SYNTHESIS OF BIO-BASED NANOCOMPOSITES FOR CONTROLLED RELEASE OF ANTIMICROBIAL AGENTS IN FOOD PACKAGING

A Dissertation in
Food Science
by
Min Liu DeGruson

Submitted in Partial Fulfillment
of the Requirements
for the Degree of

Doctor of Philosophy

August 2014
The dissertation of Min Liu DeGruson was reviewed and approved* by the following:

Gregory R. Ziegler  
Professor of Food Science  
Dissertation Co-Advisor  
Co-Chair of Committee

John D. Floros  
Professor of Food Science  
Dissertation Co-Advisor  
Co-Chair of Committee  
Dean of College of Agriculture and Director of  
Research and Extension at Kansas State University

Ramaswamy C. Anantheswaran  
Professor of Food Science

John Coupland  
Professor of Food Science

Evangelos Manias  
Professor of Polymer Science

Robert F. Roberts  
Interim Department Head  
Professor and Head of Food Science

*Signatures are on file in the Graduate School
ABSTRACT

The utilization of bio-based polymers as packaging materials has attracted great attention in both scientific and industrial areas due to the non-renewable and non-degradable nature of synthetic plastic packaging. Polyhydroxyalkanoate (PHA) is a bio-based polymer with excellent film-forming and coating properties, but exhibits brittleness, insufficient gas barrier properties, and poor thermal stability. Recently, reinforcement of such biopolymer matrices with nanoparticles was proven a promising option for improving their properties. Furthermore, modifying the nanoparticles with specific organic compounds may increase the compatibility between the bio-based polymer and nanoparticles resulting in better polymer/film properties. Antimicrobial agents (AM) incorporated into packaging materials could be used to prevent the growth of microorganisms on food surfaces and thus lead to a shelf life extension and better safety of foods. However, some properties of the AM packaging, such as mechanical, or barrier properties, may be sacrificed, because of the incorporation of antimicrobial agents and the migration and the release of AM cannot be easily predicted or controlled. The overall goal of the project was to develop the polyhydroxyalkanoate-based bio-nanocomposite films modified by antimicrobial agents with improved mechanical and gas barrier properties, along with a controlled release rate of antimicrobial agents for the inhibition of foodborne pathogens and fungi in food. The specific objectives were: a) to develop layered double hydroxide (LDH) nanoparticles modified and functionalized by antimicrobial agents (sodium benzoate, sodium gallate and potassium sorbate); b) to develop effective PHA-based LDH composite films with enhanced mechanical and barrier properties; c) to analyze and model the diffusion of antimicrobial agents through the PHA/LDH nanocomposites.

The ability for antimicrobial agents to intercalate into layered double hydroxides depended on the nature of the antimicrobial agents, such as size, spatial structure, and polarity, etc. Benzoate and gallate anions were successfully intercalated into LDH in the present study and different amounts of benzoate anion were loaded into LDH under different reaction conditions.
Incorporation of nanoparticles showed no significant effect on mechanical properties of polyhydroxybutyrate (PHB) films, however, significantly increased the tensile strength and elongation at break of polyhydroxybutyrate-co-valerate (PHBV) films. The effects of type and concentration of LDH nanoparticles (unmodified LDH and LDH modified by sodium benzoate and sodium gallate) on structure and properties of PHBV films were then studied. The arrangement of LDH in the bio-nanocomposite matrices ranged from exfoliated to phase-separated depending on the type and concentration of LDH nanoparticles. Intercalated or partially exfoliated structures were obtained using modified LDH, however, only phase-separated structures were formed using unmodified LDH. The mechanical (tensile strength and elongation at break) and thermo-mechanical (storage modulus) properties were significantly improved with low concentrations of nanoparticles incorporated into the polymer. The incorporation of LDH modified by sodium benzoate further improved the mechanical properties in comparison with unmodified LDH, which may be due to the increased compatibility between PHBV and nanoparticles and the larger basal distance between nanolayers after modification. The concentration of benzoate anions in LDH nanoparticles was another factor which affected the properties of PHBV composite films. The PHBV film with 2% modified LDH with 20.9 % w/w of benzoate anions in LDH had the best mechanical and thermo-mechanical properties. Apparent glass transition temperature increased with the addition of modified LDH but did not change with the addition of unmodified LDH. Moreover, the effect of nanoparticles on thermal properties as well as crystallization of PHBV composites was dependent on the type of nanoparticles. Unmodified LDH and LDH modified by sodium gallate had limited influence on thermal properties. However, LDH nanoparticles modified by sodium benzoate increased the crystallization temperature and enhanced recrystallization during melting, which may due to the nucleation effect by nanoparticles. The temperature at 10 % weight loss was decreased with the addition of nanoparticles, which indicated the reduction of thermal stability, but the residue left from the polymer at 300 °C was increased and the mass loss rate was decreased. Water vapor permeability was reduced with the increase of unmodified LDH due to the barrier effect of nanolayers providing a tortuous pathway for small molecules to diffuse out of the film.
Due to the hydrophilic nature of the modifiers, the water vapor permeability was increased with the addition of modified LDH.

A comparison of mechanical properties and release kinetics of antimicrobial agents directly dispersed in PHBV and modified in LDH and then dispersed in PHBV was made. The results indicated that mechanical properties increased and release rate decreased in the latter case. The release of benzoate and gallate into DI water from PHBV composite films with LDH modified by benzoate and gallate followed pseudo-Fickian behavior fitted with a power law model. The release of benzoate from PHBV composite films with LDH modified by benzoate was also fitted with a Weibull model indicating Fickian behavior in fractal substrate morphologically similar to the percolation cluster. The concentration of modified LDH and the loading of benzoate in modified LDH showed a significant effect on the release kinetics of benzoate. The diffusivities of benzoate at 21 °C ranged from 3.41 to $14.97 \times 10^{16}$ m$^2$/s. The slowest release rate was achieved by the PHBV film containing 5 % w/w of modified LDH with medium loading of benzoate (21 % w/w of benzoate) in nanoparticles. The release of gallate from PHBV was much faster than that of benzoate. The effective diffusivity of benzoate increased with increase of temperature and the activation energy $E_a$ for benzoate diffusion was calculated as 66.4 kJ/mol. It will be thus possible to design biodegradable polymeric nanocomposites with a tunable release of active molecules for various applications.

All in all, the results obtained in this work showed that mechanical and barrier properties of PHBV were increased with very low concentration (2%) of unmodified LDH and LDH modified by antimicrobial agents, which may extend the application of this bio-based polymer. Moreover, these PHBV/modified-LDH nanocomposite films containing antimicrobial agents can release different amounts of antimicrobial agents at different release rates when parameters such as incorporation methods, the nature of the interaction between the intercalated antimicrobial agents and the LDH layers, the amount of the filler in the polymer, and the amount of antimicrobial agents in the LDH are taken into consideration.
TABLE OF CONTENTS

LIST OF TABLES ........................................................................................................... x

LIST OF FIGURES ................................................................................................. xiii

ACKNOWLEDGMENTS .......................................................................................... xx

Chapter 1 ................................................................................................................. 1
INTRODUCTION AND OBJECTIVES ......................................................................... 1
1.1. Introduction ..................................................................................................... 1
1.2. Hypothesis and Objectives ........................................................................... 6
1.3. References ..................................................................................................... 6

Chapter 2 ................................................................................................................. 12
LITERATURE REVIEW ............................................................................................. 12
2.1. Bio-based Polymer Packaging Materials ..................................................... 12
    2.1.1 Polyhydroxyalkanoates (PHA) ................................................................ 12
        2.1.1.1. Synthesis ...................................................................................... 12
        2.1.1.2. Properties ................................................................................... 14
        2.1.1.3. Production and Application .......................................................... 15
    2.2. Nanotechnology and Bio-nanocomposites .................................................. 17
        2.2.1. Structure and Properties of Layered Inorganic Nanoparticles .......... 18
        2.2.2. Nanocomposite Synthesis and Characterization ............................. 21
        2.2.3. Bio-nanocomposites for Food Packaging Applications .................. 24
            2.2.3.1. Polysaccharide Based Bio-nanocomposites .............................. 24
            2.2.3.2. Protein-based Bio-nanocomposites .......................................... 28
            2.2.3.3. Lipid-based Bio-nanocomposites ............................................. 32
            2.2.3.4. PLA-based Bio-nanocomposites ............................................. 33
            2.2.3.5. PHA-based Bio-nanocomposites ............................................. 34
    2.3. Antimicrobial Nanocomposites for Food Packaging Application .............. 35
        2.3.1. Metallic-based Antimicrobial Nanocomposites ............................... 37
        2.3.2. Montmorillonite-based Antimicrobial Nanocomposites ................. 39
        2.3.3. Layered Double Hydroxide-based Antimicrobial Nanocomposites .... 40
    2.4. References .................................................................................................. 41

Chapter 3 ................................................................................................................. 59
MODIFICATION AND CHARACTERIZATION OF LAYERED DOUBLE HYDROXIDE
NANOPARTICLES WITH DIFFERENT ANTIMICROBIAL AGENTS ......................... 59
3.1. Introduction ..................................................................................................... 59
3.2. Materials and Methods .................................................................................. 61
    3.2.1. Materials .............................................................................................. 61
    3.2.2. Nanoparticle Modification .................................................................... 61
    3.2.3. Powder X-ray Diffraction (XRD) ......................................................... 62
    3.2.4. Attenuated Total Reflectance - Fourier Transform Infrared Spectroscopy.. 63
Chapter 6

MECHANICAL PROPERTIES AND RELEASE KINETICS OF
POLY(HYDROXYBUTYRATE-CO-HYDROXYVALERATE) FILMS MODIFIED
WITH DIFFERENT ANTIMICROBIAL AGENTS

6.1. Introduction................................................................................................................. 163

6.2. Materials and Methods............................................................................................... 165

6.2.1. Materials.................................................................................................................. 165

6.2.2. Preparation of Nanoparticle Modified by Antimicrobial Agents............................. 165

6.2.3. PHBV Film Preparation........................................................................................... 166

6.2.4. Design of Experiment.............................................................................................. 166

6.2.5. Mechanical Properties............................................................................................ 168

6.2.6. Antimicrobial Release Study.................................................................................... 169

6.2.6.1. Diffusion of Antimicrobial Agents........................................................................ 170

6.2.6.2. Effective Diffusivity Calculation.......................................................................... 170

6.2.6.3. Weibull Model.................................................................................................... 172

6.2.6.4. Activation Energy Determination......................................................................... 173

6.2.7. Statistical Analyses.................................................................................................. 173

6.3. Results and Discussion............................................................................................... 174

6.3.1. Sodium Benzoate.................................................................................................... 174

6.3.1.1. Effect of Incorporation Methods on Mechanical Properties................................. 174

6.3.1.2. Effect of Incorporation Methods on Release Kinetics........................................ 175

6.3.1.3. Effect of Concentrations and Types of Modified Nanoparticles in the Films on
Release Kinetics ............................................................................................................ 187

6.3.1.4. Effect of Temperature on Release Kinetics.......................................................... 197

6.3.2. Sodium Gallate........................................................................................................ 203
6.3.2.1. Effect of Incorporation Methods on Mechanical Properties ............................... 203
6.3.2.2. Effect of Incorporation Methods on Release Kinetics........................................... 204
6.3.2.3. Effect of Concentrations of Modified Nanoparticles on Release Kinetics........... 206
6.4. Conclusions ..................................................................................................................... 208
6.5. References ...................................................................................................................... 209

Chapter 7 .............................................................................................................................. 214
CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH .................. 214

Appendix A .............................................................................................................................. 218
CHAPTER 5 – SUPPLEMENTAL DATA .......................................................... 218
Appendix B .............................................................................................................................. 220
CHAPTER 6 – SUPPLEMENTAL DATA .............................................................................. 220
LIST OF TABLES

Table 3.1. Molecular properties of antimicrobial agents ........................................ 71
Table 3.2. Composition of nanoparticles containing various anions ..................... 80
Table 4.1. Mechanical properties of filtered (F) or unfiltered (UF) PHB films with 2% of unmodified LDH or LDH modified by sodium benzoate ..................................... 101
Table 4.2. Mechanical properties of filtered (F) or unfiltered (UF) PHBV films with 2% of unmodified LDH or LDH modified by sodium benzoate ................................... 102
Table 4.3. Thermal properties of filtered (F) or unfiltered (UF) PHB films with 2% of unmodified LDH or LDH modified by sodium benzoate ........................................ 110
Table 4.4. Thermal properties of filtered (F) or unfiltered (UF) PHBV films with 2% of unmodified LDH or LDH modified by sodium benzoate ........................................ 111
Table 4.5. Thermal stability of filtered (F) or unfiltered (UF) PHB films with 2% of unmodified LDH or LDH modified by sodium benzoate ........................................ 113
Table 4.6. Thermal stability of filtered (F) or unfiltered (UF) PHBV films with 2% of unmodified LDH or LDH modified by sodium benzoate ........................................ 114
Table 5.1. Composition of nanoparticles containing various anions ..................... 124
Table 5.2. Optical properties of PHBV and PHBV composite films with various concentrations of unmodified LDH, modified LDH with 12.3% benzoate (LDH-SB1), 20.9% benzoate (LDH-SB2), 34.9% benzoate (LDH-SB3), or 43.9% gallate (LDH-SG) ................................................................................................................. 138
Table 5.3. Mechanical properties of PHBV with various concentrations of unmodified LDH ......................................................................................................................... 139
Table 5.4. Effect of type (unmodified LDH, modified LDH with 12.3% benzoate (LDH-SB1), 20.9% benzoate (LDH-SB2), 34.9% benzoate (LDH-SB3), and 43.9% gallate (LDH-SG)) and concentration of nanoparticles on ultimate tensile strength of PHBV composite films .............................................................................................................................. 140
Table 5.5. Effect of type (unmodified LDH, modified LDH with 12.3% benzoate (LDH-SB1), 20.9% benzoate (LDH-SB2), 34.9% benzoate (LDH-SB3), and 43.9% gallate (LDH-SG)) and concentration of nanoparticles on elongation at break of PHBV composite films .............................................................................................................................. 140
Table 5.6. Effect of type (unmodified LDH, modified LDH with 12.3% benzoate (LDH-SB1), 20.9% benzoate (LDH-SB2), 34.9% benzoate (LDH-SB3), and 43.9% gallate (LDH-SG)) and concentration of nanoparticles on impact strength of PHBV composite films .............................................................................................................................. 140
gallate (LDH-SG)) and concentration of nanoparticles on elastic modulus of PHBV composite films .............................................................................................................. 141

Table 5.7. Effect of type (unmodified LDH, modified LDH with 12.3% benzoate (LDH-SB1), 20.9% benzoate (LDH-SB2), 34.9% benzoate (LDH-SB3), and 43.9% gallate (LDH-SG)) and concentration of nanoparticles on apparent glass transition temperature (T_g^app) of PHBV composite films ........................................................................ 146

Table 5.8. Thermal properties of PHBV and PHBV composition films with various concentrations of unmodified LDH, modified LDH with 12.3% benzoate (LDH-SB1), 20.9% benzoate (LDH-SB2), 34.9% benzoate (LDH-SB3), or 43.9% gallate (LDH-SG) ........................................................................................................................................... 150

Table 5.9. Thermal stability of PHBV and PHBV composite films with various concentrations of unmodified LDH, modified LDH with 12.3% benzoate (LDH-SB1), 20.9% benzoate (LDH-SB2), 34.9% benzoate (LDH-SB3), or 43.9% gallate (LDH-SG) ........................................................................................................................................... 154

Table 5.10. Effect of type (unmodified LDH, modified LDH with 12.3% benzoate (LDH-SB1), 20.9% benzoate (LDH-SB2), 34.9% benzoate (LDH-SB3), and 43.9% gallate (LDH-SG)) and concentration of nanoparticles on water vapor permeability (WVP) at 23 °C of PHBV composite films ........................................................................... 155

Table 6.1. Design of experiment to study the effect of incorporation methods of antimicrobial agents into PHBV on mechanical properties and release kinetics of PHBV films ................................................................................................................................. 167

Table 6.2. Design of experiment to study the effect of type and concentration of LDH modified by sodium benzoate on the release kinetics of benzoate from PHBV composite films ........................................................................................................................................... 168

Table 6.3. Mechanical properties of PHBV films with 0.42% sodium benzoate using different incorporation methods ........................................................................................................................................... 175

Table 6.4. Mechanical properties of PHBV films with 1.05% sodium benzoate using different incorporation methods ........................................................................................................................................... 175

Table 6.5. Effective diffusivity of benzoate released in DI water at 21 °C from PHBV films using different incorporation methods ........................................................................................................................................... 182

Table 6.6. Parameters α and β calculated from Weibull model and n from power law model for benzoate release in DI water at 21 °C from PHBV films using different incorporation methods ........................................................................................................................................... 185

Table 6.7. Parameter n calculated from power law model for benzoate release in DI water at 21 °C from PHBV films with different concentrations of LDH modified by sodium benzoate, LDH-SB1 with 12.3% benzoate, LDH-SB2 with 20.9% benzoate, or LDH-SB3 with 34.9% benzoate ........................................................................................................................................... 192
Table 6.8. Effect of type (modified LDH with 12.3% benzoate (LDH-SB1), 20.9% benzoate (LDH-SB2), and 34.9% benzoate (LDH-SB3)) and concentration of on effective diffusivity of benzoate release in DI water at 21°C .......................... 193

Table 6.9. Parameters $\alpha$ and $\beta$ calculated from Weibull model for benzoate release in DI water at 21°C from PHBV films with different concentrations of LDH with 12.3% benzoate (LDH-SB1), 20.9% benzoate (LDH-SB2), or 34.9% benzoate (LDH-SB3). ............................................................................................................. 197

Table 6.10. Effective diffusivity of benzoate released in DI water from PHBV film with 2% of modified LDH with 20.9% benzoate (PHBV-LDH_SB2-2%) at different temperatures .......................................................................................................................... 200

Table 6.11. Parameters $\alpha$ and $\beta$ calculated from Weibull model for the benzoate release in DI water from PHBV film with 2% of modified LDH with 20.9% benzoate (PHBV-LDH_SB2-2%) at different temperatures .................................................................................. 202

Table 6.12. Mechanical properties of PHBV films with 0.88% sodium gallate using different incorporation methods .......................................................................................... 204

Table 6.13. Mechanical properties of PHBV films with 2.2% sodium gallate using different incorporation methods .......................................................................................... 204

Table 6.14. Effect of concentration of nanoparticles modified by gallate in the films on gallate effective diffusivity ............................................................................................................ 208
LIST OF FIGURES

Figure 2.1. Classification of the bio-based polymers. .......................................................... 13

Figure 2.2. Schematic representations comparing the crystal structure of brucite (A) and LDH (B) .................................................................................................................. 19

Figure 2.3. Polymer-nanoparticles morphologies ................................................................ 22

Figure 2.4. Schematic illustration of the tortuosity for a diffusing penetrant introduced on exfoliated solid layered in a polymer matrix. A. Filled polymer and B. Unfilled polymer. .................................................................................. 23

Figure 3.1. XRD patterns of unmodified LDH (LDH-CO$_3$), LDH in chloride form (LDH-Cl), and LDH in nitrate form (LDH-NO$_3$) ........................................................................... 65

Figure 3.2. XRD patterns of LDH in nitrate form (LDH-NO$_3$), and LDH modified by sodium benzoate with different modification conditions (molar ratio of benzoate anion/NO$_3^-$; reaction temperature), LDH-SB1 (3:1; 20 °C), LDH-SB2 (18:1; 20 °C), and LDH-SB3 (18:1; 70 °C) .................................................................................. 66

Figure 3.3. The structural model showing three arrangements of benzoate anions between the LDH layers ................................................................. 70

Figure 3.4. XRD patterns of LDH in nitrate form (LDH-NO$_3$), and LDH modified by sodium gallate (LDH-SG3) under the third reaction condition (molar ratio of gallate anion/NO$_3^-$ = 18:1 and reaction temperature = 70 °C) ...................... 70

Figure 3.5. Ball-and-stick structures of antimicrobial agents generated by Material Studio v.4.1 ......................................................................................................................... 71

Figure 3.6. FTIR spectra of unmodified LDH (LDH-CO$_3$), LDH in chloride form (LDH-Cl), and LDH in nitrate form (LDH-NO$_3$) ........................................................................... 72

Figure 3.7. FTIR spectra of LDH in nitrate form (LDH-NO$_3$), LDH modified by sodium benzoate with different modification conditions (molar ratio of benzoate anion/NO$_3^-$; reaction temperature), LDH-SB1 (3:1; 20 °C), LDH-SB2 (18:1; 20 °C), and LDH-SB3 (18:1; 70 °C), and sodium benzoate (SB) .............................................. 74

Figure 3.8. FTIR spectra of LDH in nitrate form (LDH-NO$_3$), and LDH modified by sodium gallate (LDH-SG3) under the third reaction condition (molar ratio of gallate anion/NO$_3^-$ = 18:1 and reaction temperature = 70 °C) ...................... 75

Figure 3.9. TGA-MS patterns of a. unmodified LDH (LDH-CO$_3$); b. LDH in chloride form (LDH-Cl); c. LDH in nitrate form (LDH-NO$_3$); d-e, LDH modified by sodium benzoate with different modification conditions (molar ratio of benzoate
anion/NO$_3^-$; reaction temperature), d. LDH-SB1 (3:1; 20 °C), e. LDH-SB2 (18:1; 20 °C), and f. LDH-SB3 (18:1; 70 °C); and g. LDH modified by sodium gallate (LDH-SG3) under the third reaction condition (molar ratio of gallate anion/NO$_3^-$ = 18:1 and reaction temperature = 70 °C).

Figure 3.10. Benzoate release in DI water at 21 °C from LDH modified by sodium benzoate with different modification conditions (molar ratio of benzoate anion/NO$_3^-$; reaction temperature), LDH-SB1 (3:1; 20 °C), LDH-SB2 (18:1; 20 °C), and LDH-SB3 (18:1; 70 °C).

Figure 3.11. Gallate release in DI water at 21 °C from LDH modified by sodium gallate (LDH-SG3) under the third reaction condition (molar ratio of gallate anion/NO$_3^-$ = 18:1 and reaction temperature = 70 °C).

Figure 4.1. XRD patterns of filtered (F) and unfiltered (UF) PHB films with 2% of unmodified LDH (LDH-CO$_3$). The number indicates the distance between nanolayers.

Figure 4.2. XRD patterns of filtered (F) and unfiltered (UF) PHB films with 2% of LDH modified by benzoate (LDH-SB). The number indicates the distance between nanolayers.

Figure 4.3. XRD patterns of filtered (F) and unfiltered (UF) PHBV films with 2% of unmodified LDH (LDH-CO$_3$). The number indicates the distance between nanolayers.

Figure 4.4. XRD patterns of filtered (F) and unfiltered (UF) PHBV films with 2% of LDH modified by benzoate (LDH-SB). The number indicates the distance between nanolayers.

Figure 4.5. SEM images of PHB/PHBV composite films with 2% of unmodified LDH or LDH modified by sodium benzoate (LDH-SB) films with filtration.

Figure 4.6. SEM images of the filtered PHBV film with 2% of LDH modified by sodium benzoate at (a) low magnification and (b) high magnification. The white arrows indicate the nanoparticles.

Figure 4.7. Effect of temperature on storage modulus of filtered (F) or unfiltered (UF) PHB films with 2% of unmodified LDH or LDH modified by sodium benzoate.

Figure 4.8. Effect of temperature on tan δ of filtered (F) or unfiltered (UF) PHB films with 2% of unmodified LDH or LDH modified by sodium benzoate.

Figure 4.9. Effect of temperature on storage modulus of filtered (F) or unfiltered (UF) PHBV films with 2% of unmodified LDH or LDH modified by sodium benzoate.
Figure 4.10. Effect of temperature on tan δ of filtered (F) or unfiltered (UF) PHBV films with 2% of unmodified LDH or LDH modified by sodium benzoate. .......................... 105

Figure 4.11. DSC cooling cycle of filtered (F) or unfiltered (UF) PHB films with 2% of unmodified LDH or LDH modified by sodium benzoate. ................................. 107

Figure 4.12. DSC heating cycle of filtered (F) or unfiltered (UF) PHB films with 2% of unmodified LDH or LDH modified by sodium benzoate. ................................. 107

Figure 4.13. DSC cooling cycle of filtered (F) or unfiltered (UF) PHBV films with 2% of unmodified LDH or LDH modified by sodium benzoate. ................................. 108

Figure 4.14. DSC heating cycle of filtered (F) or unfiltered (UF) PHBV films with 2% of unmodified LDH or LDH modified by sodium benzoate. ................................. 108

Figure 4.15. DSC heating cycle of unfiltered PHBV film (PHBV-UF) with different heating rates. ........................................................................................................... 111

Figure 4.16. TGA patterns of filtered (F) or unfiltered (UF) PHB films with 2% of unmodified LDH or LDH modified by sodium benzoate. ................................. 113

Figure 4.17. TGA patterns of filtered (F) or unfiltered (UF) PHBV films with 2% of unmodified LDH or LDH modified by sodium benzoate. ................................. 114

Figure 5.1. XRD patterns of PHBV composite films with various concentrations of unmodified LDH. ................................................................................................... 129

Figure 5.2. XRD patterns of PHBV composite films with various concentrations of modified LDH with 12.3% benzoate (LDH-SB1). ................................................. 130

Figure 5.3. XRD patterns of PHBV composite films with various concentrations of modified LDH with 20.9% benzoate (LDH-SB2). ................................................. 131

Figure 5.4. XRD patterns of PHBV composite films with various concentrations of modified LDH with 34.9% benzoate (LDH-SB3). ................................................. 131

Figure 5.5. XRD patterns of PHBV composite films with various concentrations of modified LDH with 43.9% gallate (LDH-SG). ................................................... 132

Figure 5.6. TEM images of PHBV and PHBV composite films with various concentrations of unmodified LDH. Scale bars = 5000 nm. ................................. 134

Figure 5.7. TEM images of PHBV and PHBV composite films with various concentrations of modified LDH with 12.3% benzoate (LDH-SB1), or 20.9% benzoate (LDH-SB2). Scale bars = 2000 nm. .................................................. 135
Figure 5.8. TEM images of PHBV and PHBV with various concentrations of modified LDH with 34.9% benzoate (LDH-SB3), or 43.9% gallate (LDH-SG). Scale bars = 2000 nm. ................................................................. 136

Figure 5.9. Representative TEM images of PHBV containing unmodified (a&b) or modified (c&d) LDH. Scale bars = 100 nm. The white arrow in (a) indicates the LDH in hexagonal shape. The white square in (b) indicates the stacked nanolayers. The white arrows in (c&d) indicate individual nanolayers or disordered stacked nanolayers with swollen gap. ................................................................. 137

Figure 5.10. Effect of type (unmodified LDH, modified LDH with 12.3% benzoate (LDH-SB1), 20.9% benzoate (LDH-SB2), 34.9% benzoate (LDH-SB3), and 43.9% gallate (LDH-SG)) and concentration of nanoparticles on storage modulus (E’) of PHBV composite films. ................................................................. 143

Figure 5.11. Effect of type (unmodified LDH, modified LDH with 12.3% benzoate (LDH-SB1), 20.9% benzoate (LDH-SB2), 34.9% benzoate (LDH-SB3), and 43.9% gallate (LDH-SG)) and concentration of nanoparticles on apparent glass transition temperature of PHBV composite films ................................................................. 145

Figure 5.12. DSC cooling cycle for PHBV and PHBV composite films with various concentrations of unmodified LDH, modified LDH with 12.3% benzoate (LDH-SB1), 20.9% benzoate (LDH-SB2), 34.9% benzoate (LDH-SB3), or 43.9% gallate (LDH-SG). ................................................................................................. 147

Figure 5.13. DSC heating cycle for PHBV and PHBV composite films with various concentrations of unmodified LDH, modified LDH with 12.3% benzoate (LDH-SB1), 20.9% benzoate (LDH-SB2), 34.9% benzoate (LDH-SB3), or 43.9% gallate (LDH-SG). ................................................................................................. 148

Figure 5.14. TGA (left) and DTG (right) patterns of PHBV and PHBV composite films with various concentrations of unmodified LDH, modified LDH with 12.3% benzoate (LDH-SB1), 20.9% benzoate (LDH-SB2), 34.9% benzoate (LDH-SB3), or 43.9% gallate (LDH-SG). TGA: thermogravimetric analysis; DTG: Derivative thermogravimetry.................................................................................................. 153

Figure 5.15. Schematic illustration of the tortuosity for a diffusing penetrant introduced on exfoliated solid layered in a polymer matrix. A. Filled film with unmodified LDH, B. Unfilled film, and C. Filled film with modified LDH. ................................................................. 156

Figure 6.1. Release of benzoate in DI water at 21°C as: (a) release fraction of \( M_t/M_0 \); and (b) release fraction of \( M_t/M_\infty \) from PHBV films with 0.42% sodium benzoate using different incorporation methods of benzoate either directly incorporated into PHBV without LDH or with LDH or modified onto LDH nanolayers first and then incorporated in PHBV. \( M_0, M_t, \) and \( M_\infty \) are initial amount of benzoate in the film before release, and amounts of benzoate released at time \( t \) and equilibrium, respectively. ........................................................................................................ 177
Figure 6.2. Release of benzoate in DI water at 21°C as: (a) release fraction of $M_t/M_0$; and (b) release fraction of $M_t/M_\infty$ from PHBV films with 1.05% sodium benzoate using different incorporation methods of benzoate either directly incorporated into PHBV without LDH or with LDH or modified onto LDH nanolayers first and then incorporated in PHBV. $M_0$, $M_t$, and $M_\infty$ are initial amount of benzoate in the film before release, and amounts of benzoate released at time t and equilibrium, respectively. .................................................................................................................. 178

Figure 6.3. Plot of $\ln (M_t/M_\infty)$ vs $\ln (t)$ for the early portion release of benzoate in DI water at 21°C from PHBV films with 0.42% sodium benzoate using different incorporation methods of benzoate either directly incorporated into PHBV without LDH or with LDH or modified onto LDH nanolayers first and then incorporated in PHBV. $M_t$ and $M_\infty$ are amounts of benzoate released at time t and equilibrium, respectively. Symbols represent the experimental data and lines show the trend. The slope of the curve represented by n (power law function shown in the inset) indicates the mechanism of release. ............................................................................. 179

Figure 6.4. Plot of $\ln (M_t/M_\infty)$ vs $\ln (t)$ for the early portion release of benzoate in DI water at 21°C from PHBV films with 1.05% sodium benzoate using different incorporation methods of benzoate either directly incorporated into PHBV without LDH or with LDH or modified onto LDH nanolayers first and then incorporated in PHBV. $M_t$ and $M_\infty$ are amounts of benzoate released at time t and equilibrium, respectively. Symbols represent the experimental data and lines show the trend. The slope of the curve represented by n (power law function shown in the inset) indicates the mechanism of release. ............................................................................. 180

Figure 6.5. Plot of $M_t/M_\infty$ vs $\sqrt{t/h}$ for early portion of benzoate release ($M_t/M_\infty < 0.6$) in DI water at 21°C from PHBV films with 0.42% sodium benzoate using different incorporation methods of benzoate either directly incorporated into PHBV without LDH or with LDH or modified onto LDH nanolayers first and then incorporated in PHBV. $M_t$ and $M_\infty$ are amounts of benzoate released at time t and equilibrium, respectively. Symbols represent experimental data and lines show the trend. .................................................................................................................. 181

Figure 6.6. Plot of $M_t/M_\infty$ vs $\sqrt{t/h}$ for early portion of benzoate release ($M_t/M_\infty < 0.6$) in DI water at 21°C from PHBV films with 1.05% sodium benzoate using different incorporation methods of benzoate either directly incorporated into PHBV without LDH or with LDH or modified onto LDH nanolayers first and then incorporated in PHBV. $M_t$ and $M_\infty$ are amounts of benzoate released at time t and equilibrium, respectively. Symbols represent experimental data and lines show the trend. .................................................................................................................. 182

Figure 6.7. Experimental (symbols) and Weibull predicted (lines) benzoate release in DI water at 21°C as release fraction of $M_t/M_\infty$ from PHBV films using different incorporation methods of benzoate either directly incorporated into PHBV without LDH or with LDH or modified onto LDH nanolayers first and then
incorporated in PHBV. \( M_t \) and \( M_\infty \) are amounts of benzoate released at time \( t \) and equilibrium, respectively.

Figure 6.8. Linear regression of parameters \( n \) from power law model and \( \beta \) from Weibull model for benzoate release from PHBV films.

Figure 6.9. Release of benzoate in DI water at 21°C as release fraction of \( M_t/M_0 \) from PHBV films with various concentrations of modified LDH with 12.3% benzoate (LDH-SB1). \( M_0 \) and \( M_t \) are initial amount of benzoate in the film before release and amount of benzoate released at time \( t \), respectively.

Figure 6.10. Release of benzoate in DI water at 21°C as release fraction of \( M_t/M_0 \) from PHBV films with various concentrations of modified LDH with 20.9% benzoate (LDH-SB2). \( M_0 \) and \( M_t \) are initial amount of benzoate in the film before release and amount of benzoate released at time \( t \), respectively.

Figure 6.11. Release of benzoate in DI water at 21°C as release fraction of \( M_t/M_0 \) from PHBV films with various concentrations of modified LDH with 34.9% benzoate (LDH-SB3). \( M_0 \) and \( M_t \) are initial amount of benzoate in the film before release and amount of benzoate released at time \( t \), respectively.

Figure 6.12. Release of benzoate in DI water at 21°C as release fraction of \( M_t/M_\infty \) from PHBV films with various concentrations of modified LDH with 12.3% benzoate (LDH-SB1). \( M_t \) and \( M_\infty \) are amounts of benzoate released at time \( t \) and equilibrium, respectively.

Figure 6.13. Release of benzoate in DI water at 21°C as release fraction of \( M_t/M_\infty \) from PHBV films with various concentrations of modified LDH with 20.9% benzoate (LDH-SB2). \( M_t \) and \( M_\infty \) are amounts of benzoate released at time \( t \) and equilibrium, respectively.

Figure 6.14. Release of benzoate in DI water at 21°C as release fraction of \( M_t/M_\infty \) from PHBV films with various concentrations of modified LDH with 34.9% benzoate (LDH-SB3). \( M_t \) and \( M_\infty \) are amounts of benzoate released at time \( t \) and equilibrium, respectively.

Figure 6.15. Experimental (symbols) and Weibull predicted (lines) benzoate release in DI water at 21°C from PHBV films with different concentrations of modified LDH with 12.3% benzoate (LDH-SB1) (A-C), 20.9% benzoate (LDH-SB2) (D-F), or 34.9% benzoate (LDH-SB3) (G-I). \( M_t \) and \( M_\infty \) are amounts of benzoate released at time \( t \) and equilibrium, respectively.

Figure 6.16. Benzoate release in DI water from PHBV film with 2% of modified LDH with 20.9% benzoate (PHBV-LDH_SB2-2%) at different temperatures. \( M_t \) and \( M_\infty \) are amounts of benzoate released at time \( t \) and equilibrium, respectively.

Figure 6.17. Plot of \( \ln (M_t/M_\infty) \) vs \( \ln (t) \) for the early portion release of benzoate from PHBV film with 2% of modified LDH with 20.9% benzoate (PHBV-LDH_SB2-
2%) at different temperatures. Symbols represent the experimental data and lines show the trend. $M_t$ and $M_\infty$ are amounts of benzoate released at time $t$ and equilibrium, respectively.

Figure 6.18. Arrhenius plot of $\ln$ (effective diffusivity) versus $1/T$ for benzoate release in DI water at temperature range of 4 to 35 °C from PHBV film with 2% of modified LDH with 20.9% benzoate.

Figure 6.19. Experimental (symbols) and Weibull predicted (lines) benzoate release in DI water from PHBV film with 2% of modified LDH with 20.9% benzoate (PHBV-LDH_{SB2-2%}) at different temperatures.

Figure 6.20. Arrhenius plot of $\ln$ (kinetic constant from Weibull model) versus $1/T$ for benzoate release in DI water at temperature range of 4 to 35 °C from PHBV film with 2% of modified LDH with 20.9% benzoate.

