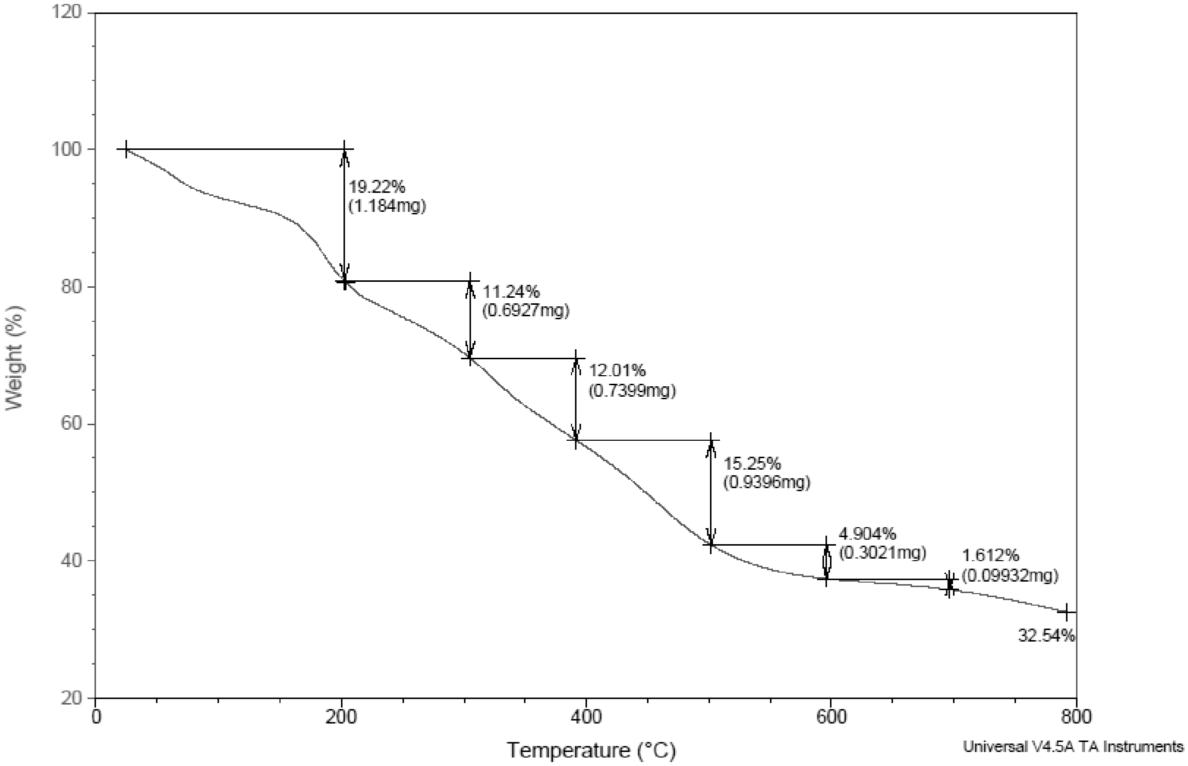

Figure 18. Thermogravimetric analysis for modified layered double hydroxide with Natamycin (LDH-Nat.1).