Figure 6.21. Release of gallate in DI water at 21°C as release fraction of $M_t/M_\infty$ from PHBV films with 0.88% sodium gallate using different incorporation methods of gallate either directly incorporated into PHBV without LDH or with LDH or modified onto LDH nanolayers first and then incorporated in PHBV. $M_t$ and $M_\infty$ are amounts of gallate released at time $t$ and equilibrium, respectively.

Figure 6.22. Release of gallate in DI water at 21°C as release fraction of $M_t/M_\infty$ from PHBV films with 2.2% sodium gallate using different incorporation methods of gallate either directly incorporated into PHBV without LDH or with LDH or modified onto LDH nanolayers first and then incorporated in PHBV. $M_t$ and $M_\infty$ are amounts of gallate released at time $t$ and equilibrium, respectively.

Figure 6.23. Release of gallate in DI water at 21°C as release fraction of $M_t/M_\infty$ from PHBV films with various concentrations of modified LDH with 43.9% gallate (LDH-SG). $M_t$ and $M_\infty$ are amounts of gallate released at time $t$ and equilibrium, respectively.

Figure 6.24. Plot of $\ln (M_t/M_\infty)$ vs $\ln (t)$ for the early portion release of gallate in DI water at 21°C as release fraction of $M_t/M_\infty$ from PHBV films with various concentrations of modified LDH with 43.9% gallate (LDH-SG). $M_t$ and $M_\infty$ are amounts of gallate released at time $t$ and equilibrium, respectively. Symbols represent the experimental data and lines show the trend. The slope of the curve represented by $n$ (power law function shown in the inset) indicates the mechanism of release.
ACKNOWLEDGMENTS

I would like to thank my advisors, Dr. John Floros and Dr. Greg Ziegler, for their invaluable guidance and advice, constant support and patience during my research and graduate studies at Penn State University. I would also like to thank my committee members, Dr. Ramaswamy Anantheswaran, Dr. John Coupland, and Dr. Evangelos Manias, for their valuable suggestions.

I would like to thank the faculty members, staff, and students in Food Science Department. The friendly work environment they create has made the research much easier and enjoyable. My special thanks go to Dr. Lingyan Kong for his assistance with the instruments in his lab and his help during the last year of my graduate study when I was away from the campus. I would also like to thank the staff in Materials Research Institute at Penn State for material characterizations, Dr. Joshua Stapleton for FTIR, Ms. Julie Anderson for TGA-MS, and Ms. Missy Hazen for TEM. My deep appreciation goes to Dr. Nicole Brown, associate professor of Agricultural and Biological Engineering, for letting me use instruments, DMA and TGA, in her lab.

I would like to express my deepest appreciation to my husband, Darin, for his endless support and valuable suggestions, and my one-year-old daughter, Juliette, for being an inspiration. I would also like to thank my parents and parents-in-law for their help and encouragement.
Chapter 1
INTRODUCTION AND OBJECTIVES

1.1. Introduction

The utilization of bio-based polymers as packaging materials has attracted great attention in both scientific and industrial areas due to the non-degradable and non-renewable nature of synthetic plastic packaging. Polyhydroxyalkanoates (PHA) represent an interesting alternative to synthetic polymers due to many advantages. Not only are they biodegradable and biocompatible, but they can also be produced by bacterial fermentation of renewable resources like cane sugar (Bordes and others 2010). Controlled fermentation of carbon feedstock and nitrogen limitation in the presence of suitable bacteria yields up to 70% of dry cell weight (Ward and others 1977). Poly (3-hydroxybutyrate) (PHB), the most common PHA, was first discovered in 1926 by Lemoigne and is now produced on an industrial scale. Depending on the carbon substrates and the metabolism of the microorganism, different monomers, and thus (co)polymers, can be obtained. Besides the main polymer PHB, different copolymesters exist such as poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV), and poly(hydroxybutyrate-co-hydroxyoctanoate) (PHBO). PHB is a highly crystalline biopolyester (above 55%) with a glass transition temperature ($T_g$) just above 0 °C. PHB is relatively stiff and brittle. Young’s modulus reaches 3.5GPa, and the elongation break is less than 5% (Bordes and others 2010). The PHB melting point ($T_m = 170$ to $180$ °C) is rather high compared to other biodegradable polyesters. Its degradation temperature is close to the $T_m$ and thermal degradation occurs according to a one-step process – namely, a random chain scission reaction. Packaging materials made from PHA possess excellent film forming and coating properties. PHAs have properties close to those of polypropylene (PP) (Brandl and Puchner 1991). The properties of the film can be adjusted by changing the ratio of hydroxybutyrate (HB) and hydroxyvalerate (HV). A high content of polyhydroxybutyrate (PHB) gives a strong and stiff material whereas polyhydroxyvalerate (PHV) has improved flexibility and toughness (Bordes and others 2010). Properties of PHBV can be improved further by using plasticizers (Kotnis and
PHAs are more hydrophobic than polysaccharide-based materials resulting in better moisture barrier properties. PHAs are also biodegradable in soil and have excellent processability. However, the high cost of production, brittleness, and poor gas barrier properties limit the use of PHAs (Petersen and others 1999). Several processes for producing PHA from cheap carbon sources have been developed which have been reviewed by Choi and Lee (1999). In order to improve the properties of the polymers, many methods have been used, such as chemical modification, blending with rubber or barrier polymers, coatings, adding glass fiber as micro-scale fillers, and a new method to form nanocomposite materials using nanoparticles. Nanocomposites, as a novel class of materials, have recently been extensively studied. Nanocomposites consist of a polymer matrix (bio-nanocomposite if the matrix is biopolymer) reinforced with nanoparticles having at least one dimension in the nanometer range (1-100 nm). Significant improvements including mechanical, thermal, gas-barrier, flame-retardant properties and tunable biodegradability have been observed in the many synthetic polymer-based nanocomposites due to the high aspect ratios and the high surface areas of the nanoparticles (Kojima and others 1993; Usuki and others 1993; Krishnamoorti and others 1996; Alexandre and Dubois 2000; Leroux and Besse 2001; Ray and Okamoto 2003; Manias and others 2007). It is important to point out that all these improvements are obtained at very low filler concentrations (generally lower than 5%).

Up to now only layered inorganic solids like clay have attracted some attention by the packaging industry. This is not only due to their availability and low cost, but also to their significant enhancements and relatively simple processability. The nanoplates can either be cation- or anion-exchanging. Layered silicate, commonly investigated cation-exchanged clay, including montmorillonite (MMT), hectorite, and saponite, are the most common nano-fillers used in the bio-nanocomposites due to their high aspect ratio and commercial availability. Very recently, interest is being devoted to layered double hydroxides (LDHs), a promising new class of inorganic layered materials. Layered double hydroxides (LDH), or so-called hydrotalcite-like, are a family of layered solids with structurally positively charged outer layers and interlayers of balancing anions (Costantino and others 1997). The general chemical formula for LDH is written as
\[ \text{M}^{II_{1-x}} \text{M}^{III_x} (\text{OH})_2 \]^{x+} x/n \cdot y\text{H}_2\text{O}, \text{ where } \text{M}^{II} \text{ is a divalent metal ion, such as } \text{Mg}^{2+}, \text{Ca}^{2+}, \text{Zn}^{2+}, \text{etc.}, \text{ M}^{III} \text{ is a trivalent metal ion, such as } \text{Al}^{3+}, \text{Cr}^{3+}, \text{Fe}^{3+}, \text{Co}^{3+}, \text{etc.} \text{ and } \text{A}^{n-} \text{ is an anion, such as } \text{CO}_3^{2-}, \text{Cl}^-, \text{NO}_3^-, \text{etc.} \text{ (Evans and Xue 2006). Anions occupy the interlayer region of these layered crystalline materials. They can be synthesized easily and have the advantage of various sheet compositions with tunable interlayer species. In addition, the aspect ratio of LDH is similar to that of MMT.}

For nanocomposite synthesis, polymer chains must diffuse into the galleries between clay layers to produce structures ranging from intercalated to exfoliated. Intercalation occurs when a small amount of polymer penetrates into the galleries, resulting in finite expansion of the clay layers. This leads to a well-ordered multilayered structure with a repeat distance of a few nanometers, and is observed in systems with limited miscibility. Extensive polymer penetration leads to exfoliation or delamination of clay layers. An exfoliated nanocomposite consists of nanometer thick platelets distributed homogeneously throughout the polymer matrix. In contrast, when the polymer and silicate are immiscible, the layers do not separate and exist as agglomerates or tactoids. Intercalation and exfoliation are the desirable arrangement for improving the properties of nanocomposites. Due to the hydrophilic surface properties of pristine LDH, LDHs modified by organic molecules are normally required for miscibility with synthetic polymers. These modified LDH-based nanocomposites have shown improved mechanical properties, barrier properties, heat stability and flame retardancy (Nyambo and others 2008; Costa and others 2008; Bugatti and others 2010). Moreover, additional properties can be brought to the nanocomposites due to the organic molecules (Costantino and others 2009; Tammaro and others 2009; San Roman and others 2013).

Food spoilage due to undesirable microbial growth is a common factor which shortens the shelf life of food products and can cause food borne illness outbreaks. The addition of antimicrobial agents (active compounds that kill or prevent the growth of microorganisms) to foods or food surfaces during processing is an effective technique that is frequently employed to eliminate food borne pathogens. However, the antimicrobial activity may be rapidly lost due to inactivation of the antimicrobials by
components in foods or the antimicrobials are diluted below active concentrations due to migration into the bulk food matrix (Robertson 2006). Therefore, antimicrobial agents have been incorporated into packaging materials, called antimicrobial food packaging (AM food packaging), to prevent the growth of microorganisms on the food surface and thus lead to an extension in shelf life or improved microbial safety of the food (Collins-Thompson and Hwang 2000; Suppakul and others 2003). AM food packaging has received great interest from the food industry attributed to the increasing consumer demand for minimally processed, preservative-free, “fresh” foods, because a major advantage arising from the use of AM packaging is that only low levels of preservative come into contact with the food, compared to the direct addition of preservative to the food (Suppakul and others 2003; Robertson 2006). Various polymers have been studied as potential candidates for incorporation of an antimicrobial substance in food packaging applications (Bastarrachea and others 2011). Synthetic polymers derived from petroleum have thus far dominated in AM food packaging, such as low-density and high-density polyethylene, polypropylene, polystyrene, polyethylene terephthalate, ethylene-vinyl acetate, poly(vinyl chloride), and poly(butylene adipate-co-terephthalate) (Joerger 2007; Bastarrachea 2011). A number of edible films and coatings have also been studied for antimicrobial applications (Ozdemir and Floros 2001; Chacko 2008; Valencia-Chamorro and others 2011; Robertson 2013) including starch, cellulose derivates, chitosan, alginate, fruit-puree, whey protein isolate, soy protein isolate, corn zein, wheat gluten, egg albumen, gelatin, and sodium caseinate. Only a few biodegradable polymers have been used for the production of antimicrobial films (Liu and others 2007; Jin and Zhang 2008; Rhim and others 2009; Bugatti and others 2011), including polycaprolactone (PCL), and poly (lactic acid) (PLA).

The properties of food-packaging films are influenced by the incorporation of antimicrobial substances, such as mechanical, gas barrier, thermal, and morphological properties (Bastarrachea and others 2011). The mechanical properties of polymeric films for food packaging applications are important since the packaging materials are under various stresses that occur during the processing, handling, and storage of packaged foods (Marcos and others 2010). A significant change can be obtained in the tensile properties
of polymeric films after the incorporation of antimicrobials. Many studies have found that a decrease in film strength with an increase in concentration of the antimicrobial incorporated in the polymer (Limjaroen and others 2003; Pranoto and others 2005; Pires and others 2008; Tippayatum and others 2009; Ture and others 2009; and Bastarrachea and others 2010). A few cases have found that the tensile properties were not significantly affected by the addition of antimicrobial agents (Jin and others 2009; Sanchez-Valdez and others 2009), whereas Marcos and others (2010) found that the tensile properties of PVOH films were improved with the incorporation of the antimicrobial enterocin. Besides the possible negative effect on mechanical properties, the incorporation of low molecular weight antimicrobial agents into the polymer matrix has another disadvantage, the migration and the release of the AM cannot be easily predicted or controlled. Due to the polymeric structure and the interaction between the polymer and antimicrobials, the antimicrobials are either completely bound in the polymeric matrix or suddenly released into the aqueous system (Chacko 2008). To face these problems, a method for fixing active molecules (anti-inflammatory, antibiotic, and antimicrobial) into an inorganic compound able to hold them has been proposed to obtain a very slow and controlled release in selected conditions (Tammaro and others 2005, 2007, 2009; Sammartino and others 2004; Bugatti and others 2011). In particular, layered double hydroxide (LDH) has been used as an active molecule delivery vehicle.

Based on this previous work on nanocomposites and antimicrobial food packaging, I hypothesized that incorporation of LDH nanoparticles modified by organic antimicrobial agents into PHA matrix will provide greater mechanical, thermal, and barrier properties and meanwhile provide extended antimicrobial effects due to more controlled release of antimicrobial agents. The LDH nanoparticles in the bio-nanocomposites play dual functions from two parts of the LDH, the inorganic nano-scale part improves the mechanical, thermal, and gas-barrier properties, and the organic moiety brings a functional group capable of providing new properties, antimicrobial in this research. At the same time, the proper selection of antimicrobial agents to modify the nanoparticle can increase the compatibility between the nanoparticle and biopolymer.
1.2. Hypothesis and Objectives

*The overall goal* of the project was to develop the polyhydroxyalkanoate-based bio-nanocomposite films modified by antimicrobial agents with improved mechanical and gas barrier properties, along with a controlled release rate of antimicrobial agents for the inhibition of foodborne pathogens and fungi in food. *The hypothesis* was that incorporation of layered double hydroxide (LDH) nanoparticles modified by antimicrobial agents into bio-based polymer (polyhydroxyalkanoates) matrices can markedly improve the mechanical, thermal, and barrier properties and control the release rate of antimicrobial agents into food systems.

*The specific objectives* were: a) to develop LDH nanoparticles modified and modified by antimicrobial agents (sodium benzoate, sodium gallate and potassium sorbate); b) to develop effective PHA-based LDH composite films with enhanced mechanical and barrier properties; c) to analyze and model the diffusion of antimicrobial agents through the PHA/LDH nanocomposites.

1.3. References


Tammaro, L.; Tortora, M.; Vittoria, V.; Costantino, U.; Marmottini, F., Methods of preparation of novel composites of poly(epsilon-caprolactone) and a modified Mg/Al hydrotalcite. *Journal of Polymer Science Part a-Polymer Chemistry* 2005, 43, (11), 2281-2290.


2.1. Bio-based Polymer Packaging Materials

The utilization of bio-based polymers as packaging materials has attracted great attention in both scientific and industrial areas due to the non-degradable and non-renewable nature of synthetic plastic packaging. Bio-based polymers are produced from renewable resources and can be classified into three different categories depending on the synthesis (Stevens 2002; Averous 2004; Bordes and others 2009; Morris and Harding 2009):

(a) nature’s polymers, which are from biomass such as the agro-polymers from agro-resources, e.g., starch, cellulose, protein;
(b) polymers produced by micro-organisms, e.g. polyhydroxyalkanoates;
(c) synthetic biopolymers, which are chemically synthesized using monomers obtained from agro-resources, e.g. poly (lactic acid).

Figure 2.1 shows these three different categories with representative polymers. Of these, polyhydroxyalkanoates (PHA) represents an interesting alternative to synthetic polymers as packaging materials due to many advantages. Not only are they biodegradable and biocompatible, but they also possess excellent film forming and coating properties (Bordes and others 2010).

2.1.1 Polyhydroxyalkanoates (PHA)

2.1.1.1 Synthesis

Polyhydroxyalkanoates are naturally produced by micro-organisms from various carbon substrates as a carbon or energy reserve. They serve as energy and carbon storage materials in bacteria. PHAs accumulate when carbon is in excess but some other nutrient limits growth, while they are consumed when no external carbon source is available.
PHAs are now produced commercially from micro-organisms through fermentation. In bioreactors, micro-organisms are fed a carbon source substrate, such as glucose or sucrose for polyhydroxybutyrate, or propionic acid for polyhydroxyvalerate (Stevens 2002). Propionic acid can be produced by the fermentation of wood pulp or from petroleum. A wide variety of bacteria both Gram negative and Gram positive such as bioreactors, micro-organisms are fed a carbon source substrate, such as glucose or sucrose for polyhydroxybutyrate, or propionic acid for polyhydroxyvalerate (Stevens 2002). Propionic acid can be produced by the fermentation of wood pulp or from petroleum. A wide variety of bacteria both Gram negative and Gram positive such as *Pseudomonas, Bacillus, Ralstonia, Aeromonas, Rhodobacter* and certain Archaea, especially members of the Halobactericeae, like *Haloferaxsulfurifontis*, synthesizes PHAs (Philip and others 2007). Controlled fermentation of carbon feed-stock and nitrogen limitation in the presence of suitable bacteria yields PHAs from 30% to 90% of dry cell
weight (Ward and others 1977; Stevens 2002). To harvest the polymers the cell wall is ruptured and the polymers are collected and purified. Poly (3-hydroxybutyrate) (PHB), the most common PHA, was first discovered in 1926 by Lemoigne and is now produced on an industrial scale. Depending on the carbon substrates and the metabolism of the microorganism, different monomers, and thus (co)polymers, can be obtained. Besides the main polymer PHB, different copolymers exist such as poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV), or poly(hydroxybutyrate-co-hydroxyhexanoate) (PHBHx), poly(hydroxybutyrate-co-hydroxyoctanoate) (PHBO) and poly(hydroxybutyrate-co-hydroxyoctadecanoate) (PHBOd).

2.1.1.2. Properties

PHB is a highly crystalline (above 55%) biopolyester with a glass transition temperature ($T_g$) just above 0°C. PHB is relatively stiff and brittle. Young’s modulus reaches 3.5GPa, and the elongation at break is less than 5% (Bordes and others 2010). The PHB melting point ($T_m = 170$ to $180$ ºC) is rather high compared to other biodegradable polyesters. PHAs can be degraded under both aerobic and anaerobic conditions. They can also be degraded by thermal means or by enzymatic hydrolysis. In a biological system, PHAs can be degraded using microbial depolymerases as well as by nonenzymatic and enzymatic hydrolysis in animal tissues (Gogolewski and others 1993). The thermal degradation temperature is close to the $T_m$ and thermal degradation occurs according to a one-step process – namely, a random chain scission reaction. Under extrusion, increasing the shear level, the temperature, or the residence time leads to fast decrease in the PHB viscosity and in the molecular weight due to chain cleavage. Packaging materials made from PHA possess excellent film forming and coating properties. PHAs have properties close to that of polypropylene (PP) (Brandl and Puchner 1991). The properties of the film can be adjusted by changing the ratio of hydroxybutyrate (HB) and hydroxyvalerate (HV). A high content of polyhydroxybutyrate (PHB) gives a strong and stiff material whereas polyhydroxyvalerate (PHV) has improved flexibility and toughness (Bordes and others 2010). Properties of PHBV can be improved further by using plasticizers (Kotnis and others 1995). PHAs are more hydrophobic than polysaccharide-based materials resulting in better moisture barrier
properties. PHAs are also biodegradable in soil and have excellent processability. However, high cost of production, brittleness, and poor gas barrier properties limit the use of PHAs (Petersen and others 1999). Several processes for producing PHA from cheap carbon sources have been developed which have been reviewed by Choi and Lee (1999).

2.1.1.3. Production and Application

The copolymer PHBV, with HV contents reaching 20%, has been industrially produced by Monsanto under the Biopol® trade mark. The production was stopped at the end of 1999. Metabolix then bought the license from Monsanto in 2001. Telles™, a joint venture between Metabolix and Archer Daniels Midlands Company (ADM), has marketed a new bio-based biodegradable polymer using renewable carbon based feedstocks under the name Mirel™. The products include Metabolix DP 9002 for denitrification in aquariums, Mirel™ P1003, P1004, F1005, and F1006 for injection molding, and Mirel™P4001 for cast sheet extrusion (Metabolix, USA). Compostable films made from Mirel™ resins are also produced by Metabolix, named Mvera™, which offer solutions to divert organic food and packaging waste from landfills to composters (Metabolix, Inc.). There are other companies that are currently producing bacterial PHAs as well, e.g., Trade name Biomer® (Biomer, Germany) and logo registered worldwide in 1994 and the annual production of PHB is now around 500 to 1000 tons. Founded in 2003, Tianjin Green Bio-science Co., Ltd (China) produces PHA under the trade name of GreenBio™. In 2008, Royal DSM N.V. participated in a $20 million financing round in Tianjin Green Bio-science to build what they say will be the country’s largest plant for PHA. The plant was expected to have the annual production capacity of 10,000 tons. Bio-on (Italy) was founded in 2007 and produces polyhydroxyalkanoates (PHAs) with trade name MINERV-PHA™. It uses sugar beet and cane processing waste materials for the production of PHAs. In 2012, Bio-on chose to collaborate with Techint Engineering & Construction. Due to the collaboration, plants on an industrial scale will be built and able to produce 10 thousand tons/year of PHAs (Bio-on). PHB Industrial S.A. (Brazil) produces PHB and PHBV (HV = 12%) 45% crystalline, from sugar cane molasses as brand, Biocycle® (Biocycle, Brazil). It had the plant with a capacity of 50 tons/year in
2001. Goodfellow Corporation (Coraopolis, PA, USA) produces PHB and PHBV (HV = 8% or 12%) granules, rods, and films.

Initially, PHAs were used to make everyday articles such as shampoo bottles and packaging materials (Hocking and Marchessault 1994). The first consumer product made out of PHA was launched in April 1990 by WellaAG. They tested their Sanara range of biodegradable shampoos in bottles made of Biopol (ICI, UK) (Weiner 1997). Over the last decade, applications have increased in many areas, including industrial, medical, and agricultural areas. A detailed discussion of applications has been reviewed by Philip and others (2007). In the industrial area, PHA latex can be used to cover paper or cardboard to make water-resistant surfaces (Lauzier and others 1993). The PHB produced by Biomer, is used to make articles such as combs, pens, and bullets (Chen 2005). Industrial production of poly-3-hydroxybutyrate-co-3-hydroxyhexanoate P(3HB-3HHx) is used to make flushables, nonwovens, binders, flexible packaging, thermoformed articles, synthetic paper, and medical devices (Chen and others 2001). PHAs are also used for medical device development, tissue repair, artificial organ construction, drug delivery, and nutritional/therapeutic uses (Wu and others 2009). Simioni and others (2008) have prepared PHBV/PCL microspheres as biodegradable drug delivery for photodynamic therapy. PHB and PHBHHx were used to prepare nanoparticles as drug release vectors (Xiong and others 2010). PHAs have also been used as mulch films for agricultural purposes (Hocking and Marchessault 1994). Procter & Gamble have produced Nodax™, a copolymer containing mainly 3(HB) and small quantities of medium-chain-length (MCL) monomers, and used it as a coating for urea fertilizers or for herbicides and insecticides. Another application of PHA is to integrate insecticides into P(3HB-3HV) pellets that are then sown along with the farmer’s crops. The insecticides would be released at a rate related to the level of pest activity since the bacteria breaking down the polymer would be affected by the same environmental conditions as that of the soil pests (Holmes 1985).

Although the applications of PHAs have increased, the high cost of production, brittleness, and poor gas barrier properties limit their use (Petersen and others 1999). In
order to improve the properties of these polymers, many methods have been used, such as chemical modification, blending with rubber or barrier polymers, coatings, adding glass fiber as micro-scale fillers, and a new method to form nanocomposite materials using nanoparticles. Nanocomposites, as a novel class of materials, have recently been extensively studied. Nanocomposites consist of a polymer matrix (bio-nanocomposite if the matrix is biopolymer) reinforced with nanoparticles having at least one dimension in the nanometer range (1-100 nm). Significant improvements including mechanical, thermal, gas-barrier, flame-retardant properties and tunable biodegradability have been observed in many synthetic polymer-based nanocomposites due to the high aspect ratios and the high surface area of the nanoparticles (Kojima and others 1993; Usuki and others 1993; Krishnamoorti and others 1996; Alexandre and others 2000; Leroux and Besse 2001; Ray and Okamoto 2003a; Manias and others 2007). PHA based nanocomposites have been synthesized by several research groups and among them layered inorganic solids like clay nanocomposites are well known since they are economical with high aspect ratio and exceptional barrier properties (Pandey and others 2005). It is hoped that nanocomposites will enable PHAs to compete more effectively with synthetic plastics. These nanocomposites will be summarized in the following section.

### 2.2. Nanotechnology and Bio-nanocomposites

Nanotechnology involves the study and use of materials at a scale of 1 to 100 nanometers. The use of nanofillers is leading to the development of polymer nanocomposites and represents a radical alternative to conventional polymer composites. The first successful polymer layered silicate nanocomposite technology was developed for automotive applications by Toyota Central Research Laboratories in 1986, and Toyota was the first company to commercialize the nanocomposites (nylon 6-clay nanocomposites) (Kawasumi 2004). In the following years, nanocomposites have attracted increasing interest as a means for improving the properties of synthetic polymers. The new generation of composites exhibits significant improvements in modulus, dimensional stability and solvent or gas resistance with respect to the pristine polymer. Nanocomposites also offer extra benefits like low density, transparency, good flow, better surface properties and recyclability. In particular, they show great promise in
providing excellent barrier properties, due to the presence of nanoparticles that delay molecular motion by making the diffusive path more tortuous (Serrentino, 2006). It is important to point out that all these improvements are obtained at very low filler contents (generally lower than 5%). Research on nanocomposites using bio-based matrices are increasing due to extraordinary success of the nanocomposite concept in the area of synthetic polymers.

2.2.1. Structure and Properties of Layered Inorganic Nanoparticles

Up to now only the layered inorganic solids like clay have attracted some attention by the packaging industry. This is not only due to their availability and low cost but also due to their significant enhancements and relatively simple processability. The nanoplates can either be cation exchanging or anion exchanging. Layered silicate, for instance, is a commonly investigated cation-exchanged clay. Layered silicates, including montmorillonite (MMT), hectorite, and saponite, are the most common nanofillers used in bio-nanocomposites due to their high aspect ratio and commercial availability. Very recently, interest is being devoted to layered double hydroxides (LDHs), a promising new class of inorganic layered materials. LDHs are composed of positively charged brucite-like layers and interlamellar exchangeable anions. They can be synthesized easily and have the advantage of various sheet compositions with tunable interlayer species. In addition, the aspect ratio of LDH is similar to that of MMT.

The general chemical formula for LDHs is written as [M\text{II}_{1-x}M\text{III}_x(OH)_2]^{x+}(A^{n-})_{x/n},yH_2O, where M\text{II} is a divalent metal ion, such as Mg\textsuperscript{2+}, Ca\textsuperscript{2+}, Zn\textsuperscript{2+}, etc., M\text{III} is a trivalent metal ion, such as Al\textsuperscript{3+}, Cr\textsuperscript{3+}, Fe\textsuperscript{3+}, Co\textsuperscript{3+}, etc. and A\textsuperscript{n-} is an anion, such as Cl\textsuperscript{-}, CO\textsubscript{3}\textsuperscript{2-}, NO\textsubscript{3}\textsuperscript{-}, etc. (Evans and Xue 2006). Anions occupy the interlayer region of these layered crystalline materials. Although a wide range of values for x is claimed to provide LDH structure, the pure phase of LDH clays is usually obtained for a limited range as 0.2 < x < 0.33. The structure of LDHs can best be explained by drawing an analogy with the structural features of the metal hydroxide layers in mineral brucite or simply the Mg(OH)\textsubscript{2} crystal structure. Brucite consists of a hexagonal close packing of hydroxyl ions with alternate octahedral sites occupied by Mg\textsuperscript{2+} ions. The
metal hydroxide sheets in brucite crystal are neutral in charge and stack one upon another by Van der Waals interaction. The interlayer distance or the basal spacing in brucite has a value of about 0.48 nm. In LDH, some of the divalent cations of these brucite-like sheets are isomorphously substituted by a trivalent cation and the mixed metal hydroxide layers, \([M^{II}_{1-x}M^{III}_x(OH)_2]^{x+}\), thus forming a net positive charge. This excess charge on the metal hydroxide layers is neutralized by the anions accumulated in the interlayer region. The interlayer anions can be exchanged by other inorganic, organic, or metallorganic anions and even by biomolecules containing ionizable acidic groups to obtain novel materials (Choy and others 2000; Hwang and others 2001; Desigaux and others 2006). The interlayer region in LDHs also contains some water molecules for the stabilization of the crystal structure. The presence of anions and water molecules leads to an enlargement of the basal spacing from 0.48 nm in brucite to about 0.77 nm in Mg-Al-LDH. A schematic representation comparing the crystal structure of brucite and LDH structures is shown in Figure 2.2.

Figure 2.2. Schematic representations comparing the crystal structure of brucite (A) and LDH (B)

LDHs are well known for their catalytic activities in organic synthesis (Tichit and Coq 2003). LDHs and their modified forms also have been used in the fields of photophysics and photochemistry (Ogawa and Kuroda 1995; Latterini and others 2007;
Tian and others 2007), wastewater treatment (Lv and others 2006; Mohan and Pittman 2007), gene therapy (Choy and others 1999; Del Hoyo 2007), and drug storage and release. LDHs as a drug delivery vehicle have received considerable attention in recent years because of their biocompatibility, anion-exchange property, nontoxicity, etc. A variety of drugs, such as diclofenac, 4-biphenylacetic acid, ibuprofen, tolefenamic acid, low molecular weight heparin, fenbufen and 5-fluorocytosine, have been incorporated into LDHs (Khan and others 2001; Li and others 2004; Costantino and others 2008; Gu and others 2008; Liu and others 2008; Rojas and others 2012). After administration of the intercalation compound, the drug may be released via a deintercalation process, occurring because of ion-exchange or displacement reactions. The rate of drug diffusion out of the LDH intercalation compound depends on the strength of the host-guest interaction, the rigidity of the layers and the diffusion path length.

LDHs, as a kind of layered inorganic solid, are also gaining importance as nanofiller for the synthesis of polymer nanocomposites. The pristine layered materials show hydrophilic surface properties. In this pristine state, layered materials may be only miscible with relatively hydrophilic polymers. To render layered materials miscible with hydrophobic polymers, one must convert the normally hydrophilic surface to an organophilic one. Generally, the possibility to replace the counter-balancing anions, located in the interlamellar region, with other anions by ion-exchange, makes LDHs a unique class of layered solids to be used as polymer fillers. The organic anions lower the surface energy and result in a larger interlayer spacing. The interlayer anions can be exchanged by organic or metallorganic anions and even by biomolecules containing ionizable acidic groups to obtain novel materials of interest in the fields of photophysics and photochemistry, electrochemistry, catalysis, drug storage and release, pharmaceutical care, and environmental protection. Additionally, the organic anions may contain various functional groups that react with the polymer to improve adhesion between the inorganic phase and the matrix. These modified LDH-based nanocomposites have showed improved mechanical properties, barrier properties, heat stability and flame retardancy (Nyambo and others 2008; Costa and others 2008; Bugatti and others 2009). Moreover, the organic molecules can bring additional properties to the nanocomposites, such as
controlled drug delivery, and antimicrobial properties (Costantino and others 2009; Tammaro and others 2009; San Roman and others 2013).

2.2.2. Nanocomposite Synthesis and Characterization

For nanocomposite synthesis, polymer chains must diffuse into the galleries between clay layers to produce structures ranging from intercalated to exfoliated (Figure 2.3). Intercalation occurs when a small amount of polymer penetrates into the galleries, resulting in finite expansion of the clay layers. This leads to a well-ordered multilayered structure with a repeat distance of a few nanometers, and is observed in systems with limited miscibility. Extensive polymer penetration leads to exfoliation or delamination of clay layers. An exfoliated nanocomposite consists of nanometer thick platelets distributed homogeneously throughout the polymer matrix. In contrast, when the polymer and silicate are immiscible, the layers do not separate and exist as agglomerates or tactoids. Exfoliation is the desirable arrangement for improving the properties of nanocomposites.

Whether a mixture of polymer and organic modified nano-particles produces an exfoliated or intercalated nanocomposite critically depends on the characteristics of the polymer matrix and the organic modifiers. These characteristics include the nature of the polymer as well as the type, packing density, and the size of the organic modifiers on the inorganic surface (Alexandre and Dubois, 2000; Pantoustier, 2001). The organic component of the organo-clay or modified hydrotalcite, in fact, increases significantly the compatibility between the polymer and the filler. The surface of the silicate or hydrotalcite layers, therefore, becomes accessible to the polymer chains and intercalation or full delamination (exfoliation) of the filler particles can occur during the mixing of the filler with the polymer.

The complete dispersion of clay platelets in a polymer optimizes the number of available reinforcing elements for carrying an applied load and deflecting cracks. The coupling between the tremendous surface area of the clay and the polymer matrix
facilitates stress transfer to the reinforcement phase, allowing for such mechanical improvements. In addition, the impermeable clay layers mandate a tortuous pathway for a permeant to transverse the nanocomposites (Figure 2.4). The enhanced barrier characteristics, chemical resistance, reduced solvent uptake and flame retardancy of polymer-clay nanocomposites all benefit from the hindered diffusion pathway through the nanocomposites (Neilsen 1967; Bharadwaj 2001; Sorrentino and others 2007).

Nanocomposites can be obtained by several methods which include in-situ polymerization, solution exfoliation, and melt intercalation. In the in-situ polymerization method, monomers are intercalated into layered clays and subsequently polymerized via heat, radiation, or catalyst. In solution exfoliation, layered clays are exfoliated into single platelets using a solvent and the polymer is adsorbed onto the platelets by mixing in the
clay suspension. In melt intercalation, layered clays are mixed with the polymer matrix in molten state (Zeng and others 2005). In the case of catalogs (a) and (b) of bio-based polymers, the choice of the suitable method for the preparation of nanocomposites is limited by the processing possibilities of the natural materials. Therefore, the *in-situ* polymerization is not applicable for bio-based polymers. Besides the melt and solvent intercalation routes for bio-based polymers, a new method has also been used to synthesize nanocomposites, which involves a solid-state mixing at room temperature using ball milling with the considerable advantage of not requiring the use of high temperature or solvent treatments (Mangiacapra and others 2005; Sorrentino and others 2005).

![Diagram](image)

**Figure 2.4.** Schematic illustration of the tortuosity for a diffusing penetrant introduced on exfoliated solid layered in a polymer matrix. A. Filled polymer and B. Unfilled polymer.

Generally, the structure of nanocomposites can be characterized by two complementary analytical techniques, namely, X-ray diffraction (XRD) and transmission electron microscopy (TEM). Due to its ease of use and availability, simple Bragg-reflection powder XRD is most commonly used to probe the structure of nanocomposites, especially for polymer/layered-inorganic filler hybrids where the $d_{001}$ basal reflection is indicative of filler-filler separation. Intercalation of the polymer chains increases the
interlayer spacing and according to Bragg’s law, it should cause a shift of the diffraction peak towards a lower angle (McGlashan and Halley 2003). However, the XRD can only detect the distance of periodically stacked layers; disordered (bunched together but not parallel stacked) or exfoliated layers cannot be detected, and large $d$-spacings (higher than 50 nm) are sometimes not detectable by powder XRD. Therefore, XRD can be highly misleading when employed as a single tool for quantifying nanocomposite structure or even filler dispersion (Manias and others 2007). TEM is also widely employed, in its simplest bright-field mode, as a tool for direct visualization of the nanocomposite structure. Although TEM does not suffer from the same limitations as XRD, since it directly visualizes the nano-scale fillers without the need for parallel stacking, it has other disadvantages. The TEM image is taken in a very small area of the nanocomposites; therefore, it is difficult to obtain representative and quantitative images to describe the nanocomposite morphology. Despite the limitations, informative TEMs should, at lease, complement XRD or other morphology studies, even if only to capture the hierarchical structures of the hybrid qualitatively at various length scales (Manias and others 2007). TEM is probably crucial when accompanying featureless XRD structures such as silent (no basal reflections) polymer/layered-nanofiller nanocomposites, polymer-nanotube hybrids, and polymer-nanoparticulate composite. Besides XRD and TEM, there are still some other techniques used to gain greater insight into the nanocomposite structure, such as small-angle X-ray scattering (SAXS) (Manias and others 2007), differential scanning calorimetry (DSC) (Giannelis 1996), nuclear magnetic resonance (NMR) (VanderHart and others 2001), and Fourier transform infrared spectroscopy (FTIR) (Loo and Gleason 2003).