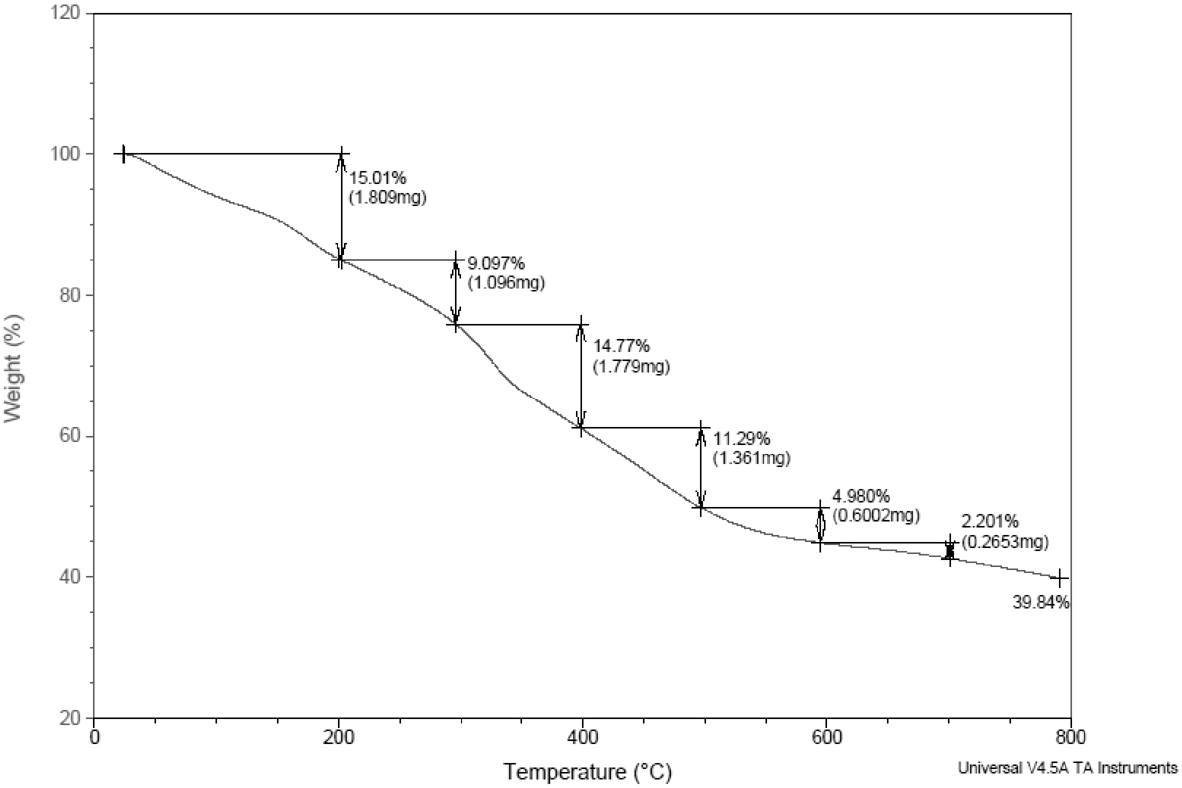

Figure 19. Thermogravimetric analysis for modified layered double hydroxide with Natamycin (LDH-Nat.2)**.**