2.2.3. Bio-nanocomposites for Food Packaging Applications

2.2.3.1. Polysaccharide Based Bio-nanocomposites

2.2.3.1.1. Starch-based Bio-nanocomposites

Starch has attracted a lot of attention in the production of films for food packaging applications due to its complete biodegradability, wide availability and low cost. As packaging material, starch needs to be plasticized or chemically modified to form films
with appropriate mechanical properties. However, starch with plasticizers, so-called thermoplastic starch (TPS), often cannot meet all requirements for food packaging, therefore, starch based nanocomposites have been widely studied to find ways to improve the properties. De Carvalho and others (2001) first prepared thermoplastic cornstarch with kaolin as a filler reinforcement in order to improve its mechanical properties. After that, many different starch/nanoclay systems, such as plasticized potato starch with unmodified montmorillonite (Cloisite Na+) and organically modified MMT by different ammonium cations (Cloisite 30B, 10A, and 6A) (Park and others 2002; Park and others 2003; Avella and others 2005; Chen and others 2005; Badgi and others 2006), glycerol plasticized Cará root starch with Ca++-hectorite (Wilhelm and others 2003), glycerol plasticized corn starch with MMT (Pandey and others 2005), acetylated starch with MMT (Qiao and others 2005), urea and ethanolamine-plasticized thermoplastic cornstarch with ethanolamine-activated montmorillonite (Huang and Yu 2006), wheat starch with MMT (Cloisite Na+ and 30B) (Chiou and others 2006), high-content corn starch with MMT and fluromica (Dean and others 2007), have been studied. More studies are reviewed by Zhao et al. (2008). The improvement in mechanical properties and barrier properties can be achieved depending on type and content of the nanoclay used, type and content of plasticizer, interaction between starch, clay surface modifications and plasticizers and process conditions. Tang et al. (2008a; 2008b) investigated the effects of chemical compatibility of starch, plasticizer and nanoclay and melt extrusion conditions on the structure and properties of the composite films. Nanocomposite film with 5% glycerol exhibited the lowest water vapor permeability and highest tensile strength. It was also found that barrier and mechanical properties of nanocomposite films did not vary significantly with different starch sources (corn, wheat and potato starch), starch-unmodified MMT hybrids have higher tensile strength and better water vapor barrier properties as compared to pristine starch, as well as starch-organically modified MMT hybrids. The starch-layered double hydroxide nanocomposites were prepared by Chung and Lai (2010), and they reported that acid-modified corn starch with LDH displayed an increase in modulus by as much as 37%. The LDH modified by carboxymethyl-cellulose sodium (LDH-CMC) has also been incorporated into glycerol plasticized-starch (GPS) by Wu and others (2011), and improved mechanical properties and water vapor barrier
properties were reported due to good interaction between LDH-CMC and GPS matrix. Besides nanoclays, nanotubes, such as carbon and halloysite nanotubes, have also been used to enhance the properties of thermoplastic starch (Fama and others 2011; Liu and others 2011; Fama and others 2012; Schmitt and others 2012; Swain and others 2013). Fama et al. (2011) reported that significant improvements in all uniaxial tensile and biaxial impact properties were obtained for low contents of multi-wall carbon nanotubes (0.027 wt %). The oxygen permeability of TPS/functionalized multi-walled carbon nanotubes was reduced by half as compared to virgin TPS reported by Swain et al. (2013).

Meanwhile, starch blends with other polymers reinforced by nanoclays have also been reported. McGlashan and Halley (2003) prepared starch/polyester/organically modified clay nanocomposites using melt extrusion. The results exhibited an increase in tensile strength of 40%, Young’s modulus of 275%, and strain at break of 40% on addition of 5% clay. Starch/polycaprolactone (PCL) / MMT nanocomposites were made by reactive extrusion (Kalambur and Rizvi 2004; Kalambur and Rizvi 2005). They found that addition of modified nanoclay at 3 wt % level increased elongation almost fourfold over that of pristine starch–PCL blends. Starch/poly vinyl alcohol (PVOH)/MMT (Ali and others 2011) nanocomposite films were produced with a range of 11.6 and 22.4 MPa of tensile strength and 28.9% and 211.4% of elongation at break. Tang and Alavi (2012) prepared starch/PVOH/laponite nanocomposite films with tensile strength ranged from 6.5 to 13.3 MPa and elongation at break ranged from 144% to 312%.

2.2.3.1.2. Cellulose-based Bio-nanocomposites

Cellulose alone forms films with poor water vapor barriers because of the inherent hydrophilic nature of polysaccharides, therefore, cellulose derivatives, such as cellulose acetate (CA), cellulose acetate propionate (CAP), and cellulose acetate butyrate (CAB), attract interests for edible coatings or films for packaging. Park et al. (2004a) first prepared plasticized CA with organically modified MMT (Cloisite 30B) hybrid nanocomposites. Nanocomposites with 20 wt % of eco-friendly triethyl citrate plasticizer exhibited the best intercalation and exfoliation of clays as well as the best physical and mechanical properties. Water vapor permeability (WVP) reduced by a factor of two. In
their later work (Park and others 2004b) they investigated the effect of a compatibilizer maleic anhydride grafted with cellulose acetate butyrate (CAB-g-MA) on the nanostructure of the nanocomposites. A better exfoliated structure was formed by the nanocomposites with 5 wt % compatibilizer compared to those without the compatibilizer. Hydroxypropyl methylcellulose (HPMC) nanocomposites with chitosan (CS) nanoparticles were synthesized (De Moura and others 2008; De Moura and others 2009). Scanning electron microscope (SEM) analysis revealed that chitosan nanoparticles tended to fill porous spaces in the HPMC matrix. Tensile strength was significantly increased and water vapor and oxygen permeability were significantly reduced with addition of chitosan poly(methacrylic acid) (CS-PMAA) nanoparticles. Starch nanocrystal-reinforced pullulan films were reported by Kristo and Billiaderis (2007). The crystallinity and glass transition temperature of the composite biopolymer films were increased with increasing starch nanocrystal content. Water uptake decreased with increasing of starch nanocrystal content whereas water vapor permeability remained constant up to 20% (w/w) and, then decreased significantly with further addition of nanocrystals. Moreover, the addition of nanocrystals caused strong enhancement of the Young’s modulus and the tensile strength, but led to a drastic decrease of the strain at break in samples conditioned at different environments (from 43% to 75% RH).

Cellulose in the form of nanofibers, has been incorporated into other polymer matrices to improve properties. The application of cellulose nanofibers as reinforcing agents has been reviewed (Eichhorn and others 2010; Siro and Plackett 2010; Klemm and others 2011). Trovatti et al. (2012) used nanofibrillated cellulose to reinforce the properties of films made from pullulan. It is reported that the pullulan-based nanocomposite films were homogeneous, translucent, and showed considerable improvements in thermal stability (increments of up to 20 °C in the degradation temperature) and mechanical properties (increments of up to 5500% and 8000% in the Young’s modulus and tensile strength, respectively, for films plasticized with glycerol) when compared to the unfilled pullulan films.
2.2.3.1.3. Other Polysaccharide-based Bio-nanocomposites

Besides starch and cellulose which have been studied in the field of nanocomposites, other polysaccharide-based nanocomposites have been prepared and improved properties have been reported. Agar/MMT nanocomposites (Rhim 2011; Rhim and others 2011) showed enhanced mechanical properties, WVP, and the nanocomposites with organically modified MMT (Cloisite 30B) showed a bacteriostatic function against *Listeria*. Mechanical and thermal properties of alginate are improved with addition of chitin whiskers (Watthanaphanit and others 2008). Montmorillonite intercalated with vitamin B<sub>1</sub> and vitamin B<sub>6</sub> was incorporated into alginate to form a drug delivery system (Kevadiya and others 2010). The controlled release of vitamin B<sub>1</sub>/B<sub>6</sub> (VB<sub>1</sub>/VB<sub>6</sub>) from VB<sub>1</sub>/VB<sub>6</sub>-MMT-alginate nanocomposite beads was observed to be controlled as compared to their release from VB<sub>1</sub>/VB<sub>6</sub>-MMT hybrid and VB<sub>1</sub>/VB<sub>6</sub>-alginate beads. Mangiacapra et al. (2006) prepared pectin/MMT nanocomposites using high energy ball milling and found increased elastic modulus, delayed oxidation, and slower diffusion of water vapor and oxygen compared to the pure pectin. Rhim et al. (2006) prepared four different types of chitosan-based nanocomposite films using a solvent-casting method with four types of nanoparticles, which are, unmodified montmorillonite (Na-MMT), organically modified montmorillonite (Cloisite 30B), nano-silver, and Ag-zeolite nanoparticles (Ag-Ion). A certain degree of intercalation was formed in the nanocomposite films, with the highest intercalation in the Na-MMT-incorporated films followed by films with Cloisite 30B and Ag-Ion. Mechanical and barrier properties of chitosan films were affected through intercalation of nanoparticles, that is, tensile strength increased by 7–16%, whereas WVP decreased by 25–30% depending on the nanoparticle material tested. In addition, chitosan-based nanocomposite films, especially silver-containing ones, showed a promising range of antimicrobial activity.

2.2.3.2. Protein-based Bio-nanocomposites

Various proteins have been utilized for industrial applications due to their film-forming ability for a long time (Cuq and others 1998). Compared with nonionic polysaccharide films, protein films have better oxygen barrier properties and lower water
vapor permeability due to their more polar nature and linear (nonring) structure, as well as lower free volume (Miller and Krochta 1997). However, low modulus, high water adsorption, and high gas permeability still limit their applications in food packaging. Nanocomposite technology, mainly using nanoclays, has been applied to various proteins to improve their properties, such as soy protein (Dean and Yu 2005; Rhim and others 2005; Chen and Zhang 2006; Kumar and others 2010a; 2010b), corn zein (Luecha and others 2010; Zhang and Wang 2012; Ozcalik and Tihminlioglu 2013), wheat gluten (Olabarrieta et al. 2006; Tunc and others 2007; Mauricio-Iglesias and others 2010), whey protein (Hedenqvist and others 2006; Sothornvit and others 2009) and gelatin (Zheng and others 2002).

### 2.2.3.2.1. Soy protein-based Bio-nanocomposites

Soy protein isolate (SPI) has attracted a lot of attention for its thermoplastic properties and its potential as a biodegradable plastic. Similar to starch, soy protein is also blended with plasticizers to make it more flexible. However, the use of plasticizers further decreases barrier properties. Therefore, SPI/MMT nanocomposites have been studied by several research groups (Dean and Yu 2005; Rhim and others 2005; Chen and Zhang 2006). Chen and Zhang (2006) concluded that the heterogeneous distribution of the surface positive charges provided the positive-charge-rich domains for the soy globulins bearing net negative charges to anchor into the negatively charged MMT galleries (unmodified MMT, Na⁺-MMT). Therefore, electrostatic attraction and hydrogen bonding interactions on the interfaces of the soy protein and MMT led to the good dispersion of the phyllosilicate layers in the protein matrix. The highly exfoliated MMT layers with a dimension of 1–2 nm in thickness were randomly dispersed in the protein matrix containing MMT lower than 12 wt %, whereas the intercalated structure was predominant when the MMT content was higher than 12 wt %. Consequently, the fine dispersion of the MMT layers and the strong interactions between SPI and MMT created a significant improvement in the mechanical strength and thermo-stability of the SPI/MMT plastics (Chen and Zhang 2006). Kumar and others (2010b) studied the effect of type and content of modified montmorillonite on the structure and properties of bio-nanocomposite films based on soy protein isolate and montmorillonite. Extrusion of SPI
and modified MMTs resulted in bio-nanocomposites with exfoliated structures at lower MMT content (5%). At higher MMT content (15%), the structure of bio-nanocomposites ranged from intercalated for Cloisite 20A to disordered intercalated for Cloisite 30B. At an MMT content of 5%, bio-nanocomposite films based on modified MMTs (Cloisite 20A and Cloisite 30B) had higher tensile strength and percentage elongation at break, higher glass transition temperature and storage modulus, and lower water vapor permeability as compared to those based on natural MMT (Cloisite Na⁺) (Kumar and others 2010a; 2010b). Another layered silicate, rectorite, was used to reinforce SPI by Yu et al. (2007). Exfoliated structure was facilely formed. Tensile strength of the nanocomposite sheet reached its maximum (12.92MPa) at a rectorite content of 12 wt %, which is almost twice that of pure SPI plastics. The percentage elongation at break of nanocomposite sheets decreased sharply with increasing rectorite content.

2.2.3.2.2. Corn zein-based Bio-nanocomposites

Zein, a major protein from corn, is obtained commercially from corn gluten meal. Zein has been proposed as a potential biodegradable packaging material among other cereal proteins. Luecha et al. (2010) made zein/MMT nanocomposites using two fabrication techniques including solvent casting and blown extrusion. Partially exfoliated nanocomposites were formed using both methods. The thermal resistance of the zein nanocomposite films fabricated from both methods improved as the MMT content increased. However, the mechanical and barrier properties showed non-linear relationships with the MMT loadings. The impact of MMT on properties of zein films strongly depended on the preparation techniques. For solvent casting nanocomposites, the critical MMT content which could result in tensile strength improvement was at 5 wt %, while it was at 3 wt % for water vapor permeability. Zhang and Wang (2012) prepared zein nanocomposites with magnetic iron oxide (Fe₃O₄) nanofiller (Fe-Zein) and highly ordered zein nanocomposites (Fe-Zein-Mag). They reported that the tensile strength, elongation, and Young’s modulus of Fe-Zein were increased by 218, 48, and 264%, respectively, while the water vapor and oxygen permeability decreased by 68 and 29%, compared to pure zein film. Furthermore, the tensile strength and elongation of Fe-Zein-Mag were increased by 10 and 48%, respectively, and a 30% decrease in Young’s
Modulus was observed, in comparison to Fe-Zein, which indicates the Fe-Zein-Mag film was more elastic. The water vapor and oxygen permeability of Fe-Zein-Mag were also decreased by an additional 48 and 17%, respectively (Zhang and Wang 2012). Corn zein nanocomposite (CZNC) coatings as an alternative to a synthetic polymer barrier layer on polypropylene (PP) films was examined by Ozcalik and Tihminlioglu (2013). Incorporation of organomodified montmorillonite (OMMT) by solution intercalation into zein matrix reduced the oxygen permeability nearly four times, while water vapor permeability reduced by 30% with 5 wt % OMMT content in 5.9 μm corn zein coating. They concluded that the tortuous permeation path formed by the fine delamination of nanoclays was found to be responsible for the barrier improvements in zein layers.

2.2.3.2.3. Wheat gluten-based Bio-nanocomposites

Currently wheat gluten was only reinforced with MMT nanoparticles. Olabarrieta et al. (2006) cast wheat gluten/MMT nanocomposite films from pH 4 or pH 11 ethanol/water solutions. Partial exfoliated structures were formed. It is shown that the film prepared from a solution at pH 11 containing unmodified MMT made the strongest, the stiffest, and the most brittle film along with showing the greatest decrease in water vapor permeability. A partially exfoliated structure of wheat/MMT nanocomposites was also obtained by Tunc et al. (2007). Significant changes in the permeability of films towards water vapor and aroma compounds were observed for MMT contents higher than 5 wt %, while O₂ and CO₂ permeability remained unchanged. A slight improvement in tensile properties was obtained for filler contents higher than 2.5 wt %. Mauricio-Iglesias et al. (2010) studied the migration of a model molecule (Uvitex OB), MMT migration, protein migration, and overall migration of wheat gluten nanocomposite films as food-contact materials. The results showed that overall migration and protein migration were high; on the contrary, MMT and vitex OB migration was low or not detectable (Mauricio-Iglesias and others 2010).

2.2.3.2.4. Animal Protein-based Bio-nanocomposites

Limited publications about animal protein-based nanocomposites are found. Only MMT has been incorporated into whey protein (Hedenqvist and others 2006; Sothornvit
and others 2009) and gelatin (Zheng and others 2002). Unmodified MMT and modified MMT (Cloisite 20A) significantly influenced the tensile and the water vapor barrier properties of whey protein-based nanocomposites, but no significant effect was found by using modified MMT (Cloisite 30B) (Sothornvit and others 2009). Zheng et al. (2002) prepared gelatin/MMT nanocomposites for the first time and found the tensile strength and Young’s modulus was increased notably depending on the content of MMT and the pH of gelatin matrix.

2.2.3.3. Lipid-based Bio-nanocomposites

Triacylglycerols, commonly called triglycerides, are the most abundant class in the family of compounds known as lipids. Polymerization of triglycerides has received attention in the context of producing new bioplastic materials (Stevens 2002). The triglycerides are first converted to a more chemically reactive form capable of being polymerized – epoxidation is one of these methods. The initial liquid resin is a low-molecular-weight polymer that is then combined with catalysts and accelerators to facilitate the cross-linking reaction. Uyama et al. (2003; 2004) prepared epoxidized soybean oil (ESO) based nanocomposites with organophilic montmorillonite. MMT layers were dispersed with a thickness of 8–20 nm in the polymer matrix, suggesting good distribution of the inorganic phase at the nanometer level. The mechanical properties were improved by the incorporation of clay in the oil-based polymer matrix and the barrier property of the hybrid towards water vapor was superior to that of the oil polymer (Uyama and others 2004). Tsujimoto et al. (2010) developed a new class of biodegradable nanocomposites from renewable oils. The acid-catalyzed curing of epoxidized triglyceride oil and oxirane-containing silane coupling agent involving the formation of covalent bonding between the organic and inorganic units produced transparent nanocomposites. The homogeneous structure of the organic polymer and silica network in nanometer scale was formed. The hardness and Young’s modulus of the nanocomposite coatings were increased, as compared with those only from the epoxidized natural oils. The dynamic viscoelasticity analysis clearly indicates the reinforcement effect by the inorganic network. The coating and mechanical properties
were controlled by the monomer structures and the feed ratio (Tsujimoto and others 2010).

2.2.3.4. PLA-based Bio-nanocomposites

Polylactic acid (PLA) has received attention as a sustainable, biocompatible, and biodegradable material. Lactic acid, the monomer of PLA, can easily be produced by fermentation of carbohydrate feedstock. However, high cost and low performance still hampers the large-scale use of PLA as packaging material. PLA nanocomposites have been prepared to improve the polymer properties and expand the applications of PLA.

PLA based composites were first prepared by Ogata et al. (1997) with nanoclay, organically modified MMT (OMMT), using a solvent casting method. A microcomposite structure was formed since MMT existed in the form of tactoids. Young's modulus of the composites increased with the addition of a small amount of the clay (Ogata and others 1997). Bandyopadhyay et al. (1999) then successfully prepared PLA – OMMT nanocomposites by the melt extrusion technique with much improved thermal and mechanical properties. Ray et al. (2002; 2003b; 2003c) also used the melt extrusion technique for the preparation of PLA/OMMT nanocomposites. XRD patterns and TEM observations showed that the silicate layers of MMT were intercalated, and randomly dispersed in the PLA matrix. The nanocomposites with intercalated structure exhibited remarkable improvement of material properties in both solid and melt states as compared to that of virgin PLA. Nanocomposites of the PLA and polycaprolactone (PCL) blends were obtained by melt blending with a properly modified kaolinite (Cabedo and others 2006). The purpose of blending PCL into PLA is to decrease the brittleness of PLA. However, the potential drawback of the blend is the increase in gas permeability undergone by PLA as a consequence of the poor gas barrier properties of PCL. Highly exfoliated nanocomposites of the PLA and PLA/PCL blends can be successfully obtained by melt-mixing with a properly modified kaolinite. All nanocomposites showed an improvement in the mechanical properties with regard to the polymers and blends without clay; but the main advantage of adding nanoclays to the polymers was the increase in gas barrier properties. Hence a blend of low content of PCL and 4% kaolinite
resulted in similar gas barrier properties than the neat PLA (Cabebo and others 2006). Ray (2012) recently reviewed the research about polylactide-based bio-nanocomposites.

2.2.3.5. PHA-based Bio-nanocomposites

PHB or PHBV/MMT nanocomposites have been the focus of most studies. The exfoliated structure was not previously reported and only intercalated or well-intercalated structures and microcomposites were obtained using organo-modified or unmodified layered silicates. Initially, Maiti et al. (2003; 2007) prepared PHB-based nanocomposites by melt extrusion. In their work, PHB was reinforced using organo-modified fluoromicas, or organo-modified MMT (OMMT) containing 2% and up to 4% of clay, respectively. X-ray diffraction (XRD) and transmission electron microscopy (TEM) showed the formation of a well-ordered intercalated nanocomposite structure. The storage modulus increased with clay content reaching an increment of 35% with 3.6 wt % of MMT. Choi et al. (2003) incorporated OMMT (Cloisite 30B) into PHBV by melt extrusion. Their research showed that the nanodispersed organoclays acted as nucleating agents, increasing the temperature and rate of crystallization. The thermal stability and tensile properties of their nanocomposites were enhanced. Bruzaud and Bourmaud (2007) studied PHBV/MMT nanocomposites prepared by the solution intercalation method. Their wide-angle X-ray scattering results showed that intercalated and/or exfoliated nanocomposites were obtained. The mechanical behaviors showed significant improvement in terms of modulus, tensile stress and hardness with increased clay loading. Wang et al. (2005) investigated PHBV and OMMT nanocomposites. The addition of the organophilic clay caused an increase in the overall crystallization rate of PHBV, but did not influence the mechanism of nucleation or the growth of PHBV crystals. Experimental results showed that the melting temperature and the enthalpy of melting of the nanocomposites decreased. The crystallinity of neat PHBV decreased and the spherulite size decreased with an increasing amount of MMT in the nanocomposites. The mechanical properties in terms of tensile strength and modulus of the PHBV nanocomposites were enhanced. With the incorporation of 3 wt % MMT, the tensile strength of the nanocomposite increased by 32% and the modulus by 2.8% over those of neat PHBV. Despite the fact that fully exfoliated structures were not obtained in
PHA/MMT systems, the mechanical properties in terms of tensile strength and modulus were increased and the thermal properties were influenced by the addition of MMT.

There has been a limited amount of research done on PHB or PHBV/LDH nanocomposites. Hsu et al. (2006; 2007) reached exfoliated structure using LDH organically modified by poly(ethylene glycol) phophonates (PMLDH). The isothermal and nonthermal crystallization behaviors were investigated. Isothermal crystallization results of PHB/PMLDH nanocomposites showed that the addition of 2 wt % PMLDH into PHB induced more heterogeneous nucleation in the crystallization, significantly increasing the crystallization rate and reducing their activation energy. By adding more PMLDH into the PHB probably causes more steric hindrance of the diffusion of PHB, reducing the transportation ability of polymer chains during crystallization, thus increasing the activation energy (Hsu and others 2006). Dagnon et al. (2009) investigated the PHBV/LDH nanocomposites. The WAXRD patterns indicated an intercalated dispersion but the TEM indicated significant agglomeration in the nanocomposites, so intercalated but aggregated dispersion was obtained. Results of thermo-mechanical tests reflected the reinforcement potential of LDH in the biopolymer matrix. They also concluded that the incorporation of LDH improved the mechanical properties of PHBV. In particular, the Young’s modulus increased by 19% and 27% with 3% and 7% LDH loading, respectively, and the tensile strength increased by 13.5% with 3% LDH loading, but decreased by 2.8% with 5% LDH loading. However, thermogravimetric analysis (TGA) results showed that the nanofiller destabilized the biopolymer matrix leading to decreased thermal stability of the nanocomposites with increasing LDH content. The decreased thermal stability could be due to the release of water from LDH which hydrolyses the ester bonds of PHBV.

2.3. Antimicrobial Nanocomposites for Food Packaging Application

Food spoilage due to undesirable microorganism growth is a common factor which shortens the shelf life of food products and can cause food borne illness outbreaks. The addition of antimicrobial agents (active compounds that kill or prevent the growth of microorganisms) to foods or food surfaces during processing is an effective technique
that is frequently employed to eliminate food borne pathogens. However, the antimicrobial activity may be rapidly lost due to inactivation of the antimicrobials by components in foods or the antimicrobials may be diluted below active concentrations due to migration into the bulk food matrix (Robertson 2006). Therefore, antimicrobial agents have been incorporated into packaging materials, called antimicrobial food packaging (AM food packaging), to prevent the growth of microorganisms on the food surface and thus lead to an extension in shelf life or improved microbial safety of the food (Collins-Thompson and Hwang 2000; Suppakul and others 2003). AM food packaging has received great interest from the food industry attributed to the increasing consumer demand for minimally processed, preservative-free, “fresh” foods, because a major advantage arising from the use of AM packaging is that only low levels of preservative come into contact with the food, compared to the direct addition of preservative to the food (Suppakul and others 2003; Robertson 2006). The properties, such as mechanical, gas barrier, thermal, and morphological properties, of food-packaging films are influenced by the incorporation of antimicrobial substances (Bastarrachea 2011). A significant change can be obtained in the tensile properties of polymeric films after the incorporation of antimicrobials. Many studies have found that a decrease in film strength and resistance with an increase in concentration of the antimicrobial incorporated in the polymer (Limjaroen and others 2003; Pranoto and others 2005; Pires and others 2008; Tippayatum and others 2009; Ture and others 2009; and Bastarrachea and others 2010). Nanocomposite systems with antimicrobial function have recently been studied for several purposes: a) to compensate for the negative effect of antimicrobial agents on the properties of the polymers; and b) to obtain a controlled release of antimicrobial agents by fixing AM on the nanoparticles. Furthermore, the nanocomposite systems with antimicrobial function are particularly effective because of the high surface-to-volume ratio and enhanced surface reactivity of the nano-sized antimicrobial agents, making them able to inactivate more microorganisms when compared to higher scale counterparts (Lloren and others 2012).
2.3.1. Metallic-based Antimicrobial Nanocomposites

Metallic-based nano-structured materials are incorporated into food contact polymers to enhance mechanical and barrier properties, and to prevent the photodegradation of plastic. Additionally heavy metals are effective antimicrobials in the form of salts, colloids, complexes such as silver zeolites, or as elemental nanoparticles (Llorens and others 2012). Silver, copper, zinc and titanium nanostructures have been incorporated into polymeric films as antimicrobial packaging.

Silver nanoparticles (AgNPs) are the most commonly used metallic-based nano-structured materials to be incorporated into polymeric films due to their strong antimicrobial activity against a broad spectrum of bacteria, viruses, and fungi as well as their unique physicochemical properties (Rhim and others 2013). Silver nanoparticles are conventionally produced by the reduction of silver ions from silver salt precursors, with silver nitrate the most frequently used. Reducing agents can be physical (such as UV irradiation, γ-ray irradiation, microwave irradiation, thermal treatment, photochemical process, and sonochemical process), chemical (such as sodium borohydride (NaBH₄), dimethyl formamide (DMF), triethanolamine, hydrazine, etc.), or mixed (hydrothermal) (Yoksan and Chirachanchai, 2010). Moreover, biological materials such as plant extracts, bacteria, fungi, and yeast (Rhim and others 2013) have been used as mediators for the synthesis of silver nanoparticles on extracellular or intracellular level. Recently, stupendous efforts are being given by researchers for the fabrication of metal nanoparticles using various saccharides such as glucose, sucrose, starch, chitosan, and marine polysaccharide (Huang and Yang 2004; Venkatpurwar and Pokharkar 2011; Shukla and others 2012). This green approach is a safe, biocompatible, nontoxic and environmentally friendly method for the synthesis of metal nanoparticles (Rhim and others 2013).

Silver nanoparticles based on silver salts or metallic silver can be readily incorporated into polymers to form antimicrobial nanocomposite films for food packaging applications (Llorens and others 2012), including both synthetic polymers and bio-based polymers. Polyethylene coated with silver-containing polyethyleneoxide was
prepared by Del Nobile et al. (2004). It is reported that the 90 nm size plasma deposited silver clusters showed high bactericidal capacity against *Alicyclobacillus acidoterrestris*, and the material prolonged the shelf-life of apple juice. The shelf-life of orange juice was extended using nanocomposites of low density polyethylene incorporated with a powder containing 95% titanium oxide doping 5% metal-nanosilver (10 nm) (Emamifar and others 2010). An et al. (2008) extended the shelf-life of asparagus by about 10 days at 2 °C by coating it with polyvinylpyrrolidone-AgNPs nanocomposites. Silver nanoparticles have also incorporated into several bio-based polymers, such as chitosan (Sanpui and others 2008), starch (Yoksan and others 2010), cellulose (Necula and others 2010; De Moura and others 2012), and agar (Rhim and others 2013). Agar-based nanocomposite films with silver nanoparticles (AgNPs), obtained by reduction of AgNO₃ using an environmentally friendly method (combined reduction of AgNO₃ by trisodium citrate solution and heating) were prepared by Rhim et al. (2013). Significant increase in water vapor barrier properties and surface hydrophobicity were observed with increase in the concentration of AgNPs without reduction in the mechanical strength, compared to the virgin agar film. In addition, the agar/AgNPs films loaded with more than 1 wt % of silver nanoparticles exhibited strong antimicrobial activity against both Gram-positive (*Listeria monocytogenes*) and Gram-negative (*Escherichia coli O157:H7*) bacterial pathogens.

Besides silver nanoparticles, copper nanoparticles (CuNPs) of elementary copper or copper oxide, zinc oxide (ZnO) and titanium dioxide (TiO₂) nanoparticles have also been incorporated into polymers as antimicrobial food packaging. Copper nanoparticles covered by copper oxide have been embedded in high-pressure polyethylene (Ushakov and others 2008). In addition, various bio-based polymers have been used as carriers of antimicrobial copper. Colloidal copper nanoparticles/chitosan composite films were prepared by Cardenas et al. (2009) for food packaging applications. The composite film reduced the microbial concentration in the liquid culture for *Staphylococcus aureus* and *Salmonella entericaserovar Typhimurium*. The oxygen permeability and water vapor permeability were also decreased with addition of copper nanoparticles. The copper
nanoparticles were also found to be effective against *Escherichia coli* in wound dressings, and could be useful for preserving hygienic conditions in food retail packaging and display cases (Mary and others 2009). The feasibility of zinc oxide nanoparticles incorporated in polymer nanocomposites intended for food packaging has been studied. Poly(vinyl chloride) films with zinc oxide nanoparticles (Li and others 2009) were reported to have antimicrobial activity against *E. coli* and *S. aureus*. The effects of a novel nano-ZnO coated poly(vinyl chloride) film on physicochemical quality and microbiological changes of fresh-cut 'Fuji' apples were evaluated (Li and others 2011). The preservation of quality indicators such as ascorbic acid and polyphenol content, and lower counts of typical altering microorganisms was improved with PVC/ZnO nanocomposite films. Titanium oxide has been positively evaluated as a food additive (Directive 94/36/EC 1994). Nano-sized TiO$_2$ particles show photocatalytic properties, being useful as self-cleaning and antibacterial agents and against UV light (Nordman and Berlin, 1986). Polypropylene films coated with TiO$_2$ nanoparticles were found to be effective in decreasing the counts of *E. coli* in *in vitro* experiments up to $3 \log_{10}$ CFU/g, but also during the storage of lettuce a reduction over $1 \log_{10}$ CFU/g was observed (Chawengkijwanich and Hayata 2008).

2.3.2. Montmorillonite-based Antimicrobial Nanocomposites

Rhim et al. (2006) found that chitosan-based nanocomposite films blended with organically modified MMT (Cloisite 30B) exhibited antimicrobial activity against Gram-positive bacteria including *Staphylococcus aureus* and *Leuconostoc monocytogenes*. They postulated that the antimicrobial action may be attributed to the quaternary ammonium salt of the organically modified nanoclay. Thereafter, other organically modified MMTs with quaternary ammonium salt were studied for antimicrobial activities. MMT-30B with modifier of methyl tallow bis(2-hydroxyethyl) quaternary ammonium and MMT-20A with a modifier of dimethyl di(hydrogenated tallow alkyl) quaternary ammonium were incorporated into whey protein isolate (Sothornvit and others 2009) and agar (Rhim and others 2011). They both reported that only Cloisite 30B-based nanocomposites showed the beneficially bacteriostatic effect again Gram-positive bacteria, *Listeria monocytogenes*. Martins et al. (2013) also found that kappa-
carrageenan/locus bean gum blends with Cloisite 30B films exhibited an inhibitory effect only against *Listeria monocytogenes*. Meng et al. (2009) modified MMT with chlorohexidine acetate (CA) by ion exchange and incorporated it into polydimethyloxane (PDMS). The PDMS/MMT-CA nanocomposite films showed strong antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* (Meng and others 2009).

**2.3.3. Layered Double Hydroxide-based Antimicrobial Nanocomposites**

LDHs, as a kind of layered inorganic solid, are gaining importance as nanofillers for the synthesis of polymer nanocomposites. Due to the hydrophilic surface properties of pristine LDH, LDH modified by organic molecules are normally required to be miscible with polymers. These modified LDH-based nanocomposites have shown improved mechanical properties, barrier properties, heat stability and flame retardancy (Nyambo and others 2008; Costa and others 2008; Bugatti and others 2009). Moreover, the organic molecules can bring additional properties to the nanocomposites, such as controlled drug delivery, and antimicrobial activity (Costantino and others 2009; Tammaro and others 2009; San Roman and others 2013).

Bugatti et al. (2011) intercalated 2,4-dichlorobenzoate and para-hydroxybenzoate, having antimicrobial activity, into Zn/Al-LDH, through anion-exchange, followed by incorporation into a polycaprolactone (PCL) matrix. The investigated release of the antimicrobial moieties “free dispersed” into the polymer was much faster than that of the molecular anions modified on the inorganic compound and occurred in one step. At variance, the release from the nanohybrids occurred in two stages: the first, rapid as a “burst“, occurring in the first few days, the second, very slow, extending up to many months (Bugatti and others 2011). The effect of the structure of the composites has been studied as well. The diffusion of the active molecules out of the microcomposite was always slower than in the case of the exfoliated nanohybrid. Anti-inflammatory drugs, diclofenac, chloramphenicol, and ketoprofen, were intercalated into LDHs and then dispersed in polylactic acid (San Roman and others 2013). The release was almost complete after 24 h for ketoprofen from the drug-LDH systems, but lower values were measured for the other drugs (60 and 80%, respectively, for diclofenac and
chloramphenicol); however, when supported on PLA the release was much slower and could be related to the degradation of the polymer.

2.4. References


Khan, A. I.; Lei, L. X.; Norquist, A. J.; O'Hare, D., Intercalation and controlled release of pharmaceutically active compounds from a layered double hydroxide. *Chemical Communications* 2001, (22), 2342-2343.


Kojima, Y.; Usuki, A.; Kawasumi, M.; Okada, A.; Kurauchi, T.; Kamigaito, O., Synthesis of nylon-6-clay hybrid by montmorillonite intercalated with epsilon-


Li, X. H.; Li, W. L.; Xing, Y. G.; Jiang, Y. H.; Ding, Y. L.; Zhang, P. P., Effects of nano-ZnO power-coated PVC film on the physiological properties and microbiological


Metabolix, Inc., accessed April 2, **2014**.


Pranoto, Y.; Rakshit, S. K.; Salokhe, V. M., Enhancing antimicrobial activity of chitosan films by incorporating garlic oil, potassium sorbate and nisin. *LWT-Food Science and Technology* 2005, 38, (8), 859-865.


Rojas, R.; Palena, M. C.; Jimenez-Kairuz, A. F.; Manzo, R. H.; Giacomelli, C. E.,
Modeling drug release from a layered double hydroxide-ibuprofen complex.
San Roman, M. S.; Holgado, M. J.; Salinas, B.; Rives, V., Drug release from layered
double hydroxides and from their polylactic acid (PLA) nanocomposites. Applied
Sanpui, P.; Murugadoss, A.; Prasad, P. V. D.; Ghosh, S. S.; Chattopadhyay, A., The
antibacterial properties of a novel chitosan-Ag-nanoparticle composite.
Schmitt, H.; Prashantha, K.; Soulestin, J.; Lacrampe, M. F.; Krawczak, P., Preparation
and properties of novel melt-blended halloysite nanotubes/wheat starch
agar-based silver nanoparticles and nanocomposite film with antibacterial
Simioni, A. R.; Vaccari, C.; Re, M. I.; Tedesco, A. C., PHBV/PCL microspheres as
biodegradable drug delivery systems (DDS) for photodynamic therapy (PDT).
Siro, I.; Plackett, D., Microfibrillated cellulose and new nanocomposite materials: a
Sorrentino, A.; Gorrasi, G.; Tortora, M.; Vittoria, V., Barrier properties of polymer/clay
Sorrentino, A.; Gorrasi, G.; Tortora, M.; Vittoria, V.; Costantino, U.; Marmottini, F.;
Padella, F., Incorporation of Mg-Al hydrotalcite into a biodegradable poly
(epsilon, caprolactone) by high energy ball milling. Polymer 2005, 46, (5), 1601-
1608.
Sorrentino, A.; Gorrasi, G.; Vittoria, V., Potential perspectives of bio-nanocomposites for
food packaging applications. Trends in Food Science & Technology 2007, 18, (2),
84-95.


3.1. Introduction

Layered double hydroxide (LDH), or the so-called hydrotalcite-like, are a family of layered solids with structurally positively charged layers and interlayer balancing anions (Costantino and others 1997). The general chemical formula for LDHs is written as 
\[ [\text{M}^{\text{II}}_{1-x}\text{M}^{\text{III}}_x\text{OH}_2]^{x^+}\text{A}^{n^-}/n \text{H}_2\text{O} \]
where \( \text{M}^{\text{II}} \) is a divalent metal ion, such as \( \text{Mg}^{2+} \), \( \text{Ca}^{2+} \), \( \text{Zn}^{2+} \), etc., \( \text{M}^{\text{III}} \) is a trivalent metal ion, such as \( \text{Al}^{3+} \), \( \text{Cr}^{3+} \), \( \text{Fe}^{3+} \), \( \text{Co}^{3+} \), etc. and \( \text{A}^{n^-} \) is an anion, such as \( \text{CO}_3^{2-} \), \( \text{Cl}^- \), \( \text{NO}_3^- \), etc. (Evans and Xue 2006). Anions occupy the interlayer region of these layered crystalline materials. Although a wide range of values for \( x \) is claimed to provide LDH structure, the pure phase of LDH clays is usually obtained for a limited range as 0.2<\( x <0.33 \). The structure of LDHs can best be explained by drawing an analogy with the structural features of the metal hydroxide layers in mineral brucite or simply the \( \text{Mg(OH)}_2 \) crystal structure. Brucite consists of a hexagonal close packing of hydroxyl ions with alternate octahedral sites occupied by \( \text{Mg}^{2+} \) ions. The metal hydroxide sheets in brucite crystal are neutral in charge and stack one upon another by Van der Waals interaction. The interlayer distance or the basal spacing in brucite has a value of about 0.48 nm. In LDH, some of the divalent cations of these brucite-like sheets are isomorphously substituted by a trivalent cation and the mixed metal hydroxide layers, \( [\text{M}^{\text{II}}_{1-x}\text{M}^{\text{III}}_x\text{(OH)}_2]^{x^+} \), thus forming a net positive charge. This excess charge on the metal hydroxide layers is neutralized by the anions accumulated in the interlayer region. The interlayer anions can be exchanged by other inorganic, organic, or metallorganic anions and even by biomolecules containing ionizable acidic groups to obtain novel materials (Choy and others 2000; Hwang and others 2001; Desigaux and others 2006). The interlayer region in LDHs also contains some water molecules for the stabilization of the crystal structure. The presence of anions and water molecules leads to an enlargement of the basal spacing from 0.48 nm in brucite to about 0.77 nm in Mg-Al-LDH.
LDHs are well known for their catalytic activities in organic synthesis (Tichit and Coq 2003). LDHs and their modified forms also have been used in the fields of photophysics and photochemistry (Ogawa and Kuroda 1995; Latterini and others 2007; Tian and others 2007), wastewater treatment (Lv and others 2006; Mohan and Pittman 2007), gene therapy (Choy and others 1999; Del Hoyo 2007), and drug storage and release. LDHs as drug delivery vehicles have received considerable attention in recent years because of their biocompatibility, anion-exchange property, nontoxicity, etc. A variety of drugs, such as diclofenac, 4-biphenylacetic acid, ibuprofen, tolefenamic acid, low molecular weight heparin, fenbufen and 5-fluorocytosine, have been incorporated into LDHs (Khan and others 2001; Li and others 2004; Costantino and others 2007; Gu and others 2008; Liu and others 2008; Rojas and others 2012). After administration of the intercalation compound, the drug may be released via a deintercalation process, occurring because of ion-exchange or displacement reactions. The rate of drug diffusion out of the LDH intercalation compound depends on the strength of the host-guest interaction, the rigidity of the layers and the diffusion path length.

LDHs, as a kind of layered inorganic solid, are also gaining importance as nanofiller for the synthesis of polymer nanocomposites. Due to the hydrophilic surface properties of pristine LDH, LDH modified by organic molecules is normally required to be miscible with polymers. These modified LDH-based nanocomposites have shown improved mechanical properties, barrier properties, heat stability and flame retardancy (Nyambo and others 2008; Costa and others 2008; Bugatti and others 2009). Moreover, the organic molecules can bring additional properties to the nanocomposites, such as controlled drug delivery, and antimicrobial activity (Sorrentino and others 2007; Costantino and others 2009; Tammaro and others 2009; San Roman and others 2013).

In this context, the present study has been conducted to prepare suitable fillers of bio-based polymers to obtain composites for “active packaging” systems. The objective of this chapter is to prepare and characterize modified Mg-Al LDH by antimicrobial agents via anion exchange. Three antimicrobial agents used in foods, sodium benzoate, sodium gallate, and potassium sorbate, are chosen as modifiers. The anion exchange is
conducted under different conditions (mole ratio of antimicrobial agent : anion in pristine LDH, and reaction temperature) in order to have various amounts of antimicrobial agents loaded on the LDH. The structure and chemical composition of modified LDH are investigated using X-ray diffraction, attenuated total reflectance-Fourier transform infrared spectroscopy, and thermogravimetric analysis-mass spectrometry. The release property of antimicrobial agents from modified LDH into DI water is studied as well.

3.2. Materials and Methods

3.2.1. Materials

Un-modified nanoparticles LDH-CO$_3$ of the formula $[\text{Mg}_{4.5}\text{Al}_2(\text{OH})_{13}](\text{CO}_3)_1 \cdot 3.5\text{H}_2\text{O}$ was kindly provided by Sechang Co. Ltd. (Jeonbuk, Korea). Sodium benzoate (SB) and potassium sorbate from J.T. Baker (Center Valley, PA, USA), gallic acid (GA) from Sigma-Aldrich, Inc (St. Louis, MO, USA) were used. All other reactants, including sodium chloride (Alfa Aesar), hydrochloric acid (VWR), sodium nitrate (VWR), and sodium hydroxide (BDH Chemicals), were purchased from VWR International LLC. (Philadelphia, PA, USA).

3.2.2. Nanoparticle Modification

*Preparation of LDH-Cl and LDH-NO$_3$:* The corresponding chloride form (LDH-Cl) was obtained by titrating, at room temperature (20 °C), the carbonate form, dispersed in a 2 mol/l NaCl solution (1 g/100ml) with 0.1 mol/l HCl by means of an automatic titrator (TitroLine® alpha plus, SI Analytics GmbH, Mainz, Germany) operating at pH stat mode to maintain a pH value of 5. In order to obtain the nitrate form (LDH-NO$_3$), LDH-Cl was suspended in a CO$_2$-free aqueous solution of 0.5 mol/l NaNO$_3$ (1 g/100ml of solution) and gently stirred for 48h at room temperature (20 °C) with continuous nitrogen purge. The slurry was then aged for 18-24 h, centrifuged at 8000 rpm for 15 min, and then the recovered solid was washed three times with CO$_2$-free deionized water and finally dried at 80 °C for 48 h. The CO$_2$-free deionized water was obtained by purging nitrogen into deionized water with stirring for 30 min.
**Preparation of LDH-SB:** The intercalation of benzoate anion was achieved by equilibrating the LDH-NO₃ with a CO₂-free aqueous solution of 0.5 mol/l sodium benzoate while gently stirring for 48 h with continuous nitrogen purge. For LDH-SB1, the molar ratio of benzoate anion/NO₃⁻ was 3 and reaction temperature was room temperature (20 °C); for LDH-SB2, the molar ratio of benzoate anion/NO₃⁻ was 18 and the reaction temperature was 20 °C; for LDH-SB3, the molar ratio of benzoate anion/NO₃⁻ was 18 and the reaction temperature was 70 °C. The slurry was then aged for 18-24 h, centrifuged at 8000 rpm for 15 min, and then the recovered solid was washed three times with CO₂-free deionized water and finally dried at 80 °C for 48 h.

**Preparation of LDH-SG:** Sodium gallate (SG) was first obtained by titrating gallic acid using sodium hydroxide by means of an automatic titrator operating at pH stat mode to maintain a pH value of 8. The resulting solution was then directly used to modify nanoparticles. To obtain sodium gallate powder, the resulting solution was then freeze dried for 48 h. The intercalation of gallate anion (LDH-SG) was achieved by equilibrating the LDH-NO₃ with a CO₂-free aqueous solution of 0.25 mol/l sodium gallate while gently stirring for 48h with continuous nitrogen purge. The molar ratio of gallate anion/NO₃⁻ was 18 and reaction temperature was 70 °C. The slurry was then aged for 18-24 h, centrifuged at 8000 rpm for 15 min, and then the recovered solid was washed three times with CO₂-free deionized water and finally dried at 80 °C for 48 h.

3.2.3. Powder X-ray Diffraction (XRD)

The X-ray diffraction patterns of the nanoparticles were taken with a X-ray diffractometer (MiniFelx II, Rigaku Corporation, Tokyo, Japan), using Ni-filtered CuKα radiation ($\lambda = 1.54$ Å) and operating 30 kV and 15 mA. The scanning angular range was $2 - 40^\circ$ with a sampling width of 0.02 and a scanning rate of 2 °/min.
3.2.4. Attenuated Total Reflectance - Fourier Transform Infrared Spectroscopy (ATR-FTIR)

A Bruker IFS 66/s FTIR spectrometer (Bruker Optics, Billerica, MA, USA) equipped with an attenuated total reflectance (ATR) accessory containing a diamond crystal was used. Spectra of the nanoparticles were obtained at room temperature (20 °C) over the wave number range of 4000–400 cm\(^{-1}\), with an accumulation of 100 scans and a resolution of 6 cm\(^{-1}\).

3.2.5. Thermogravimetric Analysis-Mass Spectrometry (TGA-MS)

Thermo-analytical investigations of the nanoparticles were carried out in a TGA Q50 (TA Instruments, New Castle, DE, USA). Samples were heated from room temperature to 1000 °C at a heating rate of 20 °C/min under a flowing argon atmosphere. To simultaneously follow the evolution of the gaseous decomposition products over the temperature range investigated, the thermobalance was connected to a downstream mass spectrometer (Thermostar GSD 301T3, Pfeiffer Vacuum Inc., Nashua, NH, USA). The thermogravimetric (TG), derivative thermogravimetric (DTG) and mass spectrometric ion intensity curves of the selected ionic species were recorded simultaneously.

3.2.6. Release Study of Modified Nanoparticles

Release profiles of antimicrobial agents from modified nanoparticles in DI water were studied at a wavelength of \(\lambda_{\text{max}} = 225\) nm for benzoate and at \(\lambda_{\text{max}} = 260\) nm for gallate using a UV-VIS spectrophotometer (Helios gamma; Thermo Spectronic, Madison, WI, USA). The release study was conducted at 21 °C by suspending modified nanoparticles into 150 ml DI water. The samples were kept agitated at 75 rpm with the help of an orbital shaker throughout the duration of the release study. At predetermined intervals, 2 milliliters of solution was withdrawn and replaced with 2 ml fresh DI water. The solution was first filtered using a 0.2 µm syringe filter followed by actual measurement of the accumulated benzoate or gallate which had been released into the solution. Calibration curves for both antimicrobial agents, relating the absorbance of the solution with concentration of the agents, were built from measurements at 225 nm and
260 nm for sodium benzoate and sodium gallate, respectively, in DI water among the expected concentration ranges. The calibration curve for benzoate at 225 nm was built with 8 points in the range of 20 µg/ml and 0.5 µg/m, while the one for gallate at 260 nm was built with 8 point in the range of 30 µg/ml and 1 µg/ml. Straight lines were obtained in both cases with regression coefficients of 0.999.

3.3. Results and Discussion

3.3.1. Powder X-ray Diffraction

The most common interlayer anionic group in LDH is CO$_3^{2-}$. However, carbonate ions are exceptionally difficult to anion-exchange because of their high affinity to LDHs. The affinity of the LDH toward various anions is in the following order (Miyata 1983):

$$\text{CO}_3^{2-} > \text{SO}_4^{2-} > \text{OH}^- > \text{F}^- > \text{Cl}^- > \text{Br}^- > \text{NO}_3^- > \Gamma.$$ 

Therefore, in order to intercalate antimicrobial agents into nanoparticles, the carbonate ions in the LDH-CO$_3$ were first deintercalated using acid-salt mixed solution to exchange carbonate ions with chloride ions (Iyi and others 2004; Costantino and others 2009), and then the LDH-Cl was modified to LDH-NO$_3$ via anion-exchange using sodium nitrate (Costantino and others 2009).

Figure 3.1 shows the XRD patterns of nanoparticles LDH-CO$_3$, LDH-Cl, and LDH-NO$_3$. The number next to the peak is the calculated basal spacing using Bragg’s Law. The nanoparticles with carbonate, chloride, and nitrate as interlayer anion clearly show one crystalline phase. In the unmodified LDH (LDH-CO$_3$), the first level reflection (003) at $2\theta = 11.6^\circ$ corresponded to an interlayer distance of 0.76 nm. Replacement of carbonate by chloride as the interlayer anion resulted in a slight expansion from 0.76 nm to 0.77 nm. The interlayer distance increased to 0.87 nm in LDH-NO$_3$ due to larger anion size of nitrate compared to carbonate or chloride, which was slightly larger compared to the value of 0.84 nm reported by Marino and Masculo (1982) and 0.81 nm by Kloprogge (2002). The difference of the interlayer distance can also be seen from the second level reflection (006) in the nanoparticles. As shown in Figure 3.1, there is a weak peak on the side of the sharp and intense peak at $2\theta = 10.7^\circ$ in LDH-NO$_3$, of which position was close
to the peak position of the first level reflection (003) of LDH-Cl and LDH-CO$_3$. A weak peak can also be seen on the side of the second level reflection (006) peak of LDH-NO$_3$, implying nitrate ions may not replace all of chloride ions, which is in agreement with the TGA-MS result (see section 3.3.3. TGA-MS).

Starting from LDH-NO$_3$, the intercalation compounds containing benzoate and gallate were prepared via anion-exchange. The exchange was conducted under three separate conditions (see section 3.2. Material and Methods) to have various amounts of benzoate or gallate anions loaded in the nanoparticles. Figure 3.2 shows the XRD patterns for the LDH modified by sodium benzoate (LDH-SBs). After exchange of the nitrate anions, new peaks appeared in the 2θ range between 2° and 10° in all three LDHs modified by benzoate but with different intensities, indicating the nitrate was exchanged with benzoate anions to varying degrees. By comparing the peak intensity (2θ <10°), more nitrate ions were replaced by benzoate anions from LDH samples SB1 through SB3, which is in agreement with TGA-MS results (see section 3.3.3. TGA-MS).

![XRD patterns of unmodified LDH (LDH-CO$_3$), LDH in chloride form (LDH-Cl), and LDH in nitrate form (LDH-NO$_3$).](image)

Figure 3.1. XRD patterns of unmodified LDH (LDH-CO$_3$), LDH in chloride form (LDH-Cl), and LDH in nitrate form (LDH-NO$_3$).
Figure 3.2. XRD patterns of LDH in nitrate form (LDH-NO₃), and LDH modified by sodium benzoate with different modification conditions (molar ratio of benzoate anion/NO₃⁻; reaction temperature), LDH-SB1 (3:1; 20 °C), LDH-SB2 (18:1; 20 °C), and LDH-SB3 (18:1; 70 °C).

With the knowledge of the basal distance, and hence of the interlayer volume available to the guest species, combining their number and anion dimension, one may give a qualitative, but reliable, description of the arrangement of the guest species in the interlayer region (Costantino and others 2009). The interlayer arrangement of LDH containing benzoate anions has been studied by several groups. Meyn and coworkers (1990) studied the anion-exchange properties of MgAl-, ZnAl-, and ZnCr-LDH with M³⁺:M²⁺ ratio of 2:1. They found the basal distance of LDH-benzoate was around 1.55 nm and therefore, deduced an orientation of the benzene rings almost perpendicular to the layer plane and water molecules were retained between the benzene ring and the surface oxygen atoms. The group at University of Cambridge (Kooli and others 1996; Newman and others 1998; Vucelic and others 1995) studied the interlayer arrangement of hydrated MgAl LDH containing guest terephthalate and benzoate anions. They pointed out that the interlayer arrangement of guest anions depended upon Mg:Al ratio (i.e., the charge density) and the degree of hydration. In the case of terephthalate a monolayer arrangement existed. Three different LDH phases were identifiable across the series of XRD patterns that corresponded to either an expanded or collapsed interlayer structure,
or what was generally thought to be an interstratification of expanded and collapsed interlayers (Newman and others 1998). The “expanded phase” was identified by an interlayer spacing of approximately 1.4 nm, which corresponded to an approximately vertical orientation of the terephthalate anion with respect to the hydroxide layers. The “collapsed phase” was identified by an interlayer spacing of approximately 0.84 nm, which corresponded to an approximately horizontal orientation of the terephthalate anion. The “interstratified phase” was identified by successive orders of basal reflections corresponding to a repeat distance of approximately 2.24 nm – the sum of the interlayer distance of the expanded and collapsed phases. For 2:1 Mg:Al ratio, the interstratified phase was formed at 9.1 and 17.7 wt % water contents, the expanded phase at higher than 17.7% water content, no collapsed phase was formed. For 3:1 Mg:Al ratio, the collapsed phase was formed at 5.5 wt % water content, interstratified phase at 12.8 wt % water content, and expanded phase at higher water content.

A similar approach can be applied to the benzoate anions. The interstratified phase consists of alternate layers containing vertical and horizontal benzoate anions. Compared to terephthalate, the vertical arrangement for benzoate was a bilayer and the horizontal a monolayer (Kooli and others 1996). Kovar and coworkers (2007) confirmed the bilayer-like vertical arrangement of benzoate anions by computer modeling. Costantino (2009) proposed the structural model for ratio of 1.9:1 Zn:Al LDH-sodium benzoate in which the benzene–COO– bond was perpendicular to the layers.

As shown in Figure 3.2, the peak position of the lowest 2θ angle of 3.92° for all three LDH-SBs is the same, which corresponded to the interlayer distance of 2.25 nm. For LDH-SB1 and LDH-SB2, both had a peak at 2θ = 7.5°, which corresponded to the interlayer distance of 1.18 nm. For LDH-SB3, the peak shifts to lower 2θ angle corresponding to the interlayer distance of 1.42 nm. According to the mechanism reported by Newman (1998), the interlayer distance of 1.42 nm could be responsible for the vertical arrangement of benzoate anions, and that of 2.25 nm could be responsible for the interstratifications of expanded and collapsed interlayers (sum of 1.42 nm and 0.83 nm). The nature of the 1.18 nm peak could be due to a higher level reflection regarding to
the peak at $2\theta = 3.92^\circ$ or to interlayer benzoate anions oriented at an oblique angle to the hydroxide layer. The relatively sharp intense peak at 0.76 nm is probably a mixture of closely spaced reflections from two or three different sources (Kukkadapu and others 1997). Firstly, it is close to the first level reflection (003) observed in LDH-CO$_3$ and thus could represent reflections from interlayers containing only carbonate. Secondly, most likely it represents reflections from interlayers containing a mixture of benzoate anions oriented parallel to the hydroxide layers and carbonate. Last, it could contain a small contribution from the higher order reflection of the benzoate anions peak at 1.42 nm.

MgAl LDH-NO$_3$ with the ratio of 2.25:1 Mg:Al in the present study was successfully modified by sodium benzoate through anion-exchange. The interstratifications phase was formed in all three LDH-SBs, which may relate to their low water contents (< 13 wt % according to TGA-MS data). The expanded phase was only seen in LDH-SB3, which had the highest amount of benzoate anions. The results followed the mechanism well proposed by Newman et al. (1998) using terephthalate as interlayer anion. The structural model of intercalated benzoate is shown in Figure 3.3.

Three separate conditions as for LDH-SB were used to conduct the anion-exchange to prepare LDH modified by sodium gallate. However, gallate anion can only be exchanged under the third condition with 18:1 of molar ratio of gallate anion: NO$_3^-$ at 70 °C, and exchange did not happen under the other two conditions (3:1 of molar ratio of gallate anion: NO$_3^-$ at 20 °C or 18:1 of molar ratio of gallate anion: NO$_3^-$ at 20 °C) (data not shown). Figure 3.4 shows the XRD patterns for the LDH modified by sodium gallate (LDH-SG3) under the third condition with 18:1 of molar ratio of gallate anion: NO$_3^-$ at 70 °C. The basal spacing for LDH-SG3 is 0.76 nm. It can then be deduced that the spatial orientation of gallate anions inside the LDH layer needed to be flat and monolayer with the phenolic hydrogen perpendicular to the ring. Hussein and coworkers (2009) found the similar interlayer arrangement of gallate anions in ZnAl LDH. The reason for the parallel orientation of gallate anions other than vertical orientation may be that the parallel orientation created more positions for stronger interactions with layer hydroxyl groups by exposing all three hydroxide groups to the hydroxide layers (Braterman, 2004).
The 2θ angle at 25.1°, corresponding to interlayer distance of 0.36 nm, could be the second level reflection of the peak at 2θ = 11.6°. The nature of the 0.60 and 0.43 nm peaks is less certain and further investigation is necessary to explain them.

It is worth mentioning that I attempted to intercalate potassium sorbate, another common antimicrobial, into LDH, but was unsuccessful under the conditions used in this study (data not shown). Spatial structure and polarity of the antimicrobial anions may affect the priority of the exchange with nitrate. Larger molecules with lower polarity may be more difficult to intercalate into nanolayers. Figure 3.5 shows the ball-and-stick structures of three antimicrobial agents, and Table 3.1 lists the molecular properties, including molecular volume and partition coefficient (log P). Log P, calculated by Software Molinspiration desktop property calculator and used to describe the polarity of the molecules, has been used in quantitative structure-activity relationship (QSAR) studies and rational drug design as a measure of molecular hydrophobicity (www.molinspiration.com). The lower log P values translate to a more polar molecule. For the benzoate anion, both the benzene ring and ester groups were lying flat. For the gallate anion, all atoms were lying flat except the hydrogens on hydroxide groups, which made the molecular volume for gallate was higher than that for benzoate. This larger volume could make the gallate harder to intercalate between nanolayers. However, gallate anions are much more polar than benzoate anions due to the presence of three hydroxide groups. Therefore, both benzoate and gallate were able to intercalate into LDH nanolayers, although gallate required harsher conditions to intercalate into LDH nanolayers. For sorbate, the molecular volume was close to that for gallate, but sorbate has a significantly lower polarity than gallate, therefore, sorbate was not able to intercalate into LDH nanolayers.
Figure 3.3. The structural model showing three arrangements of benzoate anions between the LDH layers.

Figure 3.4. XRD patterns of LDH in nitrate form (LDH-NO$_3$), and LDH modified by sodium gallate (LDH-SG3) under the third reaction condition (molar ratio of gallate anion/NO$_3^-$ = 18:1 and reaction temperation = 70 °C).
Figure 3.5. Ball-and-stick structures of antimicrobial agents generated by Material Studio v.4.1.

Table 3.1. Molecular properties of antimicrobial agents

<table>
<thead>
<tr>
<th>Antimicrobial Agents</th>
<th>Molecular Volume (Å³)*</th>
<th>Partition coefficient (log P)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzoate Anion</td>
<td>111.0</td>
<td>1.85</td>
</tr>
<tr>
<td>Gallate Anion</td>
<td>135.1</td>
<td>0.59</td>
</tr>
<tr>
<td>Sorbate Anion</td>
<td>132.3</td>
<td>0.97</td>
</tr>
</tbody>
</table>

* Molecular volumes were calculated by Software Material Studio v 4.1.
** Partition coefficients were calculated by Software Molinspiration desktop property calculator.
3.3.2. ATR-FTIR

Figure 3.6 shows a strong broad band in the 3000 – 3700 cm\(^{-1}\) range due to OH-stretching vibrations in the layer, which agree with other literature (Chatelet and others 1996; Costantino and others 2009; Kloprogge 2005).

Figure 3.6. FTIR spectra of unmodified LDH (LDH-CO\(_3\)), LDH in chloride form (LDH-Cl), and LDH in nitrate form (LDH-NO\(_3\)).

For all hydrotalcites, water HOH-bending mode was observed in the range between roughly 1580 cm\(^{-1}\) and 1655 cm\(^{-1}\) (Kloprogge 2005). Figure 3.6 shows that water HOH-bending for LDH-CO\(_3\), LDH-Cl, and LDH-NO\(_3\) is at 1584 cm\(^{-1}\), 1602 cm\(^{-1}\), and 1644 cm\(^{-1}\), respectively. The sharp and intense band at 1363 cm\(^{-1}\) for LDH-CO\(_3\) was ascribable to the asymmetric stretching vibration (\(\nu_3\)) of CO\(_3^{2-}\). The band became very broad and weak for LDH-Cl, which meant that most CO\(_3^{2-}\) was replaced by Cl\(^-\). Four vibrational modes in infrared spectrum of nitrate in hydrotalcite-like compounds have been reported assigned to symmetric stretching mode (\(\nu_1\)) at around 1049 cm\(^{-1}\), out-of-plane symmetric deformation mode (\(\nu_2\)) at around 820-840 cm\(^{-1}\), asymmetric stretching mode (\(\nu_3\)) at
around 1385 cm\(^{-1}\), and asymmetric deformation mode (\(v_a\)) at around 668 cm\(^{-1}\) (Hernandez-Moreno and others 1985; Kagunya and others 1998; Lopez and others 1997; Miyata 1975). The intense peak at 1385 cm\(^{-1}\) was not observed, instead, two peaks in the range at 1416 and 1355 cm\(^{-1}\) appeared. This may partly be due to the fact that not all carbonate was exchanged by nitrate; thereby complicating the infrared spectra due to overlapping bands (Bish and Livingstone 1981; Evana and others 1992). The weak bands around 1052 and 820 cm\(^{-1}\) can be seen, which corresponded to \(v_1\) and \(v_2\) modes for nitrate. Therefore, it can be concluded that most carbonate in LDH was replaced by nitrate.

FTIR spectra for sodium benzoate and LDH-SBs are shown in Figure 3.7. The sharp and intense bands at 1547 and 1404 cm\(^{-1}\) in sodium benzoate were ascribable to the asymmetric and symmetric stretching vibrations of the C-O bonds of COO\(^-\) groups (Costantino and others 2009). The band at 1595 cm\(^{-1}\) was assigned to the in-plane skeletal vibration for aromatic ring (Costantino and others 2009). The position of the band at 1595 cm\(^{-1}\) did not change after benzoate was intercalated into LDH layers. However, both asymmetric and symmetric stretching vibrations shifted towards lower wavenumbers, which could be due to the interaction with the surrounding interlayer water molecules causing a loss of freedom and therefore possibly a lowering of the symmetry (Klogrogge 2005). Moreover, the magnitude of the separation between of the carboxylate stretches \([\Delta = v_{as}(\text{COO}^-) - v_{s}(\text{COO}^-)]\) gave information on the mode of the carboxylate binding (Costantino and others 2009). In particular, the comparison of the \(\Delta\) value of the respective complex with the \(\Delta\) value of the particular sodium salt can be used for the assignment of the carboxylate binding (Zelenak and others 2007). The value of \(\Delta = 172\) cm\(^{-1}\) of LDH-SBs was larger than that of \(\Delta = 143\) cm\(^{-1}\) of sodium benzoate, which suggested the presence of a monodentate carboxylate coordination. Meanwhile, the intensity of these three peaks increased from sample LDH-SB1 through SB3, which indicated that more benzoate anions were loaded in the LDH from sample LDH-SB1 through LDH-SB3 as established by XRD data.
Figure 3.7. FTIR spectra of LDH in nitrate form (LDH-NO$_3$), LDH modified by sodium benzoate with different modification conditions (molar ratio of benzoate anion/NO$_3^-$; reaction temperature), LDH-SB1 (3:1; 20 °C), LDH-SB2 (18:1; 20 °C), and LDH-SB3 (18:1; 70 °C), and sodium benzoate (SB).

Figure 3.8 shows the FTIR spectrum of LDH-SG3. The strong broad band in the 3000 – 3700 cm$^{-1}$ range assigned to OH-stretching vibrations in the layer of LDH-NO$_3$ became very broad in LDH-SG3. This was also accompanied by the disappearance of the other OH modes. This was due to the hydrogen bonding taking place between the gallate molecules and the hydroxides on the surface of the LDH layers. A few more bands appeared in LDH-SG3, compared to the spectrum of LDH-NO$_3$, which indicated that gallate anions were either intercalated into LDH layers or absorbed onto the surface of LDH platelets. Both intercalation and absorption were happened in the present study as shown by the results of TGA-MS later. The band at 1613 cm$^{-1}$ was assigned to water
HOH-bending. Bands at 1544 cm\(^{-1}\), 1371 cm\(^{-1}\) and 1059 cm\(^{-1}\) were attributed to C═C stretching vibration of the benzene ring, symmetrical stretching of carboxyl group, and C-O stretching mode, respectively (Ghotbi and others 2010).

Figure 3.8. FTIR spectra of LDH in nitrate form (LDH-NO\(_3\)) and LDH modified by sodium gallate (LDH-SG3) under the third reaction condition (molar ratio of gallate anion/NO\(_3\) = 18:1 and reaction temperature = 70 °C).

3.3.3. TGA-MS

TGA-MS was used to determine the compositions of nanoparticles containing various anions. All nanoparticles showed a similar first step of weight loss between 9% and 15% up to 250 °C. This can be interpreted as being due to the loss of adsorbed water followed by the interlayer water (detected as m/z 18) (Kloprogge and others, 2003). For LDH-CO\(_3\), LDH-Cl, and LDH-NO\(_3\), the second weight loss was between 250 °C and 600 °C, which can be ascribed to the loss of the water derived from dehydroxylation of the inorganic layers and of the interlayer anions, carbonate released as CO\(_2\) (m/z 44), chloride as chlorine (m/z 36), and nitrate as NO (m/z 30). Figure 3.9 b and c show that
not all carbonate (about 76% of carbonate) was exchanged by chloride or nitrate since CO₂ peak appeared in both TGA-MS patterns. The reasons may be due to the high affinity of carbonate for the layers making it difficult to exchange or due to the contamination by carbonate from air during filtration. For three LDH-SBs, similar thermal behavior with three steps of weight loss was found. The second step between 250 °C and 400 °C was assigned to dehydroxylation of the layers and decarboxylation of benzoate (Costantino and others, 2009). The third step between 400 °C and 800 °C was assigned to removal of the benzene from benzoate (m/z 78) and carbonate released as CO₂. There was no NO peak found in any LDH-SB, which meant all nitrate was exchanged by either benzoate or carbonate. A CO₂ peak from carbonate existed to various degrees in all LDH-SBs. These carbonate anions consisted of original carbonate from LDH-NO₃ and outside carbonate from air during modification. Therefore, two things were important during modification by organic compounds via anion-exchange, which were, nitrate form should be used for modification and the reaction needed to be conducted under CO₂-free environment. For LDH-SG3, three steps of weight loss were also found. The second step was similar with the one in LDH-SB, which was assigned to dehydroxylation of the layers and decarboxylation of benzoate. The third step, due to the decomposition of gallate anions, produced CO₂, CₓHᵧ and carbon particles under nitrogen atmosphere, and occurred up to 1000 °C (Ghotbi and others, 2010). The percentage of residue varied in the nanoparticles dependent on the interlayer anions. MgO and MgAl₂O₄ were recognized as the residues (Costantino and others, 2009). The composition can be obtained with the information given by TGA-MS and the assumption of the maintained Mg : Al ratio of 2.25 : 1 during modification. The composition calculated from TGA-MS for LDH-CO₃ was in good agreement with the one given by the supplier. Table 3.2 reports the compositions for all nanoparticles. The amount of benzoate anion in LDH depended on the reaction condition. Higher temperature and ratio of benzoate : nitrate resulted in higher loading of benzoate in LDH. Composition of LDH-SG3 showed the amount of gallate loaded was 43.9%. The theoretical calculation by using LDH-gallate formula showed that less amount of gallate can intercalate into layers (around 39%) compared to that of obtained by the TGA-MS analysis. The difference may be due to the adsorbed gallate onto the surface of LDH platelets.
a. LDH-CO$_3$

![Graph showing the weight loss and ion current as a function of temperature for LDH-CO$_3$.]

b. LDH-Cl

![Graph showing the weight loss and ion current as a function of temperature for LDH-Cl.]

c. LDH-NO₃

![Graph showing weight percent vs. temperature for LDH-NO₃](image)

- Weight (%)
- Deriv. wt (%/°C)
- m/z 18
- m/z 30
- m/z 44

Temperature (°C)

- 125.14°C
- 200.63°C
- 396.12°C
- 9.28%

- Residue 51.89%

- 38.84%

- Ion Current (nA)

- Deriv. Weight (%/°C)

- 12.97%

- 24.33%

- 13.20%

- Residue 49.56%


- 40
- 60
- 80
- 100

- 0
- 200
- 400
- 600
- 800
- 1000

Temperature (°C)

- d. LDH-SB1

![Graph showing weight percent vs. temperature for LDH-SB1](image)
f. LDH-SB2

![Graph showing weight and ion current against temperature for LDH-SB2.]

f. LDH-SB3

![Graph showing weight against temperature for LDH-SB3.]

---

The graphs depict the thermal analysis of LDH-SB2 and LDH-SB3, showing weight percentage and ion current as a function of temperature. The peaks and troughs indicate different stages of degradation and transformation.
g. LDH-SG3

Figure 3.9. TGA-MS patterns of a. unmodified LDH (LDH-CO$_3$); b. LDH in chloride form (LDH-Cl); c. LDH in nitrate form (LDH-NO$_3$); d-e. LDH modified by sodium benzoate with different modification conditions (molar ratio of benzoate anion/NO$_3^-$; reaction temperature), d. LDH-SB1 (3:1; 20 °C), e. LDH-SB2 (18:1; 20 °C), and f. LDH-SB3 (18:1; 70 °C); and g. LDH modified by sodium gallate (LDH-SG3) under the third reaction condition (molar ratio of gallate anion/NO$_3^-$ = 18:1 and reaction temperature = 70 °C).