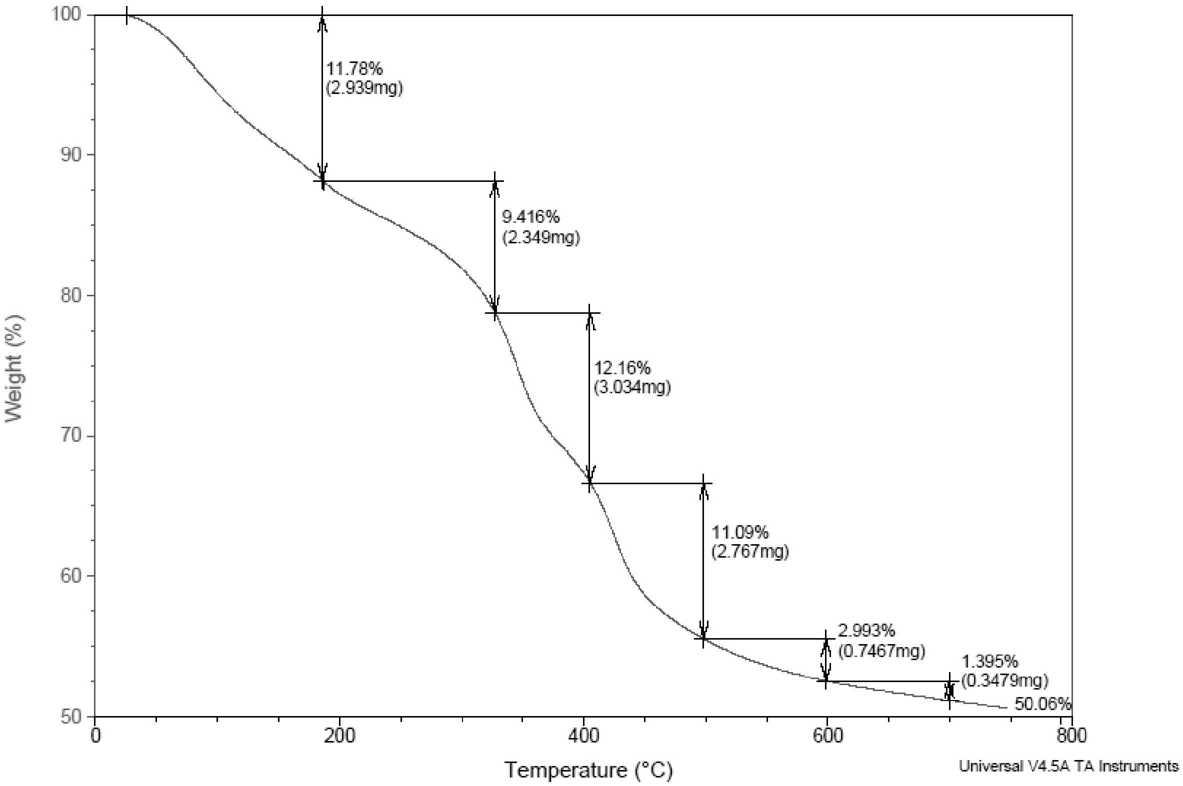

Figure 20. Thermogravimetric analysis for modified layered double hydroxide with Natamycin (LDH-Nat.3).

Table 2

Composition of Nanoparticles Containing Various Anions

Sample	Intercalated Ion	Composition	Wt. of Anion (%)
LDH-NO3	Nitrate	$[Mg_2Al(OH)_6]\ NO_3{}_{\ 0.873}\ .\ 1.476\ H_2O$	21.01
LDH-Bz1	Benzoate	$[Mg_2Al(OH)_6]\ Bz_{\ 0.33}\ .\ NO_3{}_{\ 0.66}\ .\ 1.405\ H_2O$	16.00
LDH-Bz2	Benzoate	$[Mg_2Al(OH)_6]\ Bz_{\ 0.184}\ .NO_3{}_{\ 0.815}\ .\ 1.15\ H_2O$	9.60
DH-Bz3	Benzoate	$[Mg_2Al(OH)_6]\ Bz_{\ 0.250}\ .\ NO_3{}_{\ 0.75}.\ 2.43H_2O$	12.00

Similar kind of weight loss pattern was observed by DeGruson (2014), for sodium benzoate ions, however the wt. of anion varied from 12.3 -34.9%. in previous research This might be because loading of benzoate ion in LDH depends upon the reaction condition.

Conclusion

Loading of antimicrobial agents was successfully performed into layered double hydroxides. Its loading depended upon the type of antimicrobial agent, concentration, modification condition etc. It was found that, benzoate anion can be loaded easily into layered double hydroxide matrices and its loading can be controlled by reaction condition. However, the molar concentration and the reaction time carried out for modification of benzoate did not seem to be effective for loading propionate. FTIR and XRD result illustrated that propionate anion were not successful in loading into the layered double hydroxide matrices. Mixed result was observed for Natamycin. FTIR suggested that, LDH-Nat 3, had both functional group of LDH and Natamycin. In contrast, XRD result showed that the loading of antimicrobials in the LDH matrices was not successful. However, the rest two batches, of Natamycin was loaded successfully. Therefore, it can be concluded that intercalation of antimicrobial agents like benzoate and Natamycin in LDH can be used as Nano-fillers in synthetic and bio-based packaging material to inhibit the growth of microorganisms in food.