Table 3.2. Composition of nanoparticles containing various anions

<table>
<thead>
<tr>
<th>Sample</th>
<th>Intercalated Anion</th>
<th>Composition</th>
<th>wt % of Anion</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH-CO$_3$</td>
<td>Carbonate</td>
<td>[Mg$_{4.5}$Al$<em>2$(OH)$</em>{13}$] (CO$_3$)$_1$ · 3.5H$_2$O</td>
<td>11.9%</td>
</tr>
<tr>
<td>LDH-Cl</td>
<td>Chloride</td>
<td>[Mg$<em>{4.5}$Al$<em>2$(OH)$</em>{13}$] (Cl)$</em>{1.55}$(CO$<em>3$)$</em>{0.24}$ · 2.23H$_2$O</td>
<td>11.1%</td>
</tr>
<tr>
<td>LDH-NO$_3$</td>
<td>Nitrate</td>
<td>[Mg$_{4.5}$Al$<em>2$(OH)$</em>{13}$] ( NO$<em>3$)$</em>{1.55}$(CO$<em>3$)$</em>{0.24}$ · 2.80H$_2$O</td>
<td>17.5%</td>
</tr>
<tr>
<td>LDH-SB1</td>
<td>Benzoate (Bz)</td>
<td>[Mg$<em>{4.5}$Al$<em>2$(OH)$</em>{13}$] (Bz)$</em>{0.58}$(CO$<em>3$)$</em>{0.71}$ · 4.10H$_2$O</td>
<td>12.3%</td>
</tr>
<tr>
<td>LDH-SB2</td>
<td>Benzoate (Bz)</td>
<td>[Mg$<em>{4.5}$Al$<em>2$(OH)$</em>{13}$] (Bz)$</em>{1.08}$(CO$<em>3$)$</em>{0.46}$ · 4.61H$_2$O</td>
<td>20.9%</td>
</tr>
<tr>
<td>LDH-SB3</td>
<td>Benzoate (Bz)</td>
<td>[Mg$<em>{4.5}$Al$<em>2$(OH)$</em>{13}$] (Bz)$</em>{1.97}$(CO$<em>3$)$</em>{0.015}$ · 3.40H$_2$O</td>
<td>34.9%</td>
</tr>
<tr>
<td>LDH-SG3</td>
<td>Gallate (Gl)</td>
<td>[Mg$_{4.5}$Al$<em>2$(OH)$</em>{13}$] (Gl)$_2$ · 0.46Gl · 8.30H$_2$O</td>
<td>43.9%</td>
</tr>
</tbody>
</table>
3.3.4. Release Study of Modified Nanoparticles

The release of antimicrobial agents was investigated by dispersing antimicrobial agent-intercalated nanoparticles in DI water at 21 °C. Calibration curves for both antimicrobial agents, relating the absorbance of the solution with concentration of the agents, were built from measurements at 225 nm and 260 nm for sodium benzoate and sodium gallate, respectively, in DI water among the expected concentration ranges. Straight lines were obtained in both cases with regression coefficients of 0.999.

Figure 3.10 and 3.11 show the release profiles for the modified nanoparticles. The percentage of released antimicrobial agent was calculated by:

\[ \text{Benzoate or Gallate released (\%)} = \left( \frac{\text{Released Amount (M_i)}}{\text{Total Amount Calculated from TGA - MS (M_0)}} \right) \times 100\% \]

Similar release profiles were observed in LDH-SB1 and LDH-SB2. The majority of benzoate anions (80% for LDH-SB1 and 55% for LDH-SB2) were released within 8 h, after which the release plateaued, with 100% of benzoate released after 30 h at a slower rate. However, LDH-SB3 showed a different release profile; a fast initial release, the so-called “burst effect” (Costantino and others 2008). This fast release might be explained by the release of benzoate taken up on the surface of the LDH platelets and those intercalated in the external part of the lamellar structure. After the fast initial release, the slower release was due to the exchange of benzoate anions which were in the internal part of the lamellar and had to diffuse through the nanoparticles.

The release of gallate from gallate-intercalated LDH was much faster than that of benzoate. The release profile showed a plateau after 4 h. The maximum percentage of gallate released was slightly higher than 100% (around 107%). This may be caused by the inaccurate evaluation from TGA-MS for the composition of LDH-SG3.
Figure 3.10. Benzoate release in DI water at 21 °C from LDH modified by sodium benzoate with different modification conditions (molar ratio of benzoate anion/NO$_3^-$; reaction temperature), LDH-SB1 (3:1; 20 °C), LDH-SB2 (18:1; 20 °C), and LDH-SB3 (18:1; 70 °C).

Figure 3.11. Gallate release in DI water at 21 °C from LDH modified by sodium gallate (LDH-SG3) under the third reaction condition (molar ratio of gallate anion/NO$_3^-$ = 18:1 and reaction temperature = 70 °C).
3.4. Conclusions

The intercalation of antimicrobial agents into layered double hydroxide nanoparticles was successfully carried out via anion exchange. The ability of antimicrobial agents to intercalate into LDH depended on the nature of the antimicrobial agents, such as size, spatial structure, and polarity. LDH can be easily loaded with benzoate anions and the amount of benzoate loaded in LDH can be controlled by reaction conditions. Benzoate had the lowest molecular volume, which made it easier to intercalate into LDH. Gallate had higher molecular volume compared to benzoate, but its higher polarity due to the three hydroxide groups made it still able but required harsher conditions to intercalate into LDH compared to benzoate. Sorbate had similar molecular volume with gallate but less polarity, which made it unable to intercalate into LDH at all. Intercalation of benzoate anions increased the interlayer distance in LDH. The arrangement of benzoate anions between the layers in LDH was affected by the loading of benzoate. The LDH-SBs in present study showed the interstratified structure. Besides interstratified structure, benzoate anions were in vertical bilayer arrangement in LDH-SB3, which had the highest loading of benzoate. Gallate anions were only in the horizontal arrangement in gallate-intercalated LDH probably due to the three hydroxide groups. These antimicrobial agents intercalated into LDH were able to be released in contact with DI water. The LDH intercalated by antimicrobial agents might be used as nano-fillers for synthetic or bio-based polymers to improve mechanical and barrier properties and meanwhile provide antimicrobial activity for active food packaging application.

3.5. References


Hwang, S.-H.; Han, Y.-S.; Choy, J.-H., Intercalation of functional organic molecules with pharmaceutical, cosmeceutical and neutraceutical functions into layered double


Khan, A. I.; Lei, L. X.; Norquist, A. J.; O'Hare, D., Intercalation and controlled release of pharmaceutically active compounds from a layered double hydroxide. *Chemical Communications* 2001, (22), 2342-2343.


Miyata, S., The Synthesis of hydrotalcite-like compounds and their structures and physico-chemical properties - 1: the systems Mg$^{2+}$-Al$^{3+}$-NO$_3^-$, Mg$^{2+}$-Al$^{3+}$-Cl$^-$, Mg$^{2+}$-Al$^{3+}$-ClO$_4^-$, Ni$^{2+}$-Al$^{3+}$-Cl$^-$ and Zn$^{2+}$-Al$^{3+}$-Cl$^-$. *Clays and Clay Minerals* 1975, 23, 369-375.


Newman, S. P.; Williams, S. J.; Coveney, P. V.; Jones, W., Interlayer arrangement of hydrated MgAl layered double hydroxides containing guest terephthalate anions:


Chapter 4

EFFECT OF LAYERED DOUBLE HYDROXIDE NANOPARTICLES AND PREPARATION METHOD ON PROPERTIES OF POLYHYDROXYBUTYRATE AND POLY(HYDROXYBUTYRATE-CO-VALERATE) FILMS

4.1. Introduction

The utilization of bio-based polymers as packaging materials has attracted great attention in both scientific and industrial areas due to the non-degradable and non-renewable nature of synthetic plastic packaging. Polyhydroxyalkanoates (PHA) represent an interesting alternative to synthetic polymers due to many advantages. Not only are they biodegradable and biocompatible, but they can also be produced by bacterial fermentation of renewable resources like cane sugar (Bordes and others 2010). Controlled fermentation of carbon feed-stock and nitrogen limitation in the presence of suitable bacteria yields up to 70% of dry cell weight (Ward and others 1977). Poly (3-hydroxybutyrate) (PHB), the most common PHA, was first discovered in 1926 by Lemoigne and is now produced on an industrial scale. Depending on the carbon substrates and the metabolism of the microorganism, different monomers, and thus (co)polymers, can be obtained. Besides the main polymer PHB, different copolyesters exist such as poly(hydroxybutyrate-co-hydroxyvalerate)(PHBV), and poly(hydroxybutyrate-co-hydroxyoctanoate) (PHBO). PHB is a highly crystalline biopolyester (above 55%) with a glass transition temperature (Tg) just above 0ºC. PHB is relatively stiff and brittle. Young’s modulus reaches 3.5 GPa, and the elongation break is less than 5% (Bordes and others 2010). The PHB melting point ($T_m = 170$ to 180 ºC) is rather high compared to other biodegradable polyesters. Its degradation temperature is close to the $T_m$ and thermal degradation occurs according to a one-step process – namely, a random chain scission reaction. Packaging materials made from PHA possess excellent film forming and coating properties. PHAs have properties close to that of polypropylene (PP) (Brandl and Puchner 1991). The properties of the film can be adjusted by changing the ratio of hydroxybutyrate (HB) and hydroxyvalerate (HV). A high content of polyhydroxybutyrate (PHB) gives a strong and stiff material whereas
polyhydroxyvalerate (PHV) has improved flexibility and toughness (Bordes and others 2010). Properties of PHBV can be improved further by using plasticizers (Kotnis and others 1995). PHAs are more hydrophobic than polysaccharide-based materials resulting in their higher moisture barrier properties. PHAs are also biodegradable in soil. However, high cost of production, brittleness, and poor gas barrier properties limit the use of PHAs (Petersen and others 1999). Several processes for producing PHA from cheap carbon sources have been developed which have been reviewed by Choi and Lee (1999). In order to improve the properties of the polymers, many methods have been used, such as chemical modification, blending with rubber or barrier polymers, coatings, adding glass fiber as micro-scale fillers, and a new method to form nanocomposite materials using nanoparticles. Nanocomposites, as a novel class of materials, have recently been extensively studied. Nanocomposites consist of a polymer matrix (bio-nanocomposite if the matrix is biopolymer) reinforced with nanoparticles having at least one dimension in the nanometer range (1-100 nm). Significant improvements including mechanical, thermal, gas-barrier, flame-retardant properties and tunable biodegradability have been observed in the many synthetic polymer-based nanocomposites due to the high aspect ratios and the high surface areas of the nanoparticles (Kojima and others 1993; Usuki and others 1993; Krishnamoorti and others 1996; Alexandre and others 2000; Leroux and Besse 2001; Ray and Okamoto 2003; Manias and others 2007). It is important to point out that all these improvements are obtained at low filler concentrations (generally lower than 5%).

Up to now layered inorganic solids like clay have attracted most attention of the packaging industry. This is not only due to their availability and low cost, but also to their significant enhancements and ease of processing. The nanoplates can either be cation exchanging or anion exchanging. Layered silicate, commonly investigated cation-exchanged clay, including montmorillonite (MMT), hectorite, and saponite, are the most common nano-fillers used in the bio-nanocomposites due to their high aspect ratio and commercial availability. PHB or PHBV/MMT nanocomposites have been prepared to improve the properties of PHB or PHBV. The exfoliated structure was not previously reported and only intercalated or well-intercalated structures and microcomposites were
obtained using organo-modified or unmodified layered silicates. Despite the fact that a
fully exfoliated structure was not obtained, the mechanical properties of the
nanocomposites in terms of tensile strength and modulus were increased with addition of
MMT and thermal properties as well as crystallization and biodegradation rates of
PHB/PHBV were also influenced by the addition of MMT (Choi and others 2003; Wang
and others 2005; Bruzaud and Bourmaud 2007; Maiti and others 2007).

Very recently, interest has being devoted to layered double hydroxides (LDHs), a
new promising class of inorganic layered materials. Layered double hydroxides (LDH),
or so-called hydrotalcite-like, are a family of layered solids with structurally positively
charged outer layers and interlayers of balancing anions (Costantino and others 1997).
The general chemical formula for LDH is written as \([M^{II}_{1-x}M^{III}_x(OH)_2]^k\)(A\(^{n-}\))\(_{x/m}\)\(y\)H\(_2\)O,
where \(M^{II}\) is a divalent metal ion, such as Mg\(^{2+}\), Ca\(^{2+}\), Zn\(^{2+}\), etc., \(M^{III}\) is a trivalent metal
ion, such as Al\(^{3+}\), Cr\(^{3+}\), Fe\(^{3+}\), Co\(^{3+}\), etc. and \(A^{n-}\) is an anion, such as CO\(_3^{2-}\), Cl\(^-\), NO\(_3^-\), etc.
(Evans and Xue 2006). Anions occupy the interlayer region of these layered crystalline
materials. They can be synthesized easily and have the advantage of various sheet
compositions with tunable interlayer species. In addition, the aspect ratio of LDH is
similar to that of MMT. There has been a limited amount of research done on PHB or
PHBV/LDH nanocomposites. Hsu and others (2007) obtained exfoliated structures using
LDH organically modified by poly(ethylene glycol) phophonates (PMLDH). Dagnonand
others (2009) investigated the PHBV/LDH nanocomposites and found improved
mechanical properties but reduced thermal stability.

For nanocomposite synthesis, polymer chains must diffuse into the galleries
between clay layers to produce structures ranging from intercalated to exfoliated.
Intercalation occurs when a small amount of polymer penetrates into the galleries,
resulting in finite expansion of the clay layers. This leads to a well-ordered multilayered
structure with a repeat distance of a few nanometers, and is observed in systems with
limited miscibility. Extensive polymer penetration leads to exfoliation or delamination of
clay layers. An exfoliated nanocomposite consists of nanometer thick platelets distributed
homogeneously throughout the polymer matrix. In contrast, when the polymer and
silicate are immiscible, the layers do not separate and exist as agglomerates or tactoids. Intercalation and exfoliation are the desirable arrangement for improving the properties of nanocomposites. Due to the hydrophilic surface properties of pristine LDH, LDH modified by organic molecules is normally required to be miscible with polymers. These modified LDH-based nanocomposites have shown improved mechanical properties, barrier properties, heat stability and flame retardancy (Nyangbo and others 2008; Costa and others 2008; Bugatti and others 2010). Moreover, the organic molecules can bring additional properties to the nanocomposites, such as controlled drug delivery, and antimicrobial activity (Costantino and others 2009; Tammaro and others 2009; San Roman and others 2013). In the present work, sodium benzoate, one of the most common food preservatives, was used as a modifier to modify LDH nanoparticles. Meanwhile, solvent casting was used to prepare PHB/PHBV films; the effect of filtration during solvent-casting was also studied due to the possible existence of impurities from fermentation process of producing PHAs. Therefore, the present work was designed to study the effect of filtration during solvent casting and LDH nanoparticles (unmodified and modified by benzoate) on mechanical and thermal properties of PHB or PHBV-based bio-nanocomposite films.

4.2. Materials and Methods

4.2.1. Materials

Un-modified nanoparticles LDH-CO$_3$ of the formula [Mg$_{4.5}$Al$_2$(OH)$_{13}$(CO$_3$)$_1$$\cdot$3.5H$_2$O was kindly provided by Sechang Co. Ltd. (Jeonbuk, Korea). Polyhydroxybutyrate (PHB) and poly(hydroxybutyrate-co-valerate) (PHBV) (PHB 88% & PHV 12%) in granulated form were purchased from Goodfellow Corporation (Coraopolis, PA, USA), sodium benzoate (SB) from J.T. Baker (Center Valley, PA, USA) and chloroform in HPLC grade from EMD Millipore (Merck KGaA, Darmstadt, Germany). All other reactants, including sodium chloride (Alfa Aesar), hydrochloric acid (VWR), and sodium nitrate (VWR), were purchased from VWR International LLC. (Philadelphia, PA, USA).
4.2.2. Nanoparticle Modification

Sodium benzoate was used to modify the nanoparticles as described in Section 3.2.2. Only LDH-SB1, which was modified under condition 1, with the molar ratio benzoate anion/NO$_3$ of 3 at room temperature (20 °C), was used in this chapter. The composition of LDH-SB1, which will be called LDH-SB here, is [Mg$_{4.5}$Al$_2$(OH)$_{13}$](Bz)$_{0.58}$(CO$_3$)$_{0.71}$·4.1H$_2$O, based on the TGA-MS data in Section 3.3.3.

4.2.3. Preparation of PHB/PHBV-LDH Composite Films

PHB/PHBV composite films containing 2 wt % unmodified nanoparticles (LDH) and modified nanoparticles (LDH-SB) were fabricated by a solvent casting technique. The polymer granules were dissolved in chloroform (20 mg/ml) by magnetic stirring for 8 h at 55 °C with reflux. The pre-calculated amount of nanoparticles to result in 2 wt % in the film was dispersed in chloroform for 24 h at room temperature (20 °C). Both polymer solution and nanoparticle solution were sonicated in an ultrasonic bath (Aquasonic Cleaner Bath, model 250D, VWR Scientific, West Chester, PA, USA) for 10 min at 35 °C. The polymer solution was either filtered using 0.45 µm filter (GMF, GD/X™ and 13mm syringe filter, Whatman, England) before mixing with nanoparticle dispersions or mixed directly without filtration. The mixture was then magnetically stirred for another 24 h at 45 °C. The resulting solution was sonicated in the ultrasonic bath for another 10 min cycle at 35 °C and then 30 ml was decanted into a glass Petri dish (100 × 15 mm). The solution was first dried in a chemical hood at room temperature (20 °C) overnight (12 h) followed by another drying cycle in an oven at 80 °C for 48 h to remove the residual solvent. The dried films were then removed from the Petri dishes and cut by a scalpel knife for characterizations.

4.2.4. Design of Experiment

Full factorial design (3 × 2) was applied in this study. The two factors were the incorporation of nanoparticles and filtration. There were three levels for the first factor, which were without nanoparticles, with 2 wt % of unmodified LDH nanoparticles, and with 2 wt % modified LDH nanoparticles. There were two levels for the second factor,
which were unfiltered and filtered. Two-way analysis of variance using general linear model procedure followed by Tukey’s multiple comparison tests at 95% confidence level was applied to study the effects of incorporation of nanoparticles and filtration on mechanical properties of the films using Minitab 15.1 (Minitab Inc., State College, PA, USA).

4.2.5. Characterizations of PHB/PHBV-LDH Composite Films

4.2.5.1. X-Ray Diffraction (XRD)

The X-ray diffraction patterns of nanoparticles and nanocomposite films were taken with an X-ray diffractometer (MiniFelix II, Rigaku Corporation, Tokyo, Japan), using Ni-filtered CuKα radiation (λ = 1.54 Å) and operating 30 kV and 15 mA. The scanning angular range was 2 – 40 ° with a sampling width of 0.02 and a scanning rate of 2 °/min.

4.2.5.2. Field Emission Scanning Electron Microscopy (FE-SEM)

The morphology of the fracture surface (cross-sectional surface) of the films was visualized using a field emission scanning electron microscope (JEOL 6700F, Japan Electron Optics Ltd., Tokyo, Japan) operating at 3 kV. Small pieces of the films were frozen in liquid nitrogen, cut using a sharp razor blade, and mounted on specimen stubs with 2 sided carbon tape. The fracture surfaces of the films were sputter-coated with a thin layer (~ 8-10 nm) of gold-palladium (Au-Pd) using a sputter-coater.

4.2.5.3. Mechanical Properties

A Texture Analyzer TA.XT2i (Texture Technologies Corporation, USA) was used to determine the ultimate tensile strength (UTS), the elastic modulus (EM) and the elongation at break (EL) of films. The tests were performed according to the Standard Test Method for tensile properties of thin plastic sheeting, ASTM D-882-02 (ASTM 2002). Film samples were cut into strips by a scalpel knife with a width of 10 mm and a length of 50 mm (Han and Floros 1997). Initial grip separation and crosshead speed were set to 20 mm and 0.1 mm/s, respectively. UTS (MPa) is the maximum tensile strength that a film can sustain and was calculated as the ratio of the maximum load and the
original minimum cross sectional area of the specimen. Elastic modulus (GPa) is the ratio of stress to strain over the linear part of stress-strain curve and it is a measure of film stiffness (Ozdemir and Floros 2008). Percentage of EL is the percent ratio between the extended length at the moment of rupture and the initial length.

4.2.5.4. Thermo-mechanical Properties

The thermo-mechanical properties of films were determined using a dynamic mechanical analyzer (Q800, TA Instruments, New Castle, Del., USA). Dynamic mechanical analysis (DMA) was performed in tension mode at a frequency of 1 Hz and 0.15% strain. The length and width of the film samples were around 20 (±1) mm and 8 (±1) mm, respectively. The samples were heated from -50 to 70 °C at a heating rate 2 °C/min. Storage modulus (E'), loss modulus (E'″), and loss tangent (E''/E') were recorded as a function of temperature. The peak value of tan δ was defined as apparent glass transition temperature (T_{g,app}) to distinguish from the glass transition temperature (T_g) measured by DSC.

4.2.5.5. Thermal Properties

Differential scanning calorimetry (DSC) was performed with a DSC Q100 (TA Instruments, New Castle, Del., USA). The sample in sealed platinum pan was instantaneously heated from room temperature to 170 °C and soaked for 5 minutes, followed by cooling to -80 °C at 5 °C/min, then re-heated from -80 °C to 190 °C at 10 °C/min. DSC experiments were performed under a nitrogen environment using ca. 3 mg sample. Melting points were determined during the re-heating cycle, crystallization temperatures (onset and peak temperature) were determined during the cooling cycle. Heat of fusions was measured during both re-heating and cooling.

4.2.5.6. Thermal Stability

The thermal stability of films was investigated using a thermogravimetric analyzer Q500 (TA Instruments, New Castle, Del., USA). The sample in platinum pan was heated from room temperature to 800 °C at a heating rate of 10 °C/min under air flow. Weight loss of the sample was measured as function of temperature. Three parameters were
determined from the TGA data: the temperature at 10% weight loss, the temperature at 50% weight loss, and the yield of charred residue at 800 °C.

4.3. Results and Discussion

4.3.1. Investigation of Morphology by XRD

Figure 4.1 and 4.2 show XRD patterns of filtered and unfiltered PHB films with unmodified and modified LDH. Several more peaks appeared in unfiltered PHB films than filtered PHB films, which indicated that filtration may be reducing impurities in the PHB granules. The impurities may be the organic residues (cell debris and membrane lipids) from the fermentation process. The XRD patterns of PHB composites containing LDH show the typical reflections of crystalline PHB, corresponding to the orthorhombic unit cell (Kunioka and others 1989; Galego and others 2000). Besides these reflections, PHB-LDH films also showed the reflections due to existence of LDH. The presence of the peak 2θ = 11.6°, corresponding to an interlayer distance of 0.76 nm, indicated that neither exfoliated nor intercalated morphology was formed. The XRD pattern of LDH-SB showed two new peaks compared to that of LDH in the 2θ range of 2° to 10°, indicating benzoate anions were intercalated into the nanolayers. However, these two peaks were absent in PHB-LDH_SB films. Due to the limitation of the XRD instrument, diffraction patterns of 2θ values smaller than 2° could not be observed. Several causes for the absence of these two peaks can be postulated: 1) the nanolayers were disordered or exfoliated in the polymer, 2) the intensity of the two peaks was too low at the low concentration of the nanoparticles in the film, and 3) the distance between nanolayers was large and beyond the instrument reading below 2°.

XRD patterns of filtered and unfiltered PHBV films with unmodified and modified LDH are shown in Figure 4.3 and 4.4, respectively. There was no difference of XRD patterns between unfiltered and filtered PHBV films. XRD patterns of PHBV composites containing nanoparticles had a trend similar to that discussed above for PHB composites. Phase-separated morphology was formed in PHBV with unmodified LDH. Intercalation or even exfoliated morphology may be formed in PHBV with LDH-SB.
Figure 4.1. XRD patterns of filtered (F) and unfiltered (UF) PHB films with 2% of unmodified LDH (LDH-CO$_3$). The number indicates the distance between nanolayers.

Figure 4.2. XRD patterns of filtered (F) and unfiltered (UF) PHB films with 2% of LDH modified by benzoate (LDH-SB). The number indicates the distance between nanolayers.
Figure 4.3. XRD patterns of filtered (F) and unfiltered (UF) PHBV films with 2\% of unmodified LDH (LDH-CO$_3$). The number indicates the distance between nanolayers.

Figure 4.4. XRD patterns of filtered (F) and unfiltered (UF) PHBV films with 2\% of LDH modified by benzoate (LDH-SB). The number indicates the distance between nanolayers.
4.3.2. Investigation of Morphology by FE-SEM

Figure 4.5. SEM images of PHB/PHBV composite films with 2% of unmodified LDH or LDH modified by sodium benzoate (LDH-SB) films with filtration.
Figure 4.6. SEM images of the filtered PHBV film with 2% of LDH modified by sodium benzoate at (a) low magnification and (b) high magnification. The white arrows indicate the nanoparticles.

Figure 4.5 presents the SEM micrographs from the fractured surface (cross-sectional surface) of filtered PHB/PHBV films containing unmodified and modified LDH
in order to study the morphology of the polymer/nanoparticle composite. It is evident that the morphology of the PHB film was different from that of the PHBV film. The morphologies of PHB films with unmodified or modified LDH are similar to that of virgin PHB. For the PHBV film with LDH, additional phases displaying particles or aggregates were discernible from the SEM images, suggesting that LDH dispersed in the polymer matrix as platelets not as delaminated nanolayers. Kumar and others (2010) reported that the white strands in the SEM images corresponded to nanolayers (Kumar and others 2010). Although aggregates were still seen in the SEM images for the PHBV-LDH_SB film, dispersed nanolayers can be clearly seen as well, especially in the SEM image at high magnification (shown by white arrows in Figure 4.6), which indicated the mixed exfoliated and phase-separated morphology of PHBV-LDH_SB may be formed. The result agreed with data from XRD patterns.

4.3.3. Mechanical Properties

Table 4.1. Mechanical properties of filtered (F) or unfiltered (UF) PHB films with 2% of unmodified LDH or LDH modified by sodium benzoate

<table>
<thead>
<tr>
<th>Sample</th>
<th>UTS(MPa)</th>
<th>EM (GPa)</th>
<th>EL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHB-UF</td>
<td>8.79±1.39(a)</td>
<td>0.79±0.08(a)</td>
<td>1.46±0.55(a)</td>
</tr>
<tr>
<td>PHB-F</td>
<td>8.78±0.64(a)</td>
<td>0.76±0.08(a)</td>
<td>1.70±0.17(a)</td>
</tr>
<tr>
<td>PHB-LDH-UF</td>
<td>9.12±1.29(a)</td>
<td>0.98±0.15(a)</td>
<td>1.07±0.06(a)</td>
</tr>
<tr>
<td>PHB-LDH-F</td>
<td>9.85±1.15(a)</td>
<td>0.93±0.13(a)</td>
<td>1.69±0.33(a)</td>
</tr>
<tr>
<td>PHB-LDH_SB-UF</td>
<td>9.93±1.31(a)</td>
<td>0.86±0.09(a)</td>
<td>1.69±0.29(a)</td>
</tr>
<tr>
<td>PHB-LDH_SB-F</td>
<td>8.80±0.67(a)</td>
<td>0.77±0.03(a)</td>
<td>1.79±0.19(a)</td>
</tr>
</tbody>
</table>

Values are the mean ± one standard deviation. Means in the same column followed by the same letter are not significantly different \((P > 0.05)\). UTS: ultimate tensile strength; EM: elastic modulus; EL: elongation at break.

The effect of filtration and incorporation of nanoparticles on mechanical properties of PHB-LDH films is shown in Table 4.1. No significant effect of filtration and type of nanoparticles was found for PHB films at 95% confidence level \((P = 0.63\) for tensile strength, \(P = 0.09\) for elastic modulus, and \(P = 0.11\) for elongation at break).
Table 4.2. Mechanical properties of filtered (F) or unfiltered (UF) PHBV films with 2% of unmodified LDH or LDH modified by sodium benzoate

<table>
<thead>
<tr>
<th>Sample</th>
<th>UTS (MPa)</th>
<th>EM (GPa)</th>
<th>EL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHBV-UF</td>
<td>16.79±0.96</td>
<td>1.25±0.02</td>
<td>2.42±0.61</td>
</tr>
<tr>
<td>PHBV-F</td>
<td>18.49±1.07</td>
<td>1.38±0.33</td>
<td>2.80±1.85</td>
</tr>
<tr>
<td>PHBV-LDH-UF</td>
<td>19.09±3.16</td>
<td>1.15±0.16</td>
<td>4.68±0.36</td>
</tr>
<tr>
<td>PHBV-LDH-F</td>
<td>20.23±0.86</td>
<td>1.07±0.07</td>
<td>5.75±0.71</td>
</tr>
<tr>
<td>PHBV-LDH_SB-UF</td>
<td>25.18±1.21</td>
<td>1.35±0.06</td>
<td>5.30±1.20</td>
</tr>
<tr>
<td>PHBV-LDH_SB-F</td>
<td>29.65±3.51</td>
<td>1.46±0.19</td>
<td>4.70±0.53</td>
</tr>
</tbody>
</table>

Values are the mean ± one standard deviation. Means in the same column followed by the same letter are not significantly different (\( P > 0.05 \)). UTS: ultimate tensile strength; EM: elastic modulus; EL: elongation at break.

Mechanical properties of filtered or unfiltered PHBV films with unmodified or modified LDH are reported in Table 4.2. Two-way ANOVA showed that both filtration and incorporation of nanoparticles had significant effect on the tensile strength at 95% confidence level (\( P = 0.03 \) for filtration and \( P = 0.001 \) for incorporation), and only incorporation of nanoparticles had significant effect on elongation at break at 95% confidence level (\( P = 0.001 \) for incorporation and \( P = 0.57 \) for filtration) and on elastic modulus (\( P = 0.03 \) for incorporation and \( P = 0.54 \) for filtration). The tensile strength was significantly increased after incorporation of nanoparticles. Furthermore, modified nanoparticles had stronger effect on tensile strength than unmodified nanoparticles. In particular, the tensile strength of PHBV filtered films was increased by 21% and 60% with incorporation of LDH and LDH_SBP, respectively. The elongation at break was increased after incorporation of nanoparticles. But modified nanoparticles did not show a stronger effect on elongation at break than unmodified nanoparticles.

4.3.4. Thermo-mechanical Properties

Figures 4.7 and 4.8 show the storage modulus (\( E' \)) and tan δ curves of filtered and unfiltered PHB films with unmodified and modified LDH. The storage modulus corresponds to the elastic response of the deformation and tan δ, that is, the ratio of
storage modulus / loss modulus, is useful for determining the occurrence of molecular mobility transition such as the glass transition temperature (Alexandre and Dubois 2000). The filtration and incorporation of nanoparticles did not show an effect on the $T_g^{\text{app}}$ (the peak of the tan δ curve) of PHB films. Surprisingly, all the filtered PHB films had lower $E'$ than corresponding unfiltered PHB films, which did not agree with the tensile strength result. For the unfiltered PHB films, the incorporation of unmodified nanoparticles did not change $E'$ above the $T_g^{\text{app}}$ (Figure 4.7 a&c) but the incorporation of modified nanoparticles increased the storage modulus over the entire temperature range (Figure 4.7 a&e). However, for the filtered PHB films, the incorporation of unmodified or modified nanoparticles decreased $E'$ over the entire temperature range. The specific reason of reduction in storage modulus for the filtered PHB/LDH is not clear at present and needs to be further investigated.

Figure 4.7. Effect of temperature on storage modulus of filtered (F) or unfiltered (UF) PHB films with 2% of unmodified LDH or LDH modified by sodium benzoate.
Figures 4.9 and 4.10 show the storage modulus (E’) and tan δ curves of unfiltered and filtered PHBV films with unmodified and modified LDH. Filtration had no effect on the E’ of PHBV films, but the incorporation of nanoparticles increased the storage modulus of PHBV films. PHBV-LDH_SB film had even higher E’ than PHBV-LDH film, which agreed with the tensile strength result. It can be seen from Figure 4.10 that there is no shift in T_g^app with incorporation of nanoparticles. Increases in T_g^app had been attributed to attractive interaction between the filler and the polymer; depression of T_g^app was found when the interface between the filler and polymer was weak (Arrighi and others 2003; Xie and others 2009). Since T_g^app did not change, there were no strong attractive or repulsive interactions between LDH or LDH-SB and PHBV.

Figure 4.8. Effect of temperature on tan δ of filtered (F) or unfiltered (UF) PHB films with 2% of unmodified LDH or LDH modified by sodium benzoate.
Figure 4.9. Effect of temperature on storage modulus of filtered (F) or unfiltered (UF) PHBV films with 2% of unmodified LDH or LDH modified by sodium benzoate.

Figure 4.10. Effect of temperature on tan δ of filtered (F) or unfiltered (UF) PHBV films with 2% of unmodified LDH or LDH modified by sodium benzoate.
4.3.5. Thermal Properties

DSC cooling and heating cycles of filtered or unfiltered PHB/PHBV films with unmodified or modified LDH are shown in Figures 4.11 to 4.14. The thermal properties of PHB/PHBV films, including crystallization onset temperature ($T_{c\text{onset}}$), crystallization peak temperature ($T_{c\text{peak}}$), crystallization enthalpy ($\Delta H_{mc}$), melting temperature ($T_m$), and melting enthalpy ($\Delta H_m$), are summarized in Table 4.3 and 4.4, respectively. The $T_m$ and $\Delta H_m$ values of PHB were higher than those of PHBV, which agreed with the results reported by Kunioka and others (1989).

DSC cooling cycle curves showed that unfiltered PHB films had higher $T_c$ than filtered PHB films. The impurities may exist in unfiltered PHB films from XRD results; therefore, the higher $T_c$ of unfiltered PHB films may be due to these impurities acting as nuclei resulting in enhancement of the nucleation step. The DSC curve for crystallization of unfiltered PHB films (cooling cycle) indicated only a small variation with the addition of nanoparticles, although it seems that the effect of nanoparticles on crystallization was slightly stronger in filtered PHB than in unfiltered films. This small variation of $T_c$ among PHB composites may due to a limited nucleation effect of nanoparticles.

XRD patterns of PHBV films (Figure 4.3 and 4.4) showed no change in the crystalline structure of PHBV, indicating both filtration and addition of nanoparticles did not modify the crystalline phase. Unlike PHB films, DSC cooling cycle curves showed that unfiltered and filtered pure PHBV films had the same $T_c$. However, for unfiltered PHBV, the addition of nanoparticles increased $T_c$, while, for filtered PHBV, the addition of nanoparticles decreased $T_c$. 
Figure 4.11. DSC cooling cycle of filtered (F) or unfiltered (UF) PHB films with 2% of unmodified LDH or LDH modified by sodium benzoate.

Figure 4.12. DSC heating cycle of filtered (F) or unfiltered (UF) PHB films with 2% of unmodified LDH or LDH modified by sodium benzoate.
Figure 4.13. DSC cooling cycle of filtered (F) or unfiltered (UF) PHBV films with 2% of unmodified LDH or LDH modified by sodium benzoate.

Figure 4.14. DSC heating cycle of filtered (F) or unfiltered (UF) PHBV films with 2% of unmodified LDH or LDH modified by sodium benzoate.
The melting process presents a more complex behavior. The melting peak at 50 °C showed in all PHB composites, but it is uncertain what resulted in such melting peak at this moment and needed to be further investigated. The unfiltered PHB showed a single melting peak, however, the filtered PHB and both unfiltered and filtered PHBV showed two melting peaks. The double melting endotherms have been reported by several groups and two different theories have been applied to explain this behavior. The first one theorized that it was caused by melting-recrystallization-melting behavior during heating scans (Hsu and others 2007; Bordes and others 2010). The others attributed this effect to the existence of bimodal size of crystallite or different types of crystallites (Wang and others 2005; Dagnon and others 2009). In order to explain this behavior, a study using DSC was conducted. Pure PHBV was first heated rapidly to 170 °C and soaked for 5 min to destroy the crystal history and then it was cooled to -80 °C at the same rate (5 °C/min). Finally, the sample was re-heated up from -80 °C to 190 °C at different rates (1, 5, 10 and 20 °C/min). Figure 4.15 shows the DSC heating cycle of pure PHBV with different heating rates. If the double melting endotherms are due to the bimodal size of crystallite or different types of crystallites, then these two melting peaks should be seen with different heating rates. However, as shown in Figure 4.15, at high heating rate of 20 °C/min, only one melting peak was observed, and two melting peaks were observed at lower heating rates, and the second melting peak becomes more predominant with the lower heating rate. This result suggested that the double melting endotherms behavior in the present case was due to the recrystallization phenomenon. The \( T_{m1} \) and \( T_{m2} \) can be attributed to the melting of the primary crystallites formed during the cooling scan and the melting of the recrystallized crystallites which were formed during the heating process, respectively. The glass transition temperature of PHBV was determined at heating rate of 20 °C/min, which was -2.6 °C. The value is close to that reported by others (Avella and others 2000; Jiang and others 2008), but it is lower than the apparent glass transition temperature determined from the peak of tan \( \delta \) in DMA, which was 13.85 °C.