Chapter IV: Effect of Layered Double Hydroxide Nanoparticles on Barrier Properties of Starch Based Films

The concern for safe and better environment, has rose in the interest for the use of bio-based packaging material. Petroleum based plastics are being replaced by natural polymers. Starch as a packaging material has pulled the attention both at academic and industrial level. Starch satisfies the principle aspects making it a promising raw material for packaging films (Shah et al., 2015). Starch is commonly available, inexpensive agricultural raw material. It is commercially available from different plant sources such as corn, potato, tapioca, wheat, rice etc., as a principal carbohydrate. This polymer is composed of glucose units joined by glyosidic bonds, with two glucan polymers, amylose and amylopectin. The ratio of the presence of these polymers depends upon the source of extraction. Amylose is a linear chain polymer, linked together by α-1,4-glucosidic bonds, whereas amylopectin is highly branched molecule that binds to the main glucose chain by α-1,6-glucosidic bonds. Proportion of amylose and amylopectin present, determines the crystalline properties of native starch. The crystalline structure is formed when the linear amylose and linear segments of amylopectin orient parallel to each other and facilitates the hydrogen bonding (Wurzburg, 1986). Linear structure of amylose provides more flexibility to the molecule and hence represents the amorphous fraction, while amylopectin is in the crystalline fraction due to restriction in the movement of highly branched chains (Hermansson & Svegmark, 1996).

Polysaccharide films possess a weak water vapor barrier because of their hydrophilic characteristic (Slavutsky & Bertuzzi, 2016) as they tend to absorb moisture from the surroundings. However, these properties can be increased by different ways. These can be plasticization (Perry & Donald, 2000), or blending with other materials (Vásconez, Flores, Campos, Alvarado, & Gerschenson, 2009) or modification (chemical, physical, and/or enzymatic) (Sriroth & Sangseethong, 2009) or combinations (Santayanon & Wootthikanokkhan, 2003). Plasticizers (e.g., glycerol, sorbitol, *etc.*) can be used along with those treatments to reduce the intermolecular hydrogen bonds of polymer chains and to provide good stability and flexibility. Hence, the main objective here is to study the different Barrier properties of Starch based films incorporated plasticizers and Modified Layered Double hydroxide with antimicrobials.

Preparation of Nano Particles Modified by Antimicrobials

Modification of Layered Double Hydroxide with antimicrobial agent was performed with one step biosynthesis Method in the laboratory. For the modification: Antimicrobial solution (0.1 M, 350 ml) was added into 3 -neck flasks. Metal nitrate salt solution (0.2 M Mg^{2+}, and 0.1 M Al^{3+} prepared in DI water, 300ml) added dropwise into Antimicrobial solution using additional funnel under continuous stirring and maintaining the reaction temperature at 50°C. During the synthesis, the pH value maintained at 10±0.2 by adding suitable amount of 1M NaOH solutions. Nitrogen was purged into the flask all the time. The slurry then continuously stirred at the same temperature for 30min and allowed to age in a heater at 60°C for 18 hr. Centrifugation of the solution for 5 min with 4000rpm to separate the solid particles and solution. Washing the solid particles for several times with CO_2- free water and then filtered the solution using 0.22µm Millipore filtration. Drying the white powder at 50°C in the vacuum oven till the weight was constant.

Starch Based Film Preparation

Corn starch of PURE-DENT® was provided by Grain Processing Corporation. Solvent cast Technique was used for fabrication of starch based films. Starch (2% in DI- water) and plasticizer (glycerol 30 wt. % of starch) was added to 3 necked round bottom flask. De-ionized was added to the mixture. The resulting mixture was heated to 85°C for 20 minutes with continuous reflux and magnetic stirring.

In order, to prepare Antimicrobial films (AM) and LDH modified with Antimicrobial films (LDH-AM), calculated amount of AM/ LDH-AM was dispersed in 50 ml DI- water and was stirred for 10 minutes under magnetic stirrer followed by sonication (Misonix Sonicator 3000) at power level 8 for 5 minutes. The resulting solution was then magnetically stirred for another 10 minutes followed with another 5 min sonication cycle. Then, it was mixed with starched solution and further gelatinized for 1 hour at 85°C and again sonicated for 5 minutes at power level 8. Thus, obtained solution mixture was poured (60 ml) on Teflon petri plates and vacuum dried at 40°C to eliminated trapped air bubbles. The films were then dried for 24 hours at 40°C and 55% RH. After the films were dried, they were stored at room temperature and 55% RH. For the study, Five starch-based films were prepared: starch without AM as control, starch with sodium benzoate, starch with natamycin, starch with LDH-Bz, and starch with LDH-Nat. The formula of these samples is listed below.

Table 3

The Formula for Starch Based Films

Sample name	**Starch**	**Glycerol**	**AM**
Starch	70%	30%	0%
Std-Bz	69%	30%	1% of sodium benzoate
LDH-Bz	68%	30%	2% of LDH-Bz
Std.Nat	69.6%	30%	0.4% of Natmycin
LDH_Nat	68%	30%	2% of LDH-Nat

Barrier Properties

Water vapor transmission rate (WVTR), oxygen transmission rate (OTR), and carbon dioxide transmission rate (CO_2TR) were tested for all films. The average thickness of the film was 0.11 mm.

Water vapor transmission rate.

Mocon Permatran- W® 3/34 (Mocon Inc., Minneapolis, MN, USA) was used. Test conditions were: temperature 23°C, test gas with 55% RH, carrier gas with 0% RH.

Oxygen transmission rate.

Mocon OX-TRAN® 2/22 (Mocon Inc., Minneapolis, MN, USA) was used. Test conditions were: temperature 23°C, test gas concentration 100% with 55% RH, carrier gas with 55% RH.

Carbon dioxide transmission rate.

Mocon PERMATRAN-CTM 4/4 (Mocon Inc., Minneapolis, MN, USA) was used. Test conditions were: temperature 23°C, test gas concentration 100% with 55% RH, carrier gas with 0% RH.

Permeability of the film was then calculated using the following equation.

$$\text{Permeability} = \text{Transmission Rate} * l / \Delta p$$

l is the thickness of the film; Δp is the difference of partial pressure. For water vapor permeability, Δp is 11.587 mmHg; for oxygen and carbon dioxide permeability, Δp is 1 atm.

Statistical Analysis

Statistical analysis was done using SPSS. One-way analysis of Variance (ANOVA) and tukey's multiple comparison test at 95% confidence level were applied to study the barrier properties of different starch based biofilms films.

Results and Discussion

Water vapor transmission rate (WVTR), oxygen transmission rate (OTR), and carbon dioxide transmission rate (CO_2TR) are listed in Table 4 for all films. WVTR, OTR and CO_2TR of starch based Control films were found out to be 165.2 g/(m^2/day), 23.3 cc/(m^2/day) and 74.5 cc/(m^2/day) respectively at 55% RH. Similar result was observed by Othman, Edwal, Risyon, Basha, and Talib (2017) in potato films with WVTR 113.36 g/(m^2/day) at 57% RH. WVTR of Control film was found slight greater than potato films. This may be because of difference in raw material. As proportion of amylose and amylopectin present, determines the crystalline properties of native starch (Wurzburg, 1986) and it varies with different materials. Upon Statistical analysis, the control films were found to be significantly different with other films namely Std Bz (1%), LDH Bz (2%), Std Nat (0.4%) and LDH Nat (2%) in terms of WVTR. Water Barrier properties seemed to increase with addition of Modified LDH Bz compared to Control films.

Table 4

Study of Barrier Properties of Different Starch Based Biofilms

Samples	WVTR g/(m^2/day)	OTR cc/(m^2/day)	CO_2TR cc/(m^2/day)
Control	165.2 ±4.4^{a}	23.3 ±0.7^{a}	74.5 ±4.9^{a}
Std Bz (1%)	110.2 ±2.2^{b}	11.7 ±0.2^{b,c}	19.0 ±1.4^{b,c}
LDH Bz (2%)	103.4 ±1.7^{b,c}	12.9 ±0.5^{b}	38.5± 3.5^{b}
Std Nat (0.4%)	92.0± 6.8^{c}	10.1 ±0.2^{c}	22.5± 0.7^{c}
LDH Nat (2%)	103.0 ± 3.5^{b,c}	10.3 ±1.0^{c}	19.0 ± 1.4^{c}

Note. Values are the mean ± standard deviation. Means in the same column followed by the same letter are not significantly different ($P > 0.05$).