As shown in Tables 4.3 and 4.4, \( T_{m1} \) and \( T_{m2} \) did not change for unfiltered PHB/PHBV LDH composites while shifted to the lower temperatures for filtered ones.
compared to the pure PHB/PHBV. The shifting of melting points in other nano-
biocomposites systems had been ascribed to nanoparticles hindering crystal growth (Di
Maio and others 2004; Chivrac and others 2007; Hsu and others 2007; Xie and others
2009; Bordes and other 2010; Dagnon and others 2012). Further investigation will be
necessary to conclude whether the shifting of the melting points in PHB/PHBV-LDH
composite systems is caused by the hindrance of crystal growth from nanoparticles.
Moreover, it was pointed out the change in the ratio of the melting peak intensities with
the addition of nanoparticles: $T_{m2}$ peak intensity became preponderant in polymer LDH
composites in comparison with pure polymers (Bordes and others 2010). This behavior,
attesting to more recrystallization during melting, was attributed to the enhancing
nucleation effect of nanoparticles on the recrystallization phenomenon.

Table 4.3. Thermal properties of filtered (F) or unfiltered (UF) PHB films with 2% of
unmodified LDH or LDH modified by sodium benzoate

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cooling Cycle</th>
<th>Heating Cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$T_c$ onset</td>
<td>$T_c$ peak</td>
</tr>
<tr>
<td>PHB-UF</td>
<td>115.6</td>
<td>111.2</td>
</tr>
<tr>
<td>PHB-F</td>
<td>110.8</td>
<td>103.4</td>
</tr>
<tr>
<td>PHB-LDH-UF</td>
<td>116.4</td>
<td>111.8</td>
</tr>
<tr>
<td>PHB-LDH-F</td>
<td>111.1</td>
<td>103.6</td>
</tr>
<tr>
<td>PHB-LDH_SB-UF</td>
<td>115.3</td>
<td>111.0</td>
</tr>
<tr>
<td>PHB-LDH_SB-F</td>
<td>107.0</td>
<td>98.4</td>
</tr>
</tbody>
</table>

$T_c$ onset: crystallization onset temperature; $T_c$ peak: crystallization peak temperature; $\Delta H_{mc}$:
crystallization enthalpy; $T_m1$ and $T_m2$: melting temperature at first and second peak, respectively; and $\Delta H_m$: melting enthalpy.
Table 4.4. Thermal properties of filtered (F) or unfiltered (UF) PHBV films with 2% of unmodified LDH or LDH modified by sodium benzoate

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cooling Cycle</th>
<th></th>
<th>Heating Cycle</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( T_c ) onset (°C)</td>
<td>( T_c ) peak (°C)</td>
<td>( \Delta H_{mc} ) (J/g)</td>
<td>( T_m1 ) (°C)</td>
</tr>
<tr>
<td>PHBV-UF</td>
<td>106.5</td>
<td>101.1</td>
<td>73.4</td>
<td>147.1</td>
</tr>
<tr>
<td>PHBV-F</td>
<td>106.8</td>
<td>101.4</td>
<td>82.2</td>
<td>146.3</td>
</tr>
<tr>
<td>PHBV-LDH-UF</td>
<td>107.8</td>
<td>102.7</td>
<td>79.2</td>
<td>144.3</td>
</tr>
<tr>
<td>PHBV-LDH-F</td>
<td>104.1</td>
<td>97.4</td>
<td>70.6</td>
<td>143.5</td>
</tr>
<tr>
<td>PHBV-LDH_SB-UF</td>
<td>109.4</td>
<td>104.5</td>
<td>71.5</td>
<td>144.5</td>
</tr>
<tr>
<td>PHBV-LDH_SB-F</td>
<td>98.6</td>
<td>86.8</td>
<td>80.6</td>
<td>142.4</td>
</tr>
</tbody>
</table>

\( T_c \) onset: crystallization onset temperature; \( T_c \) peak: crystallization peak temperature; \( \Delta H_{mc} \): crystallization enthalpy; \( T_m1 \) and \( T_m2 \): melting temperature at first and second peak, respectively; and \( \Delta H_m \): melting enthalpy.

Figure 4.15. DSC heating cycle of unfiltered PHBV film (PHBV-UF) with different heating rates.
4.3.6. Thermal Stability

Thermogravimetric analyses were performed to determine the influence of filtration and LDH nanoparticles on the thermal behavior of PHB/PHBV matrices. The PHB/PHBV degradation occurred according to a non-radical, random chain scission reaction just above the melting temperature (Liu and others 2009). Figures 4.16 and 4.17 show the typical TGA patterns for PHB and PHBV. The TGA patterns indicated that there was only one single weight loss between 230 °C and 350 °C during the thermal degradation of PHB and PHBV-based LDH composites. The temperature at 10% weight loss in both PHB and PHBV polymers shifted to lower temperatures after addition of nanoparticles, indicating the reduction of thermal stability. This may be due to the action of the bonding water in nanoparticles on the ester bonds giving rise to a base-catalyzed decomposition of the polymer. The filtration also reduced the thermal stability of PHB and PHBV. This may be due to impurities in the unfiltered polymers. These impurities could be other PHAs with higher molecular weight or impurities from fermentation processing, such as other polymers from cell walls of microorganisms. The yield of charred residue at 800 °C was also reported. The residue increased with addition of LDH nanoparticles, and the extra residue was attributed to the metal oxides of the LDH.
Figure 4.16. TGA patterns of filtered (F) or unfiltered (UF) PHB films with 2% of unmodified LDH or LDH modified by sodium benzoate.

Table 4.5. Thermal stability of filtered (F) or unfiltered (UF) PHB films with 2% of unmodified LDH or LDH modified by sodium benzoate

<table>
<thead>
<tr>
<th>Sample</th>
<th>$T_{10%}$ (°C)</th>
<th>$T_{50%}$ (°C)</th>
<th>Residue (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHB-UF</td>
<td>264.7</td>
<td>278.6</td>
<td>2.48</td>
</tr>
<tr>
<td>PHB-F</td>
<td>251.2</td>
<td>270.7</td>
<td>0.18</td>
</tr>
<tr>
<td>PHB-LDH-UF</td>
<td>252.0</td>
<td>268.5</td>
<td>3.60</td>
</tr>
<tr>
<td>PHB-LDH-F</td>
<td>235.7</td>
<td>250.8</td>
<td>1.32</td>
</tr>
<tr>
<td>PHB-LDH_SB-UF</td>
<td>253.3</td>
<td>266.2</td>
<td>4.42</td>
</tr>
<tr>
<td>PHB-LDH_SB-F</td>
<td>233.6</td>
<td>250.5</td>
<td>1.26</td>
</tr>
</tbody>
</table>

$T_{10\%}$: temperature at 10% weight loss; $T_{50\%}$: temperature at 50% weight loss; and Residue: yield of charred residue at 800 °C.
Figure 4.17. TGA patterns of filtered (F) or unfiltered (UF) PHBV films with 2% of unmodified LDH or LDH modified by sodium benzoate.

Table 4.6. Thermal stability of filtered (F) or unfiltered (UF) PHBV films with 2% of unmodified LDH or LDH modified by sodium benzoate

<table>
<thead>
<tr>
<th>Sample</th>
<th>$T_{10%}$ (°C)</th>
<th>$T_{50%}$ (°C)</th>
<th>Residue (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHBV-UF</td>
<td>250.1</td>
<td>267.6</td>
<td>0.66</td>
</tr>
<tr>
<td>PHBV-F</td>
<td>238.7</td>
<td>257.1</td>
<td>0.15</td>
</tr>
<tr>
<td>PHBV-LDH-UF</td>
<td>214.9</td>
<td>229.5</td>
<td>2.19</td>
</tr>
<tr>
<td>PHBV-LDH-F</td>
<td>216.3</td>
<td>229.7</td>
<td>2.04</td>
</tr>
<tr>
<td>PHBV-LDH_SB-UF</td>
<td>215.1</td>
<td>232.4</td>
<td>1.48</td>
</tr>
<tr>
<td>PHBV-LDH_SB-F</td>
<td>216.6</td>
<td>230.4</td>
<td>1.05</td>
</tr>
</tbody>
</table>
4.4. Conclusions

PHB and PHBV-based LDH composite films were prepared by the solvent casting technique with 2 wt% of unmodified LDH or LDH modified by sodium benzoate. Intercalated or partial exfoliated structures were obtained using modified LDH, however, only the phase-separated structure was formed using unmodified LDH. Filtration and the presence of nanoparticles significantly increased the tensile strength and elongation at break of PHBV films, but did not show significant effect on PHB films. The incorporation of 2 wt% modified LDH further increased the tensile strength in comparison with unmodified LDH, which may be due to the increased compatibility between PHBV and nanoparticles and larger basal distance between nanolayers after modification. Moreover, thermal properties as well as crystallization were affected by the filtration and addition of nanoparticles (unmodified or modified LDH) for both PHB and PHBV. Impurities in unfiltered PHB enhanced the nucleating step resulting in an increase of crystallization temperature compared to the filtered one. LDH nanoparticles enhanced recrystallization during melting, which may due to the nucleation effect by nanoparticles. Thermal stability was reduced with the addition of LDH. The incorporation of LDH affected the PHBV matrix more than PHB films in terms of mechanical and thermal properties.

The results obtained in this work showed that mechanical properties of PHBV films were increased with low concentrations (2 wt%) of unmodified and modified LDH, which may extend the application of this bio-based polymer. Moreover, the modifier, sodium benzoate, can also bring additional functionality for the bio-based nanocomposites. In addition to sodium benzoate, LDH can also be modified by a large number of other active components. Therefore, these new hybrid materials with additional functions can be used for a series of applications, such as antimicrobial food packaging and controlled drug delivery. The effect of concentrations and types of the LDH nanoparticles on the mechanical and thermal properties of PHBV films as well as barrier properties will be further investigated in Chapter 5. The release kinetics of the active component will be studied in Chapter 6.
4.5. References


Han, J. H.; Floros, J. D., Casting antimicrobial packaging films and measuring their physical properties and antimicrobial activity. *Journal of Plastic Film & Sheeting* 1997, 13, (4), 287-298.


Chapter 5
EFFECT OF TYPES AND CONCENTRATIONS OF MODIFIED LDH NANOPARTICLE ON PROPERTIES OF POLY(HYDROXYBUTYRATE-CO-VALERATE) FILMS

5.1. Introduction

The utilization of bio-based polymers as packaging materials has attracted great attention in both scientific and industrial areas due to the non-degradable and non-renewable nature of synthetic plastic packaging. Polyhydroxyalkanoates (PHA) represent an interesting alternative to synthetic polymers due to many advantages. Not only are they biodegradable and biocompatible, but they can also be produced by bacterial fermentation from renewable resources like cane sugar (Bordes and others 2010). Controlled fermentation of carbon feedstock and nitrogen limitation in the presence of suitable bacteria yields up to 70% of dry cell weight (Ward and others 1977). Poly (3-hydroxybutyrate) (PHB), the most common PHA, was first discovered in 1926 by Lemoigne and is now produced on an industrial scale. Depending on the carbon substrates and the metabolism of the microorganism, different monomers, and thus (co)polymers, can be obtained. Besides the main polymer PHB, different copolyesters exist such as poly(hydroxybutyrate-co-hydroxyvalerate)(PHBV), and poly(hydroxybutyrate-co-hydroxyoctanoate) (PHBO). In Chapter 4, the properties of two kinds of PHA, PHB and PHBV, were studied. PHBV film was easier to prepare by solvent casting technique than PHB film, and it has higher tensile strength and elongation at break for food packaging applications, meanwhile the incorporation of LDH nanoparticles affected the PHBV matrix more than PHB films in terms of mechanical and thermal properties. Therefore, in this chapter, PHBV will be the only polymer matrix to be investigated. Based on the results in Chapter 4, the effect of types and concentrations of LDH nanoparticles on the structure and properties of PHBV films will be further investigated in this chapter. Many studies have been conducted to investigate the effect of type and content of nanoparticles on the structure and properties of synthetic or bio-polymer based nanocomposites (Usuki and others 1993; Kojima and others 1993;
Krishnamoorti and others 1996; Alexandre and others 2000; Leroux and Besse 2001; Ray and Okamoto 2003; Manias and others 2007; Sorrentino and others 2007; Kumar and others 2010). Significant improvements including mechanical, thermal, gas-barrier, flame-retardant properties and tunable biodegradability have been observed, when compared with the neat polymers and conventional (micro-scale) composites. In particular, these nanocomposites show great promise in providing excellent barrier properties, because the impermeable nanoplates mandate a tortuous pathway for permeates to diffuse through (Neilsen 1967; Bharadwaj 2001; Sorrentino and others 2006; Sorrentino and others 2007).

For nanocomposite synthesis, polymer chains must diffuse into the galleries between clay layers to produce structures ranging from intercalated to exfoliated. Due to the hydrophilic surface properties of pristine LDH, modification of LDH nanoparticles by organic molecules is critical to make them miscible with polymers and thus results in better improvement of properties. The modified LDH-based nanocomposites have improved mechanical properties, barrier properties, heat stability and flame retardancy (Nyambo and others 2008; Costa and others 2008; Bugatti and others 2010). Moreover, the organic molecules can bring additional properties to the nanocomposites, such as use in controlled drug delivery, and antimicrobial properties (Costantino and others 2009; Tammaro and others 2009; San Roman and others 2013). Based on the results in Chapter 4, LDH modified by benzoate anions result in higher compatibility between nanoparticles and PHBV. Therefore, the modified LDH with different concentrations of benzoate anions will be further studied in this chapter. Besides using sodium benzoate as a modifier, another antimicrobial agent, sodium gallate, was used to modify LDH nanoparticles. Compared to the benzoate anion, the gallate anion has three more hydroxide groups on the benzene ring. Gallic acid, (3,4,5-trihydroxybenzoic acid) is a natural product of the hydrolysis of tannins. It is one of the main phenolic components present in black tea and also exists in natural products such as gallnut, sumac and other plants. It has been reported to possess anti-mutagenic, anti-carcinogenic and antimicrobial functions (Zhao and others 1997; Zuo and others 2002; Strlic and others 2002; Abdelwahed and others 2007; Chanwitheesuk and others 2007). Gallate anions
have been successfully intercalated into Zn/Al LDH in parallel orientation with 42.2% gallate anions loaded (w/w) by Ghotbi and Hussein (2010). Therefore, the present work is designed to study the effect of types (unmodified and LDH modified by sodium benzoate and sodium gallate) and concentrations of LDH nanoparticles on mechanical, thermal and barrier properties of PHBV-based bio-nanocomposite films.

5.2. Materials and Methods

5.2.1. Materials

Unmodified nanoparticles LDH-CO$_3$ of the formula $[\text{Mg}_{4.5}\text{Al}_2\text{(OH)}_{13}]\text{(CO}_3\text{)}_1\cdot3.5\text{H}_2\text{O}$ was kindly provided by Sechang Co. Ltd. (Jeonbuk, Korea). Poly(hydroxybutyrate-co-valerate) (PHBV) (PHB 88% & PHV 12%) in granulated form was purchased from Goodfellow Corporation (Coraopolis, PA, USA), sodium benzoate (SB) from J.T. Baker (Center Valley, PA, USA), gallic acid from Sigma-Aldrich (St. Louis, MO, USA), and chloroform in HPLC grade from EMD Millipore (Merck KGaA, Darmstadt, Germany). All other reactants, including sodium chloride (Alfa Aesar), hydrochloric acid (VWR), sodium nitrate (VWR), and sodium hydroxide (BDH Chemicals), were purchased from VWR International LLC. (Philadelphia, PA, USA).

5.2.2. Nanoparticle Modification

Sodium benzoate and sodium gallate were used to modify the nanoparticles as described in section 3.2.2. The compositions of nanoparticles used in this chapter are shown in Table 5.1. Since there is only one type of LDH modified by sodium gallate, the LDH-SG3 in Chapter 3 is named as LDH-SG in this chapter.

5.2.3. Preparation of PHBV-LDH Composite Films

PHBV composite films containing various concentrations of unmodified nanoparticles (LDH) and modified nanoparticles (LDH-SB or LDH-SG) were fabricated by a solvent casting technique. The polymer granules were dissolved in chloroform (20 mg/ml) by magnetic stirring for 8 h at 55 °C with reflux. The pre-calculated amount of
nanoparticles to result in proper concentrations in the film was dispersed in chloroform for 24 h at room temperature (20 °C). Both polymer solution and nanoparticle solution were sonicated in an ultrasonic bath (Aquasonic Cleaner Bath, model 250D, VWR Scientific, West Chester, PA, USA) for 10 min at 35 °C. The polymer solution was directly mixed with nanoparticle dispersion. The mixture was then magnetically stirred for another 24 h at 45 °C. The resulting solution was sonicated in the ultrasonic bath for another 10 min cycle at 35 °C and then 30 ml was decanted into a glass Petri dish (100 × 15 mm). The solution was first dried in a chemical hood at room temperature (20 °C) overnight (12 h) followed by another drying cycle in an oven at 80 °C for 48 h to remove the residual solvent. The dried films were then removed from the Petri dishes and cut by a scalpel knife for characterizations. The LDH concentrations of the produced films were 2, 5, 10, 15, and 20% named PHBV-LDH-2%, 5%, 10%, 15%, and 20%, respectively. The LDH-SB concentrations of the produced films were 2, 5, and 10% named PHBV-LDH_SB1-2%, 5% and 10%; PHBV-LDH_SB2-2%, 5% and 10%; and PHBV-LDH_SB3-2%, 5% and 10%, respectively. The LDH-SG concentrations of the produced films were 2, 5, and 10% named PHBV-LDH_SG-2%, 5% and 10%, respectively. The control is the PHBV film with no nanoparticles.

Table 5.1. Composition of nanoparticles containing various anions

<table>
<thead>
<tr>
<th>Sample</th>
<th>Intercalated Anion</th>
<th>Composition</th>
<th>wt % of Anion</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH-CO₃</td>
<td>Carbonate</td>
<td><a href="CO%E2%82%83">Mg₄.₅Al₂(OH)₁₃</a>₁·3.₅H₂O</td>
<td>11.9%</td>
</tr>
<tr>
<td>LDH-SB1</td>
<td>Benzoate (Bz)</td>
<td><a href="Bz">Mg₄.₅Al₂(OH)₁₃</a>₀.₅₈(CO₃)₀.₇₁·4.₁₀H₂O</td>
<td>12.₃%</td>
</tr>
<tr>
<td>LDH-SB2</td>
<td>Benzoate (Bz)</td>
<td><a href="Bz">Mg₄.₅Al₂(OH)₁₃</a>₁.₀₈(CO₃)₀.₄₆·₄.₆₁H₂O</td>
<td>20.₉%</td>
</tr>
<tr>
<td>LDH-SB₃</td>
<td>Benzoate (Bz)</td>
<td><a href="Bz">Mg₄.₅Al₂(OH)₁₃</a>₁.₉₇(CO₃)₀.₀₁₅·₃.₄₀H₂O</td>
<td>34.₉%</td>
</tr>
<tr>
<td>LDH-SG</td>
<td>Gallate (Gl)</td>
<td><a href="Gl">Mg₄.₅Al₂(OH)₁₃</a>₂·0.₄₆Gl·₈.₃₀H₂O</td>
<td>4₃.₉%</td>
</tr>
</tbody>
</table>

5.2.4. Design of Experiment

Full factorial design (5 × 4) was applied in this study. The two factors were the type and concentration of nanoparticles. There were five levels for the first factor, which were unmodified LDH, three types of LDH modified by sodium benzoate: LDH-SB1 with 12.₃%
benzoate, LDH-SB2 with 20.9% benzoate, and LDH-SB3 with 34.9% benzoate, and LDH modified by sodium gallate, LDH-SG with 43.9% gallate. There were four levels for the second factor, which were 0%, 2%, 5%, and 10%.

5.2.5. Characterizations of PHBV-LDH Composite Films

5.2.5.1. X-Ray Diffraction (XRD)

The X-ray diffraction patterns of nanoparticles and nanocomposite films were taken with an X-ray diffractometer (MiniFelix II, Rigaku Corporation, Tokyo, Japan), using Ni-filtered CuKα radiation (λ = 1.54 Å) and operating 30 kV and 15 mA. The scanning angular range is 2 – 40 ° with a sampling width of 0.02 and a scanning rate of 2 °/min.

5.2.5.2. Transmission Electronic Microscopy (TEM)

The morphology of the composite film was analyzed via TEM on a JEOL 1200 EXII (JEOL, Tokyo, Japan) at an accelerating voltage of 80 kV. The films were first cut to about 70 nm in thickness using a Leica UC6 cryo-ultramicrotome (Leica, Vienna, Austria), placed on 400 hex copper grids, and then imaged.

5.2.5.3. Optical Properties

Color values of the films were measured with a CR-400 Minolta Chromameter (Minolta Sensing Inc., Japan). Films were placed on the surface of a white standard plate (Hunter-Lab values of the plate are L: 96.8; a: 0.0045; and b: 1.7445) and the Hunter-Lab color scale (L: 0 [black] to 100 [white]; a: -80 [green] to 100 [red]; b: -80 [blue] to 70 [yellow]) was used to measure color.

5.2.5.4. Mechanical Properties

A Texture Analyzer TA.XT2i (Texture Technologies Corporation, USA) was used to determine the ultimate tensile strength (UTS), the elastic modulus (EM) and the elongation at break (EL) of films. The tests were performed according to the Standard Test Method for tensile properties of thin plastic sheeting, ASTM D-882-02 (ASTM 2002). Film samples were cut into strips by a scalpel knife with a width of 10 mm and a
length of 50 mm (Han and Floros 1997). Initial grip separation and crosshead speed were set to 20 mm and 0.1 mm/s, respectively. UTS (MPa) is the maximum tensile strength that a film can sustain and was calculated as the ratio of the maximum load and the original minimum cross sectional area of the specimen. Elastic modulus (GPa) is the ratio of stress to strain over the linear part of stress-strain curve and it is a measure of film stiffness (Ozdemir and Floros 2008). Percentage of EL is the percent ratio between the extended length at the moment of rupture and the initial length.

5.2.5.5. Thermo-mechanical Properties

The thermo-mechanical properties of films were determined using a dynamic mechanical analyzer (Q800, TA Instruments, New Castle, Del., USA). Dynamic mechanical analysis (DMA) was performed in tension mode at a frequency of 1 Hz and 0.15% strain. The length and width of the film samples were around 20 (±1) mm and 8 (±1) mm, respectively. The samples were heated from -50 to 70 °C at a heating rate 2 °C/min. Storage modulus (E'), loss modulus (E''), and loss tangent (E''/E') were recorded as a function of temperature. The peak value of tan δ was defined as apparent glass transition temperature (T_g^{app}) to distinguish from the glass transition temperature (T_g) measured by DSC.

5.2.5.6. Thermal Properties

Differential scanning calorimetry (DSC) was performed with a DSC Q100 (TA Instruments, New Castle, Del., USA). The sample in sealed platinum pan was instantaneously heated from room temperature to 170 °C and soaked for 5 minutes, followed by cooling to -80 °C at 5 °C/min, then re-heated from -80 °C to 190 °C at 10 °C/min. DSC experiments were performed under nitrogen using ca. 3 mg sample. Melting points were determined during the re-heating cycle, crystallization temperature was determined during the cooling cycle. Heat of fusion was measured during both re-heating and cooling.
5.2.5.7. Thermal Stability

The thermal stability of films was investigated using a thermogravimetric analyzer Q500 (TA Instruments, New Castle, Del., USA). The sample in platinum pan was heated from room temperature to 800 °C at a heating rate of 10 °C/min under air flow. Weight loss of the sample was measured as function of temperature. Five parameters were determined from the TGA data: the temperature at 10% weight loss, total residue 300 °C, residue from the polymer at 300 °C, the yield of charred residue at 600 °C, and mass loss rate.

5.2.5.8. Water Vapor Permeability

WVP determination

The test follows ASTM E96M-10. Quilted crystal 4 oz. jelly jars (Ball Mason, Daleville, IN) with regular mouth were used as test dishes. The external dimensions of the test dish were 6.2 cm diameter and 5.5 cm tall. Added to the dish was 40 mL of distilled water to make a depth of 2.5 cm and air head space of 3.0 cm. Silicon sealant (Cartridge 1-hour shower ready clear kitchen & bath silicone, GE Silicones Inc. Huntersville, NC) and a screw cap were used to seal films into test dishes. The test dishes were placed in an incubator equipped with two fans. Relative humidity was controlled at 0% by placing desiccant in the incubator and temperature was controlled at 23 °C. Within 2 hours, steady state had been achieved; five weights were then taken for each dish at > 2 hours intervals. Three replicates of each film were tested.

Calculations

Water vapor transmission (WVT) was determined using Eq (1) (ASTM E96M-10). Regression analysis of weight loss as a function of time was performed to insure that accurate steady state slopes were obtained. Regression coefficients were > 0.998 at p <0.001.

\[
WVT = \frac{(G/t)}{A} \quad (1)
\]

where:

G = weight change, g,
t = time, h,
G/t = slope of the straight line, g/h
A = test area, m², which is 2.826 × 10⁻³ m²
WVT = rate of water vapor transmission, g·h⁻¹·m⁻²

\[ \text{Permeance} = \frac{\text{WVT}}{\Delta p} = \frac{\text{WVT}}{S(R_1 - R_2)} \]  \hspace{1cm} (2)

where:
\( \Delta p = \) vapor pressure difference (Pa),
\( S = \) saturation vapor pressure at test temperature (2804 Pa for 23 °C),
\( R_1 = \) relative humidity in the dish expressed as a fraction, and
\( R_2 = \) relative humidity at the vapor sink expressed as a fraction.

\[ \text{Water Vapor permeability (WVP)} = \text{Permeance} \times \text{Thickness} \]  \hspace{1cm} (3)

where:
Thickness was averaged out from ten different locations.

5.2.6. Statistical Analyses

All statistical analysis was done using Minitab 15.1 (Minitab Inc., State College, PA, USA). One-way analysis of variance (ANOVA) and Tukey’s multiple comparison tests at 95% confidence level were applied to study the effect of concentration of unmodified LDH on mechanical properties of PHBV-LDH films. Two-way analysis of variance using general linear model procedure followed by Tukey’s multiple comparison tests at 95% confidence level was applied to study the effects of type and concentration of nanoparticles on mechanical properties, optical properties, and water vapor permeability of the films.

5.3. Results and Discussion

5.3.1. Investigation of Morphology by XRD

In Chapter 3, the structural features of the unmodified and nanoparticles modified by sodium benzoate and sodium gallate were analyzed. In this Chapter, these nanoparticles were incorporated into the bio-based polymer PHBV at different concentrations. XRD and TEM were used to investigate the degree of dispersion of the
inorganic component into the polymeric matrix. XRD patterns of these PHBV/LDH composites are shown from Figure 5.1 to 5.5.

The peak at $2\theta = 11.6^\circ$, corresponding to the basal distance of the pristine unmodified LDH ($d = 0.76$ nm), did not shift in any PHBV composites with various concentrations of unmodified LDH (Figure 5.1). The intensity of the peak at $2\theta = 11.6^\circ$ increased with the increase in concentration of LDH, and an increase of the peak intensity can also be seen for the peak at $2\theta = 23.3^\circ$ and $34.5^\circ$, corresponding the higher level reflections of the peak at $2\theta = 11.6^\circ$. Therefore, only phase-separated morphology was formed in the PHBV composites with unmodified LDH, which meant that they were microcomposites, not nanocomposites.

![Figure 5.1. XRD patterns of PHBV composite films with various concentrations of unmodified LDH.](image)
Figure 5.2. XRD patterns of PHBV composite films with various concentrations of modified LDH with 12.3% benzoate (LDH-SB1).

Figures 5.2 through 5.4 show the XRD patterns of PHBV composites with LDH-SB1, LDH-SB2, and LDH-SB3, respectively, which have different loadings of sodium benzoate in the nanoparticles. The peaks at 2θ = 3.92° and 7.5° corresponded to the basal spacing of the pristine modified LDHs due to the presence of benzoate anions in all PHBV-LDH_SB films. Because of the limitation of the XRD instrument, diffraction patterns of 2θ value smaller than 2° could not be observed. Therefore, absence of these two peaks in PHBV composites containing LDH-SB suggested the existence of exfoliated or disordered nanolayers in the polymer or the distance between nanolayers was at least 4.41 nm (corresponding to a 2θ value of 2°). However, the absence of these two peaks can also be due to low intensity of the two peaks at the low concentration of the nanoparticles in the film, which is highly possible for the films with low concentration of nanoparticles in the present study. Therefore, one cannot conclude the morphology of the PHBV/LDH-SBs composites only by XRD results, so TEM will be shown later to compensate XRD results to give the morphology determination.

Meanwhile, for LDH-SB1, which had the lowest loading of benzoate anions in LDH, the peak at 2θ = 11.6°, corresponded to basal spacing due to carbonate anions between the nanolayers, was still present in the PHBV-LDH_SB1 films. This suggested that partial phase-separated morphology was probably formed. A similar morphology was formed
for the composites with LDH-SB2. However, for LDH-SB3, which had the highest loading of benzoate anions in LDH, the peak at $2\theta = 11.6^\circ$ was absent in the PHBV-LDH_SB3 films, which indicated the exfoliated morphology was probably formed.

Figure 5.3. XRD patterns of PHBV composite films with various concentrations of modified LDH with 20.9% benzoate (LDH-SB2).

Figure 5.4. XRD patterns of PHBV composite films with various concentrations of modified LDH with 34.9% benzoate (LDH-SB3).
Figure 5.5. XRD patterns of PHBV composite films with various concentrations of modified LDH with 43.9% gallate (LDH-SG).

Figure 5.5 displays that in this case (PHBV-LDH_SG) the basal peak of the pristine nanoparticles was absent with 2% and 5% of LDH-SG, and only a small trace, of very low intensity, was visible for the composite with 10% of LDH-SG, appearing at $2\theta = 11.6^\circ$. Two reasons could cause the absence of the basal peaks from pristine nanoparticles, firstly, the nanolayers were disordered or exfoliated in the polymer, if so, then a mixed exfoliated/intercalated morphology was probably formed; secondly, the peaks were lost in the baseline due to low concentration of the nanoparticles in the film.

Overall, the modification of the nanoparticles had a significant effect on their dispersion into the polymer. Only microcomposites were formed for PHBV with unmodified LDH, nanocomposites with a mixed exfoliated/phase-separated morphology were probably formed for PHBV with low and medium loading of benzoate anions in LDH (LDH-SB1 and LDH-SB2), and nanocomposites with exfoliated morphology were probably formed for PHBV with high loading of benzoate and gallate anions in LDH (LDH-SB3 and LDH-SG).
5.3.2. Investigation of Morphology by TEM

TEM was used to further understand the dispersion of the inorganic component into the polymeric matrix, since it was widely employed as a tool for direct visualization of the nanocomposite structure of polymer nanocomposites (Manias and others 2007). Despite imaging relatively small sample regions, TEM images at low and high magnifications were used and considered to be representative of the composites. The TEM images at low magnification (Figures 5.6, 5.7, and 5.8) were used to determine the overall dispersion of the nanoparticles in the polymer, while the higher magnification images (Figure 5.9) provide more detail on the nanometer scale dispersion (e.g., intercalated or exfoliated morphologies). The low magnification images for unmodified LDH in PHBV (Figure 5.6) showed relatively poor dispersion, with sub-micrometer tactoids (agglomeration of LDH) and the area of dark entities, which represented nanoparticles, increased with the increase in the concentration of LDH. The stacked tactoid behavior was typical for polymer/LDH nanocomposites. Costache and others (2006) reported the stacked tactoid behavior for poly(methyl methacrylate) nanocomposites with LDH modified by sodium 4-styrenesulfonate and 2-aminotoluene-5-sulfonic acid (Costache and others 2006). However, TEM images at the low magnification for modified LDH in PBHV showed better dispersion, with only a few tactoids observed at 10% of modified LDH. The dark lines in the high magnification TEM image represented the LDH nanolayers and the gap between the two adjacent lines was the basal spacing. As shown in Figure 5.9 a, LDH was found to be hexagonal in shape, which agreed with Oh and others (2002). The stacked nanolayers were found in the composites with unmodified LDH (the white square in Figure 5.9 b), which indicated the phase-separated morphology. However, the individual nanolayers or disordered stacked nanolayers with the swollen gap between the nanolayers (shown by white arrows in Figure 5.9 c&d) were seen in the composites with modified LDH, which indicate a mixed exfoliated/intercalated morphology.

Based on XRD and TEM results, it can be concluded that PHBV with unmodified LDH resulted in microcomposites with phase-separated morphology and PHBV with
modified LDH resulted in nanocomposites with a mixed exfoliated/intercalated/phase-separated morphology or exfoliated morphology.

**PHBV-LDH**

Figure 5.6. TEM images of PHBV and PHBV composite films with various concentrations of unmodified LDH. Scale bars = 5000 nm.
Figure 5.7. TEM images of PHBV and PHBV composite films with various concentrations of modified LDH with 12.3% benzoate (LDH-SB1), or 20.9% benzoate (LDH-SB2). Scale bars = 2000 nm.
Figure 5.8. TEM images of PHBV and PHBV with various concentrations of modified LDH with 34.9% benzoate (LDH-SB3), or 43.9% gallate (LDH-SG). Scale bars = 2000 nm.
Figure 5.9. Representative TEM images of PHBV containing unmodified (a&b) or modified (c&d) LDH. Scale bars = 100 nm. The white arrow in (a) indicates the LDH in hexagonal shape. The white square in (b) indicates the stacked nanolayers. The white arrows in (c&d) indicate individual nanolayers or disordered stacked nanolayers with swollen gap.

5.3.3. Optical Properties

Hunter color values (L, a, and b) of PHBV films with nanoparticles are shown in Table 5.2. PHBV film without nanoparticles was translucent and white with L, a, and b values of 95.30, -0.15, and 2.96, respectively. The addition of unmodified LDH and LDH-SB had no effect on the optical properties of PHBV films. In contrast, the addition
Table 5.2. Optical properties of PHBV and PHBV composite films with various concentrations of unmodified LDH, modified LDH with 12.3% benzoate (LDH-SB1), 20.9% benzoate (LDH-SB2), 34.9% benzoate (LDH-SB3), or 43.9% gallate (LDH-SG) concentration of nanoparticles with PHBV films.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Type of nanoparticles</th>
<th>Concentration of nanoparticles</th>
<th>Hunter color values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>L</td>
<td>a</td>
</tr>
<tr>
<td>LDH</td>
<td>0%</td>
<td>95.30</td>
<td>-0.15 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>2%</td>
<td>96.48</td>
<td>-0.10 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>5%</td>
<td>96.33</td>
<td>-0.15 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>10%</td>
<td>96.18</td>
<td>-0.12 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>15%</td>
<td>96.11</td>
<td>-0.12 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>20%</td>
<td>96.00</td>
<td>-0.16 ± 0.01</td>
</tr>
<tr>
<td>LDH_SB1</td>
<td>2%</td>
<td>96.37</td>
<td>-0.09 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>5%</td>
<td>96.33</td>
<td>-0.14 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>10%</td>
<td>96.44</td>
<td>-0.10 ± 0.02</td>
</tr>
<tr>
<td>LDH_SB2</td>
<td>2%</td>
<td>96.31</td>
<td>-0.14 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>5%</td>
<td>96.57</td>
<td>-0.11 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>10%</td>
<td>96.43</td>
<td>-0.13 ± 0.02</td>
</tr>
<tr>
<td>LDH_SB3</td>
<td>2%</td>
<td>96.43</td>
<td>-0.13 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>5%</td>
<td>96.36</td>
<td>-0.14 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>10%</td>
<td>96.36</td>
<td>-0.11 ± 0.02</td>
</tr>
<tr>
<td>LDH_SG</td>
<td>2%</td>
<td>93.61</td>
<td>-1.38 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>5%</td>
<td>87.18</td>
<td>-1.38 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>10%</td>
<td>75.71</td>
<td>0.05 ± 0.04</td>
</tr>
</tbody>
</table>

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

The powder of sodium gallate was green and the color remained after incorporation into PHBV. There was a significant decrease in L and a value and increase in b value, which indicated the films with LDH-SG became darker and more yellow-green than virgin PHBV films.
5.3.4. Mechanical Properties

Table 5.3 Mechanical properties of PHBV with various concentrations of unmodified LDH

<table>
<thead>
<tr>
<th>Sample</th>
<th>UTS (MPa)</th>
<th>EM (GPa)</th>
<th>EL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHBV</td>
<td>16.79±0.96\textsuperscript{a}</td>
<td>1.25±0.02\textsuperscript{a}</td>
<td>2.42±0.61\textsuperscript{a,b}</td>
</tr>
<tr>
<td>PHBV-LDH-2%</td>
<td>19.09±3.16\textsuperscript{a,b}</td>
<td>1.15±0.16\textsuperscript{a}</td>
<td>4.68±0.36\textsuperscript{c,d}</td>
</tr>
<tr>
<td>PHBV-LDH-5%</td>
<td>25.45±2.03\textsuperscript{c}</td>
<td>1.09±0.08\textsuperscript{a}</td>
<td>6.42±1.13\textsuperscript{d}</td>
</tr>
<tr>
<td>PHBV-LDH-10%</td>
<td>21.90±0.62\textsuperscript{b,c}</td>
<td>1.26±0.14\textsuperscript{a}</td>
<td>4.64±0.55\textsuperscript{c,d}</td>
</tr>
<tr>
<td>PHBV-LDH-15%</td>
<td>21.02±0.30\textsuperscript{a,b,c}</td>
<td>1.26±0.15\textsuperscript{a}</td>
<td>4.22±0.80\textsuperscript{b,c}</td>
</tr>
<tr>
<td>PHBV-LDH-20%</td>
<td>17.95±0.82\textsuperscript{a,b}</td>
<td>1.22±0.11\textsuperscript{a}</td>
<td>2.33±0.19\textsuperscript{a}</td>
</tr>
</tbody>
</table>

Values are the mean ± one standard deviation. Means in the same column followed by the same letter are not significantly different (\(P > 0.05\)). UTS: ultimate tensile strength; EM: elastic modulus; EL: elongation at break.