Likewise, upon statistical analysis, OTR of Control films was found to be significantly different with rest of the films namely Std Bz (1%), LDH Bz (2%), Std Nat (0.4%) and LDH Nat (2%) films. From the Table 4, we can see that incorporation of LDH significantly improved Oxygen Barrier properties of the film. Moreover, OTR of Std Bz (1%) with LDH Bz (2%) films & Std Bz (1%) with Std Nat (0.4%) and LDH Nat (2%) films were not significantly different at 5 % level of significance.

Study of CO_2 barrier properties, of the starch based films showed that Control films were significantly different at 5% level of significance with rest of the films. The above result shows that LDH Nat. (2%) and Std.Bz (1%) film possess the highest barrier properties for CO_2 compared to other films.

Conclusion

Improvement in all the barrier properties namely WVTR, OTR and CO_2TR were observed in films incorporated with antimicrobials and modified LDH with antimicrobials compared with control films. However, Std. Nat (0.4%) films seemed to have better WVTR and OTR whereas Std. Bz (1%) and LDH Nat. (2%) films had higher CO_2 barrier properties compared to rest of the films.

Chapter V: Color Measurement of Starch Based Films

Color is a visually perceived, properties which is derived from the reflected and transmitted spectrum of light interacting with human eye (Jha, 2015). Color properties of packaging materials is one of the important attribute that determines product quality and sales. Food Product brands use variety of designs, colors, shape for the packaging of their products (Clive, Len, & Ian, 1998) to promote the sales of their product and attract consumer for the purchase. There are different color scales to determine the color intensity, among them Hunter L, a, b is the popular one. Hunter color lab scale, is generally used to get more uniform color readings for different samples including food to packaging films. Hunter Lab color is organized in a cube form. The L axis runs from Top to bottom. The maximum for L is 100, which indicates white. The minimum for L is 100, which indicate complete black. The a and b axes have no specific numerical limits. Positive "a" is red and negative "a" is green. Whereas, positive "b" is yellow and negative "b" is blue (Lab, 1996).

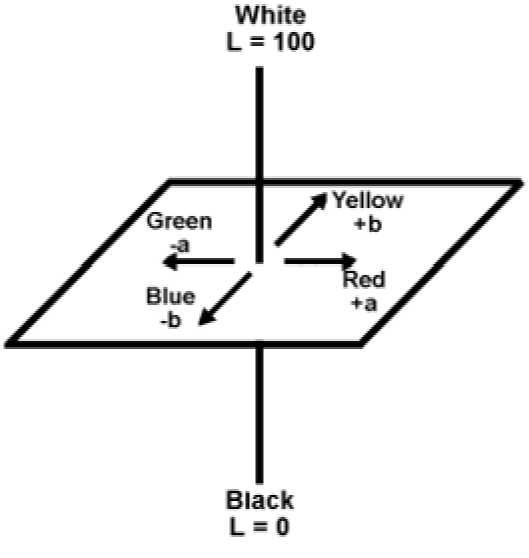

Figure 21. Hunter color lab space.

Instrumentation and Sample Preparation

Color measurement was done using Shimadzu UV- Vis spectrophotometer. For the measurement, the sample films were cut into strips 2"x 2" and placed into the sample port of the instrument. The Wavelength used in the range of 200- 900nm, triplicates readings for L, a, b were taken and the results were expressed in terms of mean values with standard deviation.

Statistical Analysis

Statistical analysis was done using SPSS. One-way analysis of Variance (ANOVA) and tukey's multiple comparison test at 95% confidence level were applied to study the color properties of different starch-based films.

Result and Discussion

Color values (L, a, b) of Starch films with nanoparticles are shown in Table 5. Starch films without nanoparticle was white and translucent with L, a and b values 93.03, 0.063 and 0.10 respectively.

Table 5

Study of Color Properties of Different Starch Based Biofilms

Sample	L	a	B
Control film	93.03±0.09 ^{a}	0.063±0.015 ^{a}	0.10±0.03 ^{a}
Unmodified LDH	94±0.33 ^{a}	0.063±0.011 ^{a}	0.23±0.11 ^{a}
Std Bz (1%)	93.96±0.56 ^{a}	0.04±0.01 ^{a}	0.063±0.11 ^{a}
LDH Bz (2%)	93.84±0.57 ^{a}	0.06±0.57 ^{a}	0.0033±0.16 ^{a}
Std Nat. Lactose (0.4%)	93.14±0.52 ^{a}	0.04±0.03 ^{a}	0.196±0.16 ^{a}
Std Nat. Salt (0.4%)	94.12±0.56 ^{a}	0.02±0.017 ^{a}	0.05±0.079 ^{a}
LDH Nat1 (2%)	93.20±0.61 ^{b}	-3.67±0.29 ^{b}	10.59±0.97 ^{b}
LDH Nat 2 (2%)	90.47±0.18 ^{c}	-2.72±0.16 ^{c}	10.46±0.51 ^{c}
LDH Nat 3 (2%)	92.53±0.27 ^{b}	-3.14±0.59 ^{b}	8.57±2.40 ^{b}

Note. Values are the mean ± standard deviation. Means in the same column followed by the same letter are not significantly different ($P > 0.05$)

The addition of unmodified LDH, LDH- Bz, Std. Bz, and Std. Natamycin (both Lactose and Salt) had no effect on optical properties. Similar results for LDH- Bz (2%) was observed by DeGruson (2014) in terms of Lightness "L" in PHBV films. In contrast addition of LDH- Nat. had significant effect on Starch films. Natamycin powder looked light yellow, after modification the color changed into darker yellow and the color of LDH-Nat. persisted even after incorporation into films at 2%. LDH- Nat. 1, LDH- Nat.2 and LDH-Nat. 3 films had high "b" value and negative "a" value that indicate the film more yellow and green respectively.

Conclusion

In terms of L a b, Control films were indifferent with Unmodified LDH, LDH Bz and the standard films of Benzoate and Natamycin. However, all the LDH- Nat. Films were found to be more yellow with low “L” value. This indicates, the use of LDH Nat. films could be restricted to use for certain colored food products like Cheddar cheese, American yellow etc. Since, the yellow color of packaging film may be unattractive from consumer point of view, when applied for all kinds of cheese packaging.

Chapter VI: Conclusion and Recommendation for Future Research

The result of the research work illustrated that intercalation of antimicrobial agent into layered double hydroxides depended upon the type of antimicrobial agent, concentration, modification condition. Benzoate and Natamycin anion was loaded successfully into layered double hydroxide matrices and its loading was controlled by reaction condition. The lowest molecular volume of benzoate made the loading of benzoate easy compared to Natamycin and propionate. However, the molar concentration and the reaction time carried out for modification of benzoate did not seem to be effective for loading propionate anion.

Likewise, barrier properties in terms of water vapor, oxygen transfer and carbon dioxide were found to be improved in films made with 2% modified LDH with antimicrobials compared to control films. In addition, Std. Nat (0.4%) films were found to have better WVTR and OTR. Std. Bz (1%) films had similar CO_2 barrier properties with respect to and LDH Nat. (2%).

A comparison of Control films in terms of L a b, was made with unmodified LDH films, antimicrobial agent modified LDH films and standard antimicrobial films. Control films were indifferent with Unmodified LDH, LDH Bz and the standard films of Benzoate and Natamycin. However, all the LDH- Nat. Films were found to be more yellow with low “L” value. This indicates, the use of LDH Nat. films could be restricted to use for certain colored food products like Cheddar cheese, American yellow etc.

Recommendations for future research include: a) Analysis of Mechanical and thermal properties of Modified LDH films; b). control release study of starch films made with modified LDH with antimicrobials; c). Study on the effect of antimicrobial properties of these packaging films on different food products.

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