The effect of concentrations of LDH on mechanical properties of PHBV/LDH composite films is shown in Table 5.3. One-way ANOVA showed that the effect of the concentration of LDH on tensile strength and elongation at break was significant (\(P < 0.05\)). However, the elastic modulus did not change with the addition of LDH (\(P = 0.42\)).

As shown in Table 5.3, the UTS and EL both increased with the increase of the concentration of LDH reaching maximum values at 5% loading, and then decreased with higher loadings, but were still higher or kept the same as these for virgin PHBV. Compared to the virgin PHBV film, a 51.6% increase in tensile strength and 165% increase in elongation at break were obtained for the 5% LDH/PHBV composites. This enhancement in mechanical properties reflected reinforcement by LDH in PHBV. This can be attributed to the interaction between the polymer chain and inorganic part of LDH, which effectively transferred the stress across the LDH interface. The limited enhancement at high loading of LDH (>10%) was likely due to the aggregation of LDH nanoparticles. Such phenomenon of nanoparticles increasing tensile strength or elongation at break and then decreasing at high concentration of nanoparticles has been observed in other nano-biocomposites systems (Yu and others 2007; Dagnonand others 2009; Xie and others 2009; Kumar and others 2010).
Table 5.4. Effect of type (unmodified LDH, modified LDH with 12.3% benzoate (LDH-SB1), 20.9% benzoate (LDH-SB2), 34.9% benzoate (LDH-SB3), and 43.9% gallate (LDH-SG)) and concentration of nanoparticles on ultimate tensile strength of PHBV composite films

<table>
<thead>
<tr>
<th>LDH content</th>
<th>Ultimate Tensile Strength (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LDH</td>
</tr>
<tr>
<td>0%</td>
<td>16.79±0.96&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2%</td>
<td>19.09±3.16&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>5%</td>
<td>25.45±2.03&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>10%</td>
<td>21.90±0.62&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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

Table 5.5. Effect of type (unmodified LDH, modified LDH with 12.3% benzoate (LDH-SB1), 20.9% benzoate (LDH-SB2), 34.9% benzoate (LDH-SB3), and 43.9% gallate (LDH-SG)) and concentration of nanoparticles on elongation at break of PHBV composite films

<table>
<thead>
<tr>
<th>LDH content</th>
<th>Elongation at Break (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LDH</td>
</tr>
<tr>
<td>0%</td>
<td>2.42±0.61&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2%</td>
<td>4.68±0.36&lt;sup&gt;bcd&lt;/sup&gt;</td>
</tr>
<tr>
<td>5%</td>
<td>6.42±1.13&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>10%</td>
<td>4.64±0.55&lt;sup&gt;bcd&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are the mean ± one standard deviation. Means followed by the same letter are not significantly different (P > 0.05).
Table 5.6. Effect of type (unmodified LDH, modified LDH with 12.3% benzoate (LDH-SB1), 20.9% benzoate (LDH-SB2), 34.9% benzoate (LDH-SB3), and 43.9% gallate (LDH-SG)) and concentration of nanoparticles on elastic modulus of PHBV composite films

<table>
<thead>
<tr>
<th>LDH content</th>
<th>Elastic Modulus (GPa)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LDH</td>
<td>LDH-SB1</td>
<td>LDH-SB2</td>
<td>LDH-SB3</td>
<td>LDH-SG</td>
</tr>
<tr>
<td>0%</td>
<td>1.25±0.02&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.25±0.02&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.25±0.02&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.25±0.02&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.25±0.02&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2%</td>
<td>1.15±0.16&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.35±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.38±0.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.12±0.06&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>0.97±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5%</td>
<td>1.09±0.08&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.27±0.17&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>0.95±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.15±0.13&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>0.99±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>10%</td>
<td>1.26±0.14&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.19±0.03&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.14±0.03&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.25±0.08&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.02±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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

The effects of types and concentrations of LDH on tensile strength, elongation at break, and elastic modulus of PHBV films are shown in Table 5.4, 5.5, and 5.6, respectively. Two-way analysis of variance (Table A.2 to A.4 in Appendix A) showed that the effects of nanoparticle type, concentration, and interaction between type and concentration on UTS, EL, and EM were significant (P < 0.05). As shown in Table 5.4, tensile strength increased with the increase in concentration of LDH-SB1, LDH-SB2, and LDH-SG reaching a maximum at 2% loading and then started to decrease. The trend was similar to that for LDH, and the difference was that the maximum value for PHBV-LDH composites was at higher loading, 5%. This can be ascribed to the fact that a mixed exfoliated/intercalated morphology was formed in PHBV/modified LDH nanocomposites compared to the phase-separated morphology formed in PBHV/unmodified LDH microcomposites. With the mixed exfoliated/intercalated morphology, more interactions between nanolayers and polymer chains were formed at the same concentration of nanoparticles compared with the phase-separated morphology. The highest tensile strength was obtained with the addition of 2% of LDH-SB2, with a 55% increase compared to the virgin PHBV film. Although exfoliated morphology was formed for PHBV-LDH_SB3 and PHBV-LDH_SG nanocomposites that should have theoretically
resulted in the highest improvement of mechanical properties, only a slight increase of tensile strength was obtained in these nanocomposites. This may be due to a too high loading of modifiers in LDH-SB3 and LDH-SG, and these modifiers can increase the mobility of the polymer chain at the polymer-nanoparticles interfacial region, thus acting as plasticizers, and thus reduce the tensile strength of the films (Muksing and others 2011). On the basis of the results collected here, it can be concluded that a higher tensile strength can be obtained with low concentrations of modified LDH having medium loading of the modifier between nanolayers. The elongation at break showed similar trends as the tensile strength. It increased with the increase of concentration up to 2% or 5% and then started to decrease. The highest EL was obtained with addition of 2% of LDH-SG, with a 212% increase compared to the virgin PHBV film. This may be due to the plasticizing effect from the gallate ions. The three hydroxide groups on the gallate ions possibly formed hydrogen bonding the polymer chain, and these interactions increased the mobility of the polymer chain, thus increased the elongation at break. The change of elastic modulus was much smaller than that of tensile strength or elongation at break.
5.3.5. Thermo-mechanical Properties

Figure 5.10. Effect of type (unmodified LDH, modified LDH with 12.3% benzoate (LDH-SB1), 20.9% benzoate (LDH-SB2), 34.9% benzoate (LDH-SB3), and 43.9% gallate (LDH-SG)) and concentration of nanoparticles on storage modulus (E') of PHBV composite films.
The storage modulus ($E'$) behavior vs. temperature of composites prepared with different types of nanoparticles with different concentrations are shown in Figure 5.10. Over the entire temperature range, $E'$ of PHBV-LDH composites was higher than that of virgin PHBV film. $E'$ increased with the increase of the concentration of nanoparticles and then started to decrease. For modified LDH, the maximum of $E'$ was reached at 2% loading, while for unmodified LDH, the maximum of $E'$ was reached at 5% loading. Storage modulus of virgin PHBV film at 20 °C was 1158 MPa. The values of storage modulus for PHBV-LDH composites with 5% LDH, 2% LDH-SB1, 2% LDH-SB2, 2% LDH-SB3, and 2% LDH-SG were 2166, 2550, 3184, 2027, and 2136 MPa, respectively. These results were in agreement with the results of tensile strength but not completely with those of elastic modulus. The trend found on $E'$ was seen for LDH-SB1 and LDH-SB2 on elastic modulus, but not for other types of nanoparticles, which could be due to the calculation error of elastic modulus. The higher $E'$ of modified LDH composites compared to the unmodified composites can be attributed to the better interaction between polymer chains and nanolayers. The degree of improvement for the nanoparticles, LDH-SB3 and LDH-SG, with high loading of modifier was reduced compared to nanoparticles with low and medium loading of the modifier, which may be due to the reduction of the interaction between polymer chains and nanolayers because the polymer chains also interact with the modifiers.
Figure 5.11. Effect of type (unmodified LDH, modified LDH with 12.3% benzoate (LDH-SB1), 20.9% benzoate (LDH-SB2), 34.9% benzoate (LDH-SB3), and 43.9% gallate (LDH-SG)) and concentration of nanoparticles on apparent glass transition temperature of PHBV composite films.
Tan δ behavior vs. temperature of composites prepared with different types of nanoparticles with different concentrations is shown in Figure 5.11. The peak of Tan δ represented the apparent glass transition temperature ($T_{g, app}$) of the PHBV film. Table 5.7 summarizes the apparent glass transition temperatures of these PHBV composites. Increase of $T_g$ has been attributed to attractive interaction between the nanoparticles and the polymer (Xie and others 2009). Apparent $T_g$ showed no change with the incorporation of unmodified LDH, which indicated the relatively weak interaction between unmodified LDH and the polymer. Apparent $T_g$ increased with the addition of modified LDH. The increases of $T_{g, app}$ indicated the stronger interaction between modified nanoparticles and PHBV. The apparent $T_g$ increased the most by the modified nanoparticles with medium loading of benzoate (LDH-SB2). The $T_{g, app}$ decreased by the modified nanoparticles with highest loading of modifier (benzoate or gallate) compared to LDH-SB2, which could be due to the plasticizing effect from the modifiers as mentioned above.

### Table 5.7. Effect of type (unmodified LDH, modified LDH with 12.3% benzoate (LDH-SB1), 20.9% benzoate (LDH-SB2), 34.9% benzoate (LDH-SB3), and 43.9% gallate (LDH-SG)) and concentration of nanoparticles on apparent glass transition temperature ($T_{g, app}$) of PHBV composite films

<table>
<thead>
<tr>
<th>LDH content</th>
<th>LDH</th>
<th>LDH-SB1</th>
<th>LDH-SB2</th>
<th>LDH-SB3</th>
<th>LDH-SG</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>13.9</td>
<td>13.9</td>
<td>13.9</td>
<td>13.9</td>
<td>13.9</td>
</tr>
<tr>
<td>2%</td>
<td>13.3</td>
<td>16.2</td>
<td>18.0</td>
<td>16.3</td>
<td>16.8</td>
</tr>
<tr>
<td>5%</td>
<td>13.2</td>
<td>16.3</td>
<td>19.7</td>
<td>15.2</td>
<td>14.7</td>
</tr>
<tr>
<td>10%</td>
<td>13.8</td>
<td>16.3</td>
<td>15.9</td>
<td>18.7</td>
<td>15.9</td>
</tr>
</tbody>
</table>
5.3.6. Thermal Properties

Figure 5.12. DSC cooling cycle for PHBV and PHBV composite films with various concentrations of unmodified LDH, modified LDH with 12.3% benzoate (LDH-SB1), 20.9% benzoate (LDH-SB2), 34.9% benzoate (LDH-SB3), or 43.9% gallate (LDH-SG).
Figure 5.13. DSC heating cycle for PHBV and PHBV composite films with various concentrations of unmodified LDH, modified LDH with 12.3% benzoate (LDH-SB1), 20.9% benzoate (LDH-SB2), 34.9% benzoate (LDH-SB3), or 43.9% gallate (LDH-SG).
DSC cooling and heating cycles of PHBV/LDH composite films with unmodified or modified LDH are shown in Figures 5.12 and 5.13. The thermal properties of these films, including crystallization temperature ($T_c$), crystallization enthalpy ($\Delta H_{mc}$), melting temperature ($T_m$), and melting enthalpy ($\Delta H_m$), are summarized in Table 5.8.

XRD patterns showed no change in the crystalline structure of PHBV, indicating the addition of nanoparticles did not significantly modify the crystalline phase. Crystallization temperature is affected in different ways, depending on the type and concentration of nanoparticles. Unmodified LDH and LDH modified by sodium gallate showed limited effect on $T_c$ of PHBV films. However, the incorporation of LDH modified by sodium benzoate resulted in higher $T_c$ compared to the virgin PHBV at low and medium concentrations and then $T_c$ decreased at high concentrations. At low and medium concentrations, the nanoparticles acted as nucleation agents, which accelerated crystallization (Xie and other 2009). However, at high concentrations, there may be free benzoate in the polymer instead of bonding on the nanolayers, and by having such small molecular weight compound in the polymer, $T_c$ could be reduced.

Double melting endotherms are shown in all PHBV-LDH composites films. This behavior has been considered as the recrystallization phenomenon in Chapter 4. The $T_{m1}$ and $T_{m2}$ can be attributed to the melting of the primary crystallites formed during the cooling scan and the melting of the recrystallized crystallites which were formed during the heating process. As shown in Table 5.8, $T_{m1}$ and $T_{m2}$ did not change much with addition of nanoparticles except with modified LDH with highest loading of benzoate (LDH-SB3). The melting point decreased with the addition of LDH-SB3 compared to the virgin PHBV. There were benzoate anions physically adsorbed on the surface of the nanoparticles in LDH-SB3, and the melting point could decrease if these benzoate anions dissolve into polymer. Moreover, it was pointed out that the change in the ratio of the melting peak intensities with the addition of nanoparticles: $T_{m2}$ peak intensity became preponderant in polymer LDH composites in comparison with pure polymers (Bordes
Table 5.8. Thermal properties of PHBV and PHBV composition films with various concentrations of unmodified LDH, modified LDH with 12.3% benzoate (LDH-SB1), 20.9% benzoate (LDH-SB2), 34.9% benzoate (LDH-SB3), or 43.9% gallate (LDH-SG).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Type of nanoparticles</th>
<th>Content of nanoparticle</th>
<th>T&lt;sub&gt;c&lt;/sub&gt; (°C)</th>
<th>ΔH&lt;sub&gt;mc&lt;/sub&gt; (J/g)</th>
<th>T&lt;sub&gt;m&lt;/sub&gt; (°C)</th>
<th>ΔH&lt;sub&gt;m&lt;/sub&gt; (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0%</td>
<td>101.8</td>
<td>66.5</td>
<td>147.0</td>
<td>155.7</td>
</tr>
<tr>
<td>LDH</td>
<td></td>
<td>2%</td>
<td>102.7</td>
<td>78.8</td>
<td>145.5</td>
<td>154.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5%</td>
<td>103.3</td>
<td>75.3</td>
<td>145.9</td>
<td>155.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10%</td>
<td>103.0</td>
<td>66.3</td>
<td>145.5</td>
<td>154.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15%</td>
<td>101.6</td>
<td>70.7</td>
<td>144.6</td>
<td>154.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20%</td>
<td>101.6</td>
<td>58.5</td>
<td>144.5</td>
<td>154.0</td>
</tr>
<tr>
<td>LDH_SB1</td>
<td></td>
<td>2%</td>
<td>106.6</td>
<td>76.0</td>
<td>147.9</td>
<td>156.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5%</td>
<td>104.2</td>
<td>72.0</td>
<td>145.8</td>
<td>154.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10%</td>
<td>102.6</td>
<td>68.0</td>
<td>145.0</td>
<td>154.1</td>
</tr>
<tr>
<td>LDH_SB2</td>
<td></td>
<td>2%</td>
<td>104.9</td>
<td>78.3</td>
<td>145.4</td>
<td>154.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5%</td>
<td>105.9</td>
<td>87.1</td>
<td>147.1</td>
<td>155.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10%</td>
<td>102.3</td>
<td>63.8</td>
<td>144.1</td>
<td>153.2</td>
</tr>
<tr>
<td>LDH_SB3</td>
<td></td>
<td>2%</td>
<td>104.2</td>
<td>71.5</td>
<td>140.1</td>
<td>149.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5%</td>
<td>103.0</td>
<td>70.7</td>
<td>136.8</td>
<td>145.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10%</td>
<td>98.4</td>
<td>62.2</td>
<td>130.9</td>
<td>141.4</td>
</tr>
<tr>
<td>LDH_SG</td>
<td></td>
<td>2%</td>
<td>103.0</td>
<td>71.8</td>
<td>147.7</td>
<td>156.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5%</td>
<td>102.0</td>
<td>66.4</td>
<td>147.0</td>
<td>156.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10%</td>
<td>102.6</td>
<td>66.5</td>
<td>147.1</td>
<td>155.8</td>
</tr>
</tbody>
</table>

T<sub>c</sub>: crystallization temperature; ΔH<sub>mc</sub>: crystallization enthalpy; T<sub>m</sub>: melting temperature; and ΔH<sub>m</sub>: melting enthalpy.
and others 2010). This behavior, attesting for more recrystallization during melting, was attributed to the enhanced nucleation effect of nanoparticles on the recrystallization phenomenon. Such phenomenon of nanoparticles enhancing nucleation step has already been observed in other nano-biocomposites systems (Di Maio and others 2004; Chivrac and others 2007; Hsu and others 2007; Xie and others 2009; Bordes and others 2010; Dagnon and others 2012). In contrast, the melting points and the intensity ratio of the two melting points did not change with the addition of nanoparticles modified by sodium gallate.

5.3.7. Thermal Stability

Thermogravimetric analyses were performed to determine the effects of types and concentrations of LDH nanoparticles on the thermal stability of PHBV matrices. The PHBV degradation occurred according to a non-radical, random chain scission reaction just above the melting temperature (Liu and others 2009). Figure 5.14 shows the typical TGA patterns for PHBV films. The TGA patterns indicate that there is only one single weight loss between 230 °C and 350 °C during the thermal degradation of PHBV-based LDH composites. The temperature at 10% weight loss for PHBV composites shifts to lower temperature after addition of nanoparticles. This may be due to the action of the bonding water in nanoparticles on the ester bonds giving rise to a base-catalyzed decomposition of the polymer. The yield of charred residue at 600 °C increases with addition of LDH nanoparticles, and the extra residue is attributed to the metal oxides from LDH. The residue at 300 °C is also reported because it is noticed that the virgin PHBV stopped losing weight after 300 °C. The total residue of PHBV-LDH composites is composed of two parts, one from the polymer and the other from the nanoparticles. The second one can be calculated combining the TGA data of the nanoparticles reported in Chapter 3. The residues of LDH, LDH-SB1, LDH-SB2, LDH-SB3, and LDH-SG at 300 °C were 83.99%, 85.27%, 84.74%, 85.12%, and 76.44%, respectively. Therefore, the residues of PHBV-LDH films from the polymer at 300 °C, which are reported in Table 5.9, can be calculated as follows:

\[
\text{Residue from the polymer} = \text{Total residue} - \text{Content of nanoparticles} \times \text{Residue of nanoparticles at 300 °C}
\]
The data showed that the residue from the polymer at 300 °C increased with the increase of nanoparticles. The increase may be due to some condensed phase mechanism taking place during thermal degradation (Nyambo and others 2008). Meanwhile, the mass loss rates for the PHBV-LDH composites were reduced, which was possibly attributed to the barrier effect of the nanolayers of LDH (Zhang and others 2012). In comparison with LDH-SB, LDH-SG had less effect on the thermal stability in terms of $T_{10\%}$ and the mass loss rate, which may be ascribed to the interaction between the polymer chain and hydrogen groups on the gallate ion. Overall, although the temperature at 10 % weight loss
(T\textsubscript{10\%}) decreased by the addition of nanoparticles, the residue left from the polymer at 300 °C was increased and the mass loss rate was decreased.

Table 5.9. Thermal stability of PHBV and PHBV composite films with various concentrations of unmodified LDH, modified LDH with 12.3% benzoate (LDH-SB1), 20.9% benzoate (LDH-SB2), 34.9% benzoate (LDH-SB3), or 43.9% gallate (LDH-SG).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Type of nanoparticles of nanoparticle</th>
<th>Concentration</th>
<th>T\textsubscript{10%} (°C)</th>
<th>Total Residue at 300 °C</th>
<th>Residue from PHBV at 300 °C</th>
<th>Charred Residue at 600 °C</th>
<th>Mass loss rate (%/°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH</td>
<td>0%</td>
<td>251.9</td>
<td>1.5%</td>
<td>1.5%</td>
<td>0.9%</td>
<td>5.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2%</td>
<td>217.5</td>
<td>4.4%</td>
<td>2.7%</td>
<td>1.6%</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5%</td>
<td>216.4</td>
<td>11.1%</td>
<td>6.9%</td>
<td>4.9%</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10%</td>
<td>215.3</td>
<td>17.5%</td>
<td>9.1%</td>
<td>4.9%</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15%</td>
<td>214.7</td>
<td>32.4%</td>
<td>19.8%</td>
<td>8.1%</td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20%</td>
<td>204.5</td>
<td>36.8%</td>
<td>20.0%</td>
<td>9.5%</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>LDH_SB1</td>
<td>2%</td>
<td>214.9</td>
<td>6.1%</td>
<td>4.4%</td>
<td>2.2%</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5%</td>
<td>211.3</td>
<td>10.0%</td>
<td>5.3%</td>
<td>3.0%</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10%</td>
<td>209.5</td>
<td>21.2%</td>
<td>12.7%</td>
<td>5.9%</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td>LDH_SB2</td>
<td>2%</td>
<td>220.5</td>
<td>4.8%</td>
<td>3.1%</td>
<td>2.2%</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5%</td>
<td>214.1</td>
<td>10.4%</td>
<td>6.1%</td>
<td>3.2%</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10%</td>
<td>208.6</td>
<td>19.6%</td>
<td>11.1%</td>
<td>5.8%</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>LDH_SB3</td>
<td>2%</td>
<td>206.5</td>
<td>5.0%</td>
<td>3.3%</td>
<td>1.7%</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5%</td>
<td>203.7</td>
<td>9.9%</td>
<td>5.7%</td>
<td>3.1%</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10%</td>
<td>201.6</td>
<td>17.1%</td>
<td>8.6%</td>
<td>4.7%</td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td>LDH_SG</td>
<td>2%</td>
<td>245.9</td>
<td>3.6%</td>
<td>2.1%</td>
<td>1.3%</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5%</td>
<td>245.4</td>
<td>6.4%</td>
<td>2.5%</td>
<td>2.2%</td>
<td>6.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10%</td>
<td>243.6</td>
<td>9.0%</td>
<td>1.3%</td>
<td>2.3%</td>
<td>6.4</td>
<td></td>
</tr>
</tbody>
</table>

T\textsubscript{10\%}: temperature at 10% weight loss.
5.3.8. Water Vapor Permeability

Table 5.10. Effect of type (unmodified LDH, modified LDH with 12.3% benzoate (LDH-SB1), 20.9% benzoate (LDH-SB2), 34.9% benzoate (LDH-SB3), and 43.9% gallate (LDH-SG)) and concentration of nanoparticles on water vapor permeability (WVP) at 23 °C of PHBV composite films.

<table>
<thead>
<tr>
<th>LDH concentration</th>
<th>WVP (g·m·h⁻¹·m⁻²·Pa⁻¹) (× 10⁻⁹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LDH</td>
</tr>
<tr>
<td>0%</td>
<td>13.91±1.52ᵃ</td>
</tr>
<tr>
<td>2%</td>
<td>10.64±2.39ᵃᵇ</td>
</tr>
<tr>
<td>5%</td>
<td>9.65±0.84ᵇ</td>
</tr>
<tr>
<td>10%</td>
<td>11.07±0.79ᵃᵇ</td>
</tr>
<tr>
<td>15%</td>
<td>7.49±0.68ᵇ</td>
</tr>
<tr>
<td>20%</td>
<td>7.76±1.28ᵇ</td>
</tr>
</tbody>
</table>

Values are the mean ± one standard deviation. Means in the same column followed by the same letter are not significantly different (P > 0.05). -, data not measured.

Water vapor permeability (WVP) of the PHBV-LDH composite films is shown in Table 5.10. The addition of unmodified LDH and modified LDH has the opposite effect on the WVP of the PHBV film. WVP decreases with the increase of unmodified LDH, while it increases with the increase of modified LDH. The decrease of the WVP is possibly due to the barrier effect of the nanolayers, which provided a torturous pathway for the water molecules to diffuse out of the film. The increase of the WVP with the addition of modified LDH may be ascribed to the hydrophilic nature of the modifier loaded in the nanolayer, which attracted the water molecules, and thus gave driving force for the water molecule to diffuse through. Although the diffusion pathway for the films with modified nanoparticles was longer than that in the films without nanoparticles, WVP increased for the former case due to the interaction between water and modifier. The mechanism is described in Figure 5.15, with the consideration of the morphologies of PHBV-LDH composite films (phase separated morphology was formed with unmodified...
LDH, and exfoliated morphology was formed with modified LDH) based on the XRD and TEM data.

![Diagram of LDH structures](image)

Figure 5.15. Schematic illustration of the tortuosity for a diffusing penetrant introduced on exfoliated solid layered in a polymer matrix. A. Filled film with unmodified LDH, B. Unfilled film, and C. Filled film with modified LDH.

5.4. Conclusions

PHBV-based composite films with different concentrations of unmodified LDH and modified LDH were prepared by solvent casting technique. The arrangement of LDH in the bio-nanocomposites matrix ranged from exfoliated to phase-separated depending on the type and concentration of LDH nanoparticles. Intercalated or partial exfoliated structures were obtained using modified LDH, however, only phase-separated structures were formed using unmodified LDH. The mechanical (tensile strength and elongation at break) and thermo-mechanical (storage modulus) properties were increased with low concentrations of nanoparticles incorporated in the polymer. The incorporation of modified LDH with sodium benzoate further improved the mechanical properties in
comparison with unmodified LDH, which may be due to the increased compatibility between PHBV and nanoparticles and larger basal distance between nanolayers after modification. The concentration of benzoate anions in LDH nanoparticles was another factor to affect the properties of PHBV composite films. The PHBV film with 2% modified LDH with medium loading of benzoate anions (20.9 % w/w of benzoate anions in LDH) had the greatest mechanical and thermo-mechanical properties. Apparent glass transition temperature increased with the addition of modified LDH but did not change with the addition of unmodified LDH. Moreover, the effect of nanoparticles on thermal properties as well as crystallization of PHBV composites was dependent on the type of nanoparticles. Unmodified LDH and LDH modified by sodium gallate had limited effect on the thermal properties. However, LDH nanoparticles modified by sodium benzoate increased the crystallization temperature and enhanced recrystallization during melting, which may due to the nucleation effect by nanoparticles. The temperature at 10 % weight loss was decreased with the addition of nanoparticles, which indicated the reduction of thermal stability, but the residue left from the polymer at 300 °C was increased and the mass loss rate was decreased. Water vapor permeability was reduced with the increase of unmodified LDH due to the barrier effect of nanolayers providing a torturous pathway for small molecules to diffuse out of the film. Due to the hydrophilic nature of the modifiers, the water vapor permeability increased with the addition of modified LDH.

The results obtained in this work showed that substantial improvement of mechanical, thermal, and barrier properties of PHBV were achieved with very low concentration (2%) of unmodified and modified LDH, which may extend the application of this bio-based polymer. Moreover, the modifiers, sodium benzoate and sodium gallate, can also bring the additional functionality to the bio-based nanocomposites. Therefore, these new hybrid materials with additional functions can be used as antimicrobial food packaging. The release kinetics of the active components will be studied in Chapter 6.
5.5. References


Costache, M. C.; Wang, D. Y.; Heidecker, M. J.; Manias, E.; Wilkie, C. A., The thermal degradation of poly(methyl methacrylate) nanocomposites with montmorillonite,


Han, J. H.; Floros, J. D., Casting antimicrobial packaging films and measuring their physical properties and antimicrobial activity. *Journal of Plastic Film & Sheeting* 1997, 13, (4), 287-298.


Chapter 6
MECHANICAL PROPERTIES AND RELEASE KINETICS OF POLY(HYDROXYBUTYRATE-CO-HYDROXYVALERATE) FILMS MODIFIED WITH DIFFERENT ANTIMICROBIAL AGENTS

6.1. Introduction

Food spoilage due to undesirable growth of microorganism is a common factor which shortens the shelf life of food products and can cause food borne illness outbreaks. The addition of antimicrobial agents (active compounds that kill or prevent the growth of microorganisms) to foods or food surfaces during processing is an effective technique that is frequently employed to eliminate food borne pathogens. However, the antimicrobial activity may be rapidly lost due to inactivation of the antimicrobials by components in foods or because the antimicrobials are diluted below active concentrations due to migration into the bulk food matrix (Robertson 2006). Therefore, antimicrobial agents have been incorporated into packaging materials, called antimicrobial food packaging (AM food packaging), to prevent the growth of microorganisms on the food surface and extend the shelf life or improve microbial safety of the food (Collins-Thompson and Hwang 2000; Suppakul and others 2003). AM food packaging has received great interest from the food industry attributed to the increasing consumer demand for minimally processed, preservative-free, “fresh” foods, because a major advantage arising from the use of AM packaging is that only low levels of preservative come into contact with the food, compared to the direct addition of preservative to the food (Suppakul and others 2003; Robertson 2006). Various polymers have been studied as potential candidates for incorporation of an antimicrobial substance in food packaging applications (Bastarrachea and others 2011). Synthetic polymers derived from petroleum, such as low-density and high-density polyethylene, polypropylene, polystyrene, polyethylene terephthalate, ethylene-vinyl acetate, poly(vinyl chloride), and poly(butylene adipate-co-terephthalate), have thus far dominated in AM food packaging, (Joerger 2007; Bastarrachea 2011). Meanwhile, a number of edible films and coatings have also been studied for antimicrobial applications (Ozdemir and Floros...
starch, cellulose derivatives, chitosan, alginate, fruit-puree, whey protein isolated, soy protein isolated, corn zein, wheat gluten, egg albumen, gelatin, and sodium caseinate. Only few biodegradable polymers have been used for the production of antimicrobial films (Liu and others 2007; Jin and Zhang 2008; Rhim and others 2009; Bugatti and others 2011), including polycaprolactone (PCL), and poly (lactic acid) (PLA). The biobased polymer, poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV), used in the present study, has not been previously studied for antimicrobial packaging.

The mechanical, gas barrier, thermal, and morphological properties of food-packaging films, are influenced by the incorporation of antimicrobial substances (Bastarrachea 2011). The mechanical properties of polymeric films for food packaging applications are important since the packaging materials are under various stresses that occur during the processing, handling, and storage of packaged foods (Marcos and others 2010). A significant change can be obtained in the tensile properties of polymeric films after the incorporation of antimicrobials. Many studies have found a decrease in film strength and resistance with an increase in concentration of the antimicrobial incorporated in the polymer (Limjaroen and others 2003; Pranoto and others 2005; Pires and others 2008; Tippayatum and others 2009; Ture and others 2009; and Bastarrachea and others 2010). A few cases have found that the tensile properties were not significantly affected by the addition of antimicrobial agents (Jin and others 2009 Sanchez-Valdez and others 2009); whereas Marcos and others (2010) found that the tensile properties of PVOH films were improved with the incorporation of the antimicrobial enterocin. Besides the possible negative effect on mechanical properties, the incorporation of low molecular weight antimicrobial agents into the polymer matrix has another disadvantage, the migration and the release of the AM cannot be easily predicted or controlled. Due to the polymeric structure and the interaction between the polymer and antimicrobials, the antimicrobials are either completely bound in the polymeric matrix or suddenly released into the aqueous system (Chacko 2008). To overcome these problems, a method for loading active molecules (anti-inflammatory, antibiotic, and antimicrobial) into inorganic compounds to obtain a very slow and
controlled release in selected conditions has been proposed (Tammaro and others 2005, 2007, 2009; Sammartino and others 2004; Bugatti and others 2011). In particular, layered double hydroxide (LDH) has been used as an active molecule delivery vehicle. In the previous chapters, LDH was modified by antimicrobial agents, sodium benzoate and sodium gallate, to increase the compatibility between the matrix and nanoparticles thus improving the mechanical, thermal, and barrier properties of polymer films. In this chapter, the release kinetics of antimicrobial agents from PHBV films will be the focus. The effects of incorporation methods of antimicrobial agents into PHBV films, either directly incorporated into PHBV or modified in LDH first and then incorporated into PHBV, on mechanical properties and release kinetics will be studied first. Secondly, the concentration and type of modified LDH used in PHBV films will be investigated to find the effect on the release kinetics of AMs.

6.2. Materials and Methods

6.2.1. Materials

Unmodified nanoparticles LDH-CO\(_3\) of the formula \([\text{Mg}_{4.5}\text{Al}_2(\text{OH})_{13}](\text{CO}_3)_1 \cdot 3.5\text{H}_2\text{O}\) was kindly provided by Sechang Co. Ltd. (Jeonbuk, Korea). Poly(hydroxybutyrate-co-valerate) (PHBV) (PHB 88\% & PHV 12\%) in granule form was purchased from Goodfellow Corporation (Coraopolis, PA, USA), sodium benzoate (SB) from J.T. Baker (Center Valley, PA, USA), gallic acid from Sigma-Aldrich (St. Louis, MO, USA), and chloroform in HPLC grade from EMD Millipore (Merck KGaA, Darmstadt, Germany). All other reactants, including sodium chloride (Alfa Aesar), hydrochloric acid (VWR), sodium nitrate (VWR), and sodium hydroxide (BDH Chemicals), were purchased from VWR International LLC. (Philadelphia, PA, USA).

6.2.2. Preparation of Nanoparticle Modified by Antimicrobial Agents

Sodium benzoate and sodium gallate were used to modify the nanoparticles as described in section 3.2.2. The compositions of nanoparticles used in this chapter are shown in Table 5.1. Since there is only one type of LDH modified by sodium gallate, the LDH-SG3 in Chapter 3 is named as LDH-SG in this chapter.
6.2.3. PHBV Film Preparation

All PHBV composite films were fabricated by a solvent casting technique as described in Section 5.2.3. The calculated amounts of nanoparticles or antimicrobial agents in each film were listed in Table 6.1 and Table 6.2.

6.2.4. Design of Experiment

Modified nanoparticles, LDH-SB2 and LDH-SG, were used to study the effects of different incorporation methods of antimicrobial agents into PHBV on mechanical properties and release kinetics of PHBV films. Three groups of films were prepared, including PHBV film with modified nanoparticles, PHBV film with antimicrobial agent, and PHBV film with antimicrobial agent and unmodified LDH. The amounts of antimicrobial agents and unmodified nanoparticles in these films were the same as in the films with modified nanoparticles. For example, the benzoate anion content is 20.9% (wt/wt) in LDH-SB2, therefore, film PHBV-LDH_S2-2% contains 0.42% benzoate and 1.58% unmodified LDH. So the two samples with direct incorporation of benzoate were prepared based on these calculated concentrations. The concentrations of each additive (modified LDH, unmodified LDH, or antimicrobial agent) in each film are shown in Table 6.1. The percentages in brackets are the calculated concentrations of antimicrobial agents and unmodified nanoparticles in the films with modified nanoparticles.
Table 6.1. Design of experiment to study the effect of incorporation methods of antimicrobial agents into PHBV on mechanical properties and release kinetics of PHBV films

<table>
<thead>
<tr>
<th>Modified Nanoparticles</th>
<th>Sample Name</th>
<th>Conc. of Antimicrobial Agents</th>
<th>Conc. of Unmodified Nanoparticles</th>
<th>Conc. of Modified Nanoparticles</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LDH-SB2</strong></td>
<td>PHBV-SB0.42%</td>
<td>0.42%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>PHBV-SB0.42%&amp;LDH1.58%</td>
<td>0.42%</td>
<td>1.58%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>PHBV-LDH_SB2-2%</td>
<td>(0.42%)</td>
<td>(1.58%)</td>
<td>2%</td>
</tr>
<tr>
<td></td>
<td>PHBV-SB1.05%</td>
<td>1.05%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>PHBV-SB1.05%&amp;LDH3.95%</td>
<td>1.05%</td>
<td>3.95%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>PHBV-LDH_SB2-5%</td>
<td>(1.05%)</td>
<td>(3.95%)</td>
<td>5%</td>
</tr>
<tr>
<td><strong>LDH-SG</strong></td>
<td>PHBV-SB0.88%</td>
<td>0.88%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>PHBV-SG0.88%&amp;LDH1.12%</td>
<td>0.88%</td>
<td>1.12%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>PHBV-LDH_SG-2%</td>
<td>(0.88%)</td>
<td>(1.12%)</td>
<td>2%</td>
</tr>
<tr>
<td></td>
<td>PHBV-SG2.2%</td>
<td>2.2%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>PHBV-SG2.2%&amp;LDH2.8%</td>
<td>2.2%</td>
<td>2.8%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>PHBV-LDH_SG-5%</td>
<td>(2.2%)</td>
<td>(2.8%)</td>
<td>5%</td>
</tr>
</tbody>
</table>

LDH-SB2: modified LDH with 20.9% benzoate; LDH-SG: modified LDH with 43.9% gallate. The percentages in brackets are the calculated concentrations of antimicrobial agents and unmodified nanoparticles in the films with modified nanoparticles.

Full factorial design \((3^2)\) was applied to study the effect of type and concentration of LDH modified by sodium benzoate on the release kinetics of benzoate. The two factors were type and concentration of nanoparticles. There were three levels for the first factor, which were modified LDH by sodium benzoate, LDH-SB1 with 12.3% benzoate, LDH-SB2 with 20.9% benzoate, and LDH-SB3 with 34.9% benzoate, with three levels for the second factor as well, which were 2%, 5%, and 10%. The experimental design is shown in Table 6.2. The effect of concentration of LDH modified by sodium gallate (LDH-SG) was also studied. Three concentrations, 2%, 5%, and 10%, were studied, with sample names of PHBV-LDH_SG-2%, PHBV-LDH_SG-5%, and PHBV-LDH_SG-10%.
Table 6.2. Design of experiment to study the effect of type and concentration of LDH modified by sodium benzoate on the release kinetics of benzoate from PHBV composite films

<table>
<thead>
<tr>
<th>Modified Nanoparticles</th>
<th>Sample Name</th>
<th>Concentration of Modified Nanoparticles</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH-SB1</td>
<td>PHBV-LDH_SB1-2%</td>
<td>2%</td>
</tr>
<tr>
<td></td>
<td>PHBV-LDH_SB1-5%</td>
<td>5%</td>
</tr>
<tr>
<td></td>
<td>PHBV-LDH_SB1-10%</td>
<td>10%</td>
</tr>
<tr>
<td>LDH-SB2</td>
<td>PHBV-LDH_SB2-2%</td>
<td>2%</td>
</tr>
<tr>
<td></td>
<td>PHBV-LDH_SB2-5%</td>
<td>5%</td>
</tr>
<tr>
<td></td>
<td>PHBV-LDH_SB2-10%</td>
<td>10%</td>
</tr>
<tr>
<td>LDH-SB3</td>
<td>PHBV-LDH_SB3-2%</td>
<td>2%</td>
</tr>
<tr>
<td></td>
<td>PHBV-LDH_SB3-5%</td>
<td>5%</td>
</tr>
<tr>
<td></td>
<td>PHBV-LDH_SB3-10%</td>
<td>10%</td>
</tr>
</tbody>
</table>

Modified nanoparticles: LDH-SB1 with 12.3% benzoate, LDH-SB2 with 20.9% benzoate, and LDH-SB3 with 34.9% benzoate.

6.2.5. Mechanical Properties

A Texture Analyzer TA.XT2i (Texture Technologies Corporation, USA) was used to determine the ultimate tensile strength (UTS), the elastic modulus (EM) and the elongation at the break (EL) of films. The tests were performed according to the Standard Test Method for tensile properties of thin plastic sheeting, ASTM D-882-02 (ASTM 2002). Film samples were cut into strips by a scalpel knife with a width of 10 mm and a length of 50 mm (Han and Floros 1997). The samples with direct incorporation of antimicrobial agents were cut into strips ready to be tested when they were still wet with some residual solvent. The reason for this is that these films became very brittle at 100% dryness which made them impossible cut without breakage. The samples with modified nanoparticles were cut after all residual solvent was removed. Initial grip separation and crosshead speed were set to 20 mm and 0.1 mm/s, respectively. UTS, (MPa) the maximum tensile strength that a film can sustain, was calculated as the ratio of the maximum load and the original minimum cross sectional area of the specimen. EM (GPa)
is the ratio of stress to strain over the linear part of stress-strain curve and it is a measure of film stiffness (Ozdemir and Floros 2008). Percentage of EL is the percent ratio between the extended length at the moment of rupture and the initial length.

6.2.6. Antimicrobial Release Study

Release profile of antimicrobial agents from PHBV films in DI water were studied at $\lambda_{\text{max}} = 225$ nm for benzoate and at $\lambda_{\text{max}} = 260$ nm for gallate using a UV-VIS spectrophotometer (Helios gamma; Thermo Spectronic, Madison, WI, USA). The films were cut into a circle with diameter of 46 mm. To ensure double sided diffusion from the film, the film was placed between two rings made from beading wire in a short wide-mouth jar of size 54×46mm. This process pushed the film to be immersed into the solution and still prevents the film from resting on the bottom of the jar. Distilled water was used as the food simulant to do the release study. The volume/surface area ratio for the double-sided diffusion experiment from polymeric films should be maintained between 0.31 to 155 ml/cm$^2$ (ASTM D4754). A volume/surface area ratio of 0.90 ml/cm$^2$ was used in this study. The study was conducted in a temperature controlled chamber maintained at 21 °C. The sample was kept agitated at 75 rpm with the help of an orbital shaker throughout the benzoate or gallate release study. Calibration curves for both antimicrobial agents, relating the absorbance of the solution with concentration of the agents, were built from measurements at 225 nm and 260 nm for sodium benzoate and sodium gallate, respectively, in DI water among the expected concentration ranges. The calibration curve for benzoate at 225 nm was built with 8 points in the range of 20 µg/ml and 0.5 µg/ml, while the one for gallate at 260 nm was built with 8 points in the range of 30 µg/ml and 1 µg/ml. Straight lines were obtained in both cases with regression coefficients of 0.999. The accumulated amount of benzoate or gallate released into the solution was measured at predetermined intervals. The release kinetics for PHBV-LDH_SB-2% at different temperatures, which were 4 °C, 10 °C, 21 °C, 35 °C, and 45 °C, was studied as well.
6.2.6.1. Diffusion of Antimicrobial Agents

The Peppas equation or the so-called power law (Eq. 1) (Crank, 1975; Langer and Peppas, 1983; Ritger and Peppas, 1987; Ozdemir and Floros, 2001) is used to fit the early portion of the release curve (where the fractional release $M_t/M_\infty < 0.6$) in order to investigate the diffusion mechanism.

$$\frac{M_t}{M_\infty} = k t^n$$  \hspace{1cm} (1)

Where, $M_t =$ amount of antimicrobial agents released at time $t$

$M_\infty =$ amount of antimicrobial agents released at equilibrium

$k$ describes the polymer solvent interaction and contact between two phases:

$k < 1$ for insufficient contact

$k = 1$ for ideal system

$k > 1$ for structural changes due to swelling

$n$ describes the transport mechanism or diffusional exponent characteristic of release mechanism

$n = 0.5$ (Case I transport) shows Fickian diffusion & release is proportional to $\sqrt{t}$

$n = 1.0$ (Case II transport) shows non-Fickian mode & release is proportional to $t$

$0.5 < n < 1.0$ (Case III transport) shows anomalous or non-Fickian diffusion

$n < 0.5$ shows a pseudo-Fickian behavior, where the sorption curves resemble Fickian diffusion curves.

6.2.6.2. Effective Diffusivity Calculation

In one dimensional diffusion, diffusivity (diffusion coefficient) is defined as the rate of transfer of the diffusing substance across unit area of a section divided by the space gradient of concentration at that section (Crank, 1975).

Fick’s second law of diffusion can be written as follows for unidirectional diffusion (Crank, 1975):

$$\frac{\partial c_a}{\partial t} = D \frac{\partial^2 c_a}{\partial x^2}$$  \hspace{1cm} (2)
Where $C_a$ is the concentration of diffusion substance in the film; $D$ is diffusivity; $x$ is coordinate dimension in the direction of transport; and $t$ is time.

Different analytical solutions are available for Eq. (2) depending on the conditions to which the thin film is subjected. For a thin film of thickness $h$ ($-h/2 < x < 2/h$) containing initially uniform concentration of additive ($C_0$), the following conditions apply:

Initial condition: $C_a = C_0$ at $t = 0$

Boundary condition 1: $C_a = 0$ at $x = -h/2$

Boundary condition 2: $C_a = 0$ at $x = h/2$.

The analytical solution for Eq. (2) can be obtained (Crank, 1975):

$$
\frac{C_a}{C_0} = \frac{4}{\pi} \sum_{n=0}^{\infty} \frac{(-1)^n}{2n+1} \exp \left\{ \frac{-D(2n+1)^2 \pi^2 t}{h^2} \right\} \cos \left( \frac{(2n+1)\pi x}{h} \right)
$$

(3)

The fractional solute desorption from the film is the same as the sorption fraction to the food or simulation solution. Therefore, from the above equation, the fractional migration, which is the ratio of the total migration to the maximum migration, is shown in Eq. (4) (Crank, 1975):

$$
\frac{M_t}{M_\infty} = 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2 \pi^2} \exp \left\{ \frac{-D(2n+1)^2 \pi^2 t}{h^2} \right\}
$$

(4)

For short time, Eq. (4) can be written:

$$
\frac{M_t}{M_\infty} = 4 \left( \frac{Dt}{h^2} \right)^{1/2} \left\{ \pi^{-1/2} + 2 \sum_{n=1}^{\infty} (-1)^n ierfc \left( \frac{nh}{2\sqrt{Dt}} \right) \right\}
$$

(5)

The ($ierfc$) is an associated function of the mathematical error function ($erfc$). Both functions are defined and calculated by Crank (1975).

When $(M_t/M_\infty) < 2/3$, the Eq. (5) can be simplified to Eq. (6) to accurately estimate the release profile (Redl and others 1996; Han and Floros 1998; Han and Floros 2000; Teerakarn and others 2002):
\[
\frac{M_t}{M_\infty} = 4 \left( \frac{Dt}{\pi h^2} \right)^{1/2} = kt^{1/2}
\] (6)

If diffusion is Fickian with constant diffusivity \(D\), a plot of \(M_t/M_\infty\) vs \(\sqrt{t}\) will yield a straight line. The slope of this line, which is represented by \(k\) is used for diffusivity calculation (Teerakarn and others 2002; Chacko 2008).

\[
D = \left( \frac{kh}{4} \right)^2 \pi
\] (7)

where, \(h\) = film thickness (cm) and \(k\) is the slope of antimicrobial agents desorption curve.

### 6.2.6.3. Weibull Model

The antimicrobial agent release profile is also modeled using a Weibull equation (Eq. 8). Kosmidis et al. (2003) and Paradopoulou et al. (2006) used this model to describe the entire drug release kinetics and found that the exponent of time \(\beta\) of the Weibull function was linearly related to the exponent \(n\) of the power law derived from the analysis of the first 60% of the release curves. Origin 8.5 is used for the mathematical modeling.

\[
\frac{M_t}{M_\infty} = 1 - \exp \left( -\frac{t}{\alpha} \right)^\beta = 1 - \exp(-k_\alpha)^\beta
\] (8)

Where, \(M_t\) = amount of antimicrobial agents released at time \(t\)

\(M_\infty\) = amount of antimicrobial agents released at equilibrium

\(\alpha\) = scale parameter

\(k_\alpha\) = kinetic constant

\(\beta\) = Weibull shape parameter.
6.2.6.4. Activation Energy Determination

To assess the effect of temperature on the diffusion behavior of antimicrobial agents from PHBV films with modified LDH into DI water, Arrhenius equation (9) or (10) was used to determine the activation energy using diffusivity calculated in Section 6.2.6.2 or kinetic constant from Weibull model in Section 6.2.6.3:

\[
D = D_0 e^{-E_a^D/RT} \quad (9) \\
\]

\[
k_\alpha = k_{\alpha 0} e^{-E_a^k/RT} \quad (10)
\]

where:
D = diffusivity
\(D_0\) = pre-exponential factor of diffusion
\(E_a^D\) = activation energy using diffusivity
\(k_\alpha\) = kinetic constant from Weibull model
\(k_{\alpha 0}\) = pre-exponential factor of diffusion
\(E_a^k\) = activation energy using kinetic constant from Weibull model
R = gas constant, 8.3145 J/mol
T = temperature, K

The Eq. (9)&(10) can be converted into:

\[
\ln D = \ln D_0 - E_a^D/R \cdot (1/T)
\]

\[
\ln k_\alpha = \ln k_{\alpha 0} - E_a^k/R \cdot (1/T)
\]

Therefore, \(E_a\) was obtained from the slope of a plot of reciprocal of temperature (1/T) versus the natural logarithm of D or \(k_\alpha\). (\(E_a = -\text{slope \times R}\))

6.2.7. Statistical Analyses

All statistical analysis was done using Minitab 15.1 (Minitab Inc., State College, PA, USA). One-way analysis of variance (ANOVA) were applied to study the effect of different incorporation methods of antimicrobial agents (benzoate and gallate) into PHBV
on mechanical properties of the films and effective diffusivity of benzoate or gallate and followed by Tukey’s tests at 95% confidence level for multiple comparisons among treatments. Two-way analysis of variance using general linear model procedure followed by Tukey’s multiple comparison tests at 95% confidence level was applied to study the effects of type and concentration of LDH modified by sodium benzoate (LDH-SBs) on effective diffusivity of benzoate, n in power law model and Weibull shape factor β.

6.3. Results and Discussion

6.3.1. Sodium Benzoate

6.3.1.1. Effect of Incorporation Methods on Mechanical Properties

Sodium benzoate was incorporated into PHBV films using three different methods, including directly incorporated into the PHBV matrix, directly incorporated into the PHBV matrix with unmodified LDH nanoparticle, and modified onto LDH nanolayers before incorporation. Mechanical properties of PHBV films prepared by these three methods with two concentrations of sodium benzoate, 0.42% and 1.05% are shown in Table 6.3 and 6.4, respectively. All three measured parameters, ultimate tensile strength, elongation at break, and elastics modulus, decreased with sodium benzoate directly incorporated into PHBV. The films with 1.05% sodium benzoate (with LDH or without LDH) became very brittle and the mechanical properties of these films decreased dramatically as shown in Table 6.4. On the contrary, the mechanical properties increased when the equivalent amount of sodium benzoate was first modified in LDH nanoparticles and then incorporated into the matrix compared to virgin PHBV film.
Table 6.3. Mechanical properties of PHBV films with 0.42% sodium benzoate using different incorporation methods

<table>
<thead>
<tr>
<th>Sample</th>
<th>UTS(MPa)</th>
<th>EM (GPa)</th>
<th>EL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHBV</td>
<td>16.79±0.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.25±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.42±0.61&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PHBV-SB0.42%</td>
<td>11.44 ± 0.97&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.73 ± 0.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.39 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PHBV-SB0.42%&amp;LDH1.58%</td>
<td>12.80 ± 0.98&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.79 ± 0.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.07 ± 0.63&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PHBV-LDH_SB2-2%</td>
<td>26.01 ± 1.69&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.38 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.75 ± 1.12&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Three incorporation methods: sodium benzoate either directly incorporated into PHBV without LDH or with LDH or modified onto LDH nanolayers first and then incorporated into PHBV.

Values are the mean ± one standard deviation. Means in the same column followed by the same letter are not significantly different (P > 0.05). UTS: ultimate tensile strength; EM: elastic modulus; EL: elongation at break.

Table 6.4. Mechanical properties of PHBV films with 1.05% sodium benzoate using different incorporation methods

<table>
<thead>
<tr>
<th>Sample</th>
<th>UTS(MPa)</th>
<th>EM (GPa)</th>
<th>EL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHBV</td>
<td>16.79±0.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.25±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.42±0.61&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PHBV-SB1.05%</td>
<td>2.27±0.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.76±0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.57±0.21&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>PHBV-SB1.05%&amp;LDH3.95%</td>
<td>6.53±1.181&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.99±0.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.04±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>PHBV-LDH_SB2-5%</td>
<td>18.97 ± 0.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.95 ± 0.04&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>7.50 ± 0.36&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Three incorporation methods: sodium benzoate either directly incorporated into PHBV without LDH or with LDH or modified onto LDH nanolayers first and then incorporated into PHBV.

Values are the mean ± one standard deviation. Means in the same column followed by the same letter are not significantly different (P > 0.05). UTS: ultimate tensile strength; EM: elastic modulus; EL: elongation at break.

6.3.1.2. Effect of Incorporation Methods on Release Kinetics

Figures 6.1 and 6.2 show the release profiles of benzoate in DI water from PHBV films prepared by the three methods with 0.42% and 1.05% of benzoate, respectively. M<sub>t</sub> and M<sub>∞</sub> are amounts of benzoate released at time t and equilibrium, respectively, and M<sub>0</sub> is the initial amount of benzoate in the film before release. Not all benzoate incorporated into the polymer was released for all films, and more benzoate was released when it was incorporated into polymer with unmodified LDH (Figure 6.1 a and Figure 6.2 a), compared to the other two methods, without unmodified LDH or with modified LDH.
Phase-separated morphology was formed for composite films with unmodified LDH according to XRD and TEM data in section 5.3.1 and 5.3.2. These unmodified nanoparticles would aggregate together and form tactoids and more tactoids are formed with higher concentration of unmodified LDH. The presence of the large tactoids resulted in damage of the network in the film, and thus less benzoate was trapped in the network of the film. Therefore, more benzoate can be released from the film with unmodified LDH. With 1.58% of unmodified LDH, 37% of initial benzoate in the film was released, and 56% of that was released from the film with 3.95% of unmodified LDH. With modified LDH, fewer tactoids existed in the films due to the formation of exfoliated/phase-separated morphology. Therefore, less percentage of initial benzoate in the films with modified LDH was released, compared to the film with unmodified LDH, but higher than the film without any nanoparticles. The release profiles as $M_t/M_\infty$ vs. time are shown in Figure 6.1 b and Figure 6.2 b. Unmodified LDH did not show effect on the release profiles. However, the release of benzoate from PHBV film with modified LDH was slower than the release of benzoate simply blended to PHBV. The difference in the release profiles among these three methods was larger with higher concentrations of benzoate (1.05%) in the matrix.

A power law model (Eq. 1) was used to determine if the benzoate diffusion is Fickian or non-Fickian. The parameters in Eq. (1) were calculated from the plot of ln ($M_t/M_\infty$) vs. ln (t) of the first 60% of benzoate release (Figures 6.3 and 6.4). When only benzoate incorporated into PHBV, the value of $n$ was lower than 0.5, which indicated that the diffusion exhibits pseudo-Fickian behavior. For Fickian diffusion, the rate of diffusion was much less than that of the polymer segment mobility (Marom 1985). Sorption equilibrium is rapidly established, leading to time-independent boundary conditions that exhibit no dependence on swelling kinetics. The values of $n$ were very close to each other when benzoate simply mixed into PHBV with or without unmodified LDH, which indicates the mechanism of diffusion did not change with addition of unmodified LDH. However, the value of $n$ became higher when benzoate first modified onto LDH nanolayers and then incorporated in PHBV. The change indicates that some other mechanisms may get introduced with LDH modified by benzoate, such as surface
sorption of the benzoate on the nanoparticles and interaction between benzoate and nanolayers.

Figure 6.1. Release of benzoate in DI water at 21°C as: (a) release fraction of $M_t/M_0$; and (b) release fraction of $M_t/M_\infty$ from PHBV films with 0.42% sodium benzoate using different incorporation methods of benzoate either directly incorporated into PHBV without LDH or with LDH or modified onto LDH nanolayers first and then incorporated in PHBV. $M_0$, $M_t$, and $M_\infty$ are initial amount of benzoate in the film before release, and amounts of benzoate released at time $t$ and equilibrium, respectively.
Figure 6.2. Release of benzoate in DI water at 21°C as: (a) release fraction of $M_t/M_0$; and (b) release fraction of $M_t/M_\infty$ from PHBV films with 1.05% sodium benzoate using different incorporation methods of benzoate either directly incorporated into PHBV without LDH or with LDH or modified onto LDH nanolayers first and then incorporated in PHBV. $M_0$, $M_t$, and $M_\infty$ are initial amount of benzoate in the film before release, and amounts of benzoate released at time $t$ and equilibrium, respectively.
Figure 6.3. Plot of $\ln \left( \frac{M_t}{M_\infty} \right)$ vs $\ln (t)$ for the early portion release of benzoate in DI water at 21°C from PHBV films with 0.42% sodium benzoate using different incorporation methods of benzoate either directly incorporated into PHBV without LDH or with LDH or modified onto LDH nanolayers first and then incorporated in PHBV. $M_t$ and $M_\infty$ are amounts of benzoate released at time $t$ and equilibrium, respectively. Symbols represent the experimental data and lines show the trend. The slope of the curve represented by $n$ (power law function shown in the inset) indicates the mechanism of release.
Figure 6.4. Plot of \( \ln \left( \frac{M_t}{M_\infty} \right) \) vs \( \ln (t) \) for the early portion release of benzoate in DI water at 21°C from PHBV films with 1.05% sodium benzoate using different incorporation methods of benzoate either directly incorporated into PHBV without LDH or with LDH or modified onto LDH nanolayers first and then incorporated in PHBV. \( M_t \) and \( M_\infty \) are amounts of benzoate released at time \( t \) and equilibrium, respectively. Symbols represent the experimental data and lines show the trend. The slope of the curve represented by \( n \) (power law function shown in the inset) indicates the mechanism of release.

6.3.1.2.1. Benzoate Effective Diffusivity (\( D_{\text{eff}} \)) Calculation

A plot of \( \frac{M_t}{M_\infty} \) vs \( t \) shows the release profile of benzoate in DI water. If diffusion is Fickian with constant diffusivity \( D \), plot of \( \frac{M_t}{M_\infty} \) vs \( \sqrt{t} \) would yield a straight line from Eq. (6). According to the power law model in previous section, the diffusion was pseudo-Fickian, therefore, Eq. (6) is used to calculate the so-called effective diffusivity (\( D_{\text{eff}} \)). In order to normalize the effect of thickness on the release profiles, \( \frac{M_t}{M_\infty} \) vs \( \sqrt{t/h} \) is plotted as shown in Figures 6.5 and 6.6. Therefore, the Eq. (6) can be expressed as

\[
\frac{M_t}{M_\infty} = 4 \left( \frac{Dt}{\pi h^2} \right)^{0.5} = k' \frac{\sqrt{t}}{h} \tag{11}
\]
The data was fitted into a linear relationship to get the slope $k'$, which was used for calculating effective diffusivity $D_{eff}$ using following equation:

$$D_{eff} = \left(\frac{k'}{4}\right)^2 \pi$$

The calculated effective diffusivity values are shown in Table 6.5.
Figure 6.6. Plot of $M_t/M_\infty$ vs $\sqrt{t/h}$ for early portion of benzoate release ($M_t/M_\infty < 0.6$) in DI water at 21°C from PHBV films with 1.05% sodium benzoate using different incorporation methods of benzoate either directly incorporated into PHBV without LDH or with LDH or modified onto LDH nanolayers first and then incorporated in PHBV. $M_t$ and $M_\infty$ are amounts of benzoate released at time $t$ and equilibrium, respectively. Symbols represent experimental data and lines show the trend.

Table 6.5. Effective diffusivity of benzoate released in DI water at 21°C from PHBV films using different incorporation methods

<table>
<thead>
<tr>
<th>Sample</th>
<th>Effective diffusivity (m²/s) × $10^{-16}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHBV-SB0.42%</td>
<td>6.71 ± 2.99&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>PHBV-SB0.42%&amp;LDH1.58%</td>
<td>7.21 ± 1.34&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>PHBV-LDH_SB2-2%</td>
<td>4.60 ± 1.48&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>PHBV-SB1.05%</td>
<td>9.55 ± 1.47&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>PHBV-SB1.05%&amp;LDH3.95%</td>
<td>14.97 ± 2.84&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PHBV-LDH_SB2-5%</td>
<td>3.41 ± 0.27&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Three incorporation methods: sodium benzoate either directly incorporated into PHBV without LDH or with LDH or modified onto LDH nanolayers first and then incorporated into PHBV. Values are the mean ± one standard deviation. Means in the same column followed by the same letter are not significantly different ($P > 0.05$).
It is hypothesized that the presence of nanoparticles provides a tortuous pathway for active molecules to diffuse out leading to a slower release rate (Sorrentino and others 2007). It is shown that the presence of unmodified LDH did not change the effective diffusivity at low concentration (0.42% of benzoate and 1.58% of LDH), but increased it at high concentration (1.05% of benzoate and 3.95% of LDH), which is opposite to the expect effect. This behavior suggested that unmodified LDH nanoparticles did not act as impermeable layers to delay the diffusion of benzoate molecules as hypothesized. The reason can be that the unmodified LDH nanoparticles were aggregated together when they were incorporated into PHBV matrix because of the incompatibility between the nanoparticles and matrix (See section 5.3.1 and 5.3.2. Investigation of Morphology). Therefore, the barrier effect of nanoparticles was limited. With higher concentration of unmodified LDH (3.95%), the effective diffusivity was higher than the film without unmodified LDH; this could be explained by the mechanism used for water vapor permeability in section 5.3.8. The increase of effective diffusivity for the film with unmodified LDH may be ascribed to increased driving force for diffusion due to the interaction between nanoparticles and benzoate anions. Moreover, the effective diffusivity for the PHBV films with benzoate modified onto LDH nanolayers decreased compared to the PHBV films with benzoate simply blended to the matrix. This effect may be caused by two factors, one is the de-intercalation process from the nanolayers and the rate of the process is dependent on the electronic and steric structure of the intercalated anions. The other was that nanoparticles were exfoliated into nanolayers because of the increased compatibility between nanoparticles and matrix, which provided the tortuous pathway for benzoate molecules to diffuse out of the film.

**6.3.1.2.2. Benzoate Release: Weibull modeling**

The Weibull model was used to describe the benzoate release profile. Compared to the power law for analysis of the first 60% of the release curve, the Weibull model can be used for analysis of the entire release process.
Figure 6.7. Experimental (symbols) and Weibull predicted (lines) benzoate release in DI water at 21°C as release fraction of $M_t/M_\infty$ from PHBV films using different incorporation methods of benzoate either directly incorporated into PHBV without LDH or with LDH or modified onto LDH nanolayers first and then incorporated in PHBV. $M_t$ and $M_\infty$ are amounts of benzoate released at time $t$ and equilibrium, respectively.
Figure 6.7 demonstrates the experimental and predicted (Weibull model) benzoate release from PHBV films with different incorporation methods plotted as a function of time. The Weibull model gave an excellent prediction of experimental data with a coefficient of determination ($R^2$) 0.98 and above. The parameters $\alpha$ and $\beta$ are shown in Table 6.6.

Table 6.6. Parameters $\alpha$ and $\beta$ calculated from Weibull model and $n$ from power law model for benzoate release in DI water at 21°C from PHBV films using different incorporation methods

<table>
<thead>
<tr>
<th>Sample</th>
<th>Weibull Model Parameters</th>
<th>Power law model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Scale parameter $\alpha$ (hours)</td>
<td>Weibull shape factor $\beta$</td>
</tr>
<tr>
<td>PHBV-SB0.42%</td>
<td>16.6</td>
<td>0.43</td>
</tr>
<tr>
<td>PHBV-SB0.42%&amp;LDH1.58%</td>
<td>31.5</td>
<td>0.44</td>
</tr>
<tr>
<td>PHBV-LDH_SB2-2%</td>
<td>41.6</td>
<td>0.54</td>
</tr>
<tr>
<td>PHBV-SB1.05%</td>
<td>13.2</td>
<td>0.28</td>
</tr>
<tr>
<td>PHBV-SB1.05%&amp;LDH3.95%</td>
<td>14.6</td>
<td>0.29</td>
</tr>
<tr>
<td>PHBV-LDH_SB2-5%</td>
<td>75.9</td>
<td>0.54</td>
</tr>
</tbody>
</table>

Three incorporation methods: sodium benzoate either directly incorporated into PHBV without LDH or with LDH or modified onto LDH nanolayers first and then incorporated into PHBV.

The scale parameter, $\alpha$, defines the rate and represents the time needed to accomplish 63% of the process (Marabi and others 2003). As seen in Table 6.6, it was taken much longer time to accomplish 63% of the release for benzoate modified onto the LDH nanolayers compared to the benzoate simply blended into the matrix. This observation demonstrated that initial release was much faster for benzoate simply blended into the matrix compared to that for benzoate modified onto the LDH nanolayers, which was in agreement with the conclusion from effective diffusivity calculation (Table 6.5).
Weibull factor $\beta$ describes the shape of the release curve and therefore could be used to describe diffusional mechanism. Papadopoulou et al. (2006) summarized the diffusional mechanism in connection with the specific $\beta$ values of the Weibull function: 

$\beta < 0.75$ indicates Fickian diffusion;

$0.75 < \beta < 1$ indicates a combined mechanism (Fickian and case II transport);

$\beta = 1$ indicates first order kinetics;

$\beta > 1$ indicates a complex mechanism.

According to the previous works (Paradopoulou and others 2006; Kosmidis and others 2003; Kosmidis and Argyrakis 2003), for values of $\beta$ lower than 0.75 the release followed Fickian diffusion either in Euclidian ($0.69 < \beta < 0.75$) or fractal space, $\beta < 0.69$. The increase of $\beta$ reflected the decrease of the disorder of the medium. However, unlike the ‘n’ component in ‘power law model’, Weibull shape factor $\beta$ did not differentiate between Fickian and pseudo-Fickian diffusion. As evident from the $\beta$ values (Table 6.5), PHBV films with benzoate indicated Fickian diffusion in fractal space. $\beta$ values for PHBV films with benzoate modified onto LDH nanolayers were higher than these for PHBV films with benzoate simply blended to the matrix, and the $\beta$ values decreased with the increase of benzoate concentration. These results indicated that the PHBV films with free benzoate molecules provide a highly disordered medium ($\beta < 0.35$), and PHBV films with benzoate modified onto LDH nanolayers provided a space in fractal substrate morphologically similar to the percolation cluster ($0.35 < \beta < 0.69$). For the film with benzoate modified onto LDH nanolayers, the benzoate was bonded with LDH nanolayers instead of bonding with polymer chains resulting in changes of the film structure.

A linear relationship ($R^2 = 0.962$) was found between the exponent $n$ of the power law model derived from the analysis of the first 60% of the release process and the Weibull shape factor $\beta$ from the analysis of the entire release process based on data for the PHBV films with benzoate using three different incorporating methods (Figure 6.8). The linear equation derived is $\beta = 1.347n + 0.031$. Similar linear relationship was reported by Papadopoulou et al (2006) based on literature data, as $\beta = 1.408n + 0.110$ ($R^2 = 0.894$). The linear relationship established indicated not only the mathematical
relevance of the exponents $\beta$ and $n$, but also the physical connection of the models’ parameters and the release mechanism. Therefore, the theories developed from power law model and Weibull model regarding to the release mechanisms may be shared.

![Figure 6.8](image)

Figure 6.8. Linear regression of parameters $n$ from power law model and $\beta$ from Weibull model for benzoate release from PHBV films.

### 6.3.1.3. Effect of Concentrations and Types of Modified Nanoparticles in the Films on Release Kinetics

PHBV films were incorporated with varying concentrations of three types of nanoparticles, LDH-SB1 with 12.3 wt % benzoate, LDH-SB2 with 20.9 wt % benzoate, and LDH-SB3 with 34.9 wt % benzoate, in order to study the effect of concentrations and types of LDH on the release profile. Figures 6.9 to 6.11 demonstrate the release profiles of benzoate as release fraction of $M_t/M_0$ from PHBV films with varying concentrations of (2%, 5%, and 10%) of three types of nanoparticles, LDH-SB1, LDH-SB2, and LDH-SB3, respectively. $M_t$ and $M_0$ are amounts of benzoate released at time $t$ and the initial amount of benzoate in the film before release. For PHBV-LDH_SB1-2%, about 25% of initial benzoate in the film was able to be released, and then more benzoate was released up to about 45% of initial benzoate in the film with increase of the loading of benzoate in the
nanoparticles and the concentration of the nanoparticles. Considering the PHBV films with different types of LDH at the same concentration level, the films with LDH-SB1 always had the lowest percentage of initial benzoate released. The only one was out of this trend was PHBV-LDH-SB2-10%, and the reason for this was unclear. LDH-SB1 had the lowest loading of benzoate in the nanoparticles, so the benzoate may be easily buried between the nanolayers, which made less benzoate able to be released. The difference of released fraction among concentrations for LDH-SB3 was getting smaller, which could be due to reaching the saturation concentration of benzoate in the polymer since LDH-SB3 has highest loading of benzoate in the nanoparticles.

![Graph showing release of benzoate](image)

Figure 6.9. Release of benzoate in DI water at 21°C as release fraction of $M_t/M_0$ from PHBV films with various concentrations of modified LDH with 12.3% benzoate (LDH-SB1). $M_0$ and $M_t$ are initial amount of benzoate in the film before release and amount of benzoate released at time $t$, respectively.
Figure 6.10. Release of benzoate in DI water at 21°C as release fraction of $M_t/M_0$ from PHBV films with various concentrations of modified LDH with 20.9% benzoate (LDH-SB2). $M_0$ and $M_t$ are initial amount of benzoate in the film before release and amount of benzoate released at time $t$, respectively.

Figure 6.11. Release of benzoate in DI water at 21°C as release fraction of $M_t/M_0$ from PHBV films with various concentrations of modified LDH with 34.9% benzoate (LDH-SB3). $M_0$ and $M_t$ are initial amount of benzoate in the film before release and amount of benzoate released at time $t$, respectively.
Figures 6.12 to 6.14 show the release profiles of benzoate as release fraction of $M_t/M_\infty$ from PHBV films with varying concentrations of (2%, 5%, and 10%) of three types of nanoparticles, LDH-SB1 with 12.3 wt % benzoate, LDH-SB2 with 20.9 wt % benzoate, and LDH-SB3 with 34.9 wt % benzoate, respectively. $M_t$ and $M_\infty$ are amounts of benzoate released at time $t$ and equilibrium, respectively. It is shown that the benzoate release profile is not significantly affected by the concentration of the nanoparticles. Equilibrium was reached in shorter time for films with LDH-SB1 than that for films with LDH-SB2 and LDH-SB3. For PHBV-LDH-SB1-2%, which had the lowest amount of benzoate in the film, it took the shortest time to reach the equilibrium, which was about 75 hours.

The power law model was also used to determine if the benzoate diffusion was Fickian or non-Fickian. The parameters in Eq. (1) were calculated from the plot of ln $(M_t/M_\infty)$ vs. ln (t) of the first 60% of benzoate release (Figures B.1 to B.3 in Appendix B). Two-way analysis of variance (Table B.1 in Appendix B) showed that the effects of nanoparticle type, concentration, and interaction between type and concentration on parameter $n$ were significant ($P < 0.05$). The values of $n$ were all lower than 0.5 (Table 6.7), which indicated that the diffusion mechanism exhibited pseudo-Fickian behavior and the type and concentration of nanoparticles did not change the overall diffusion mechanism, however, different type and concentration of nanoparticles may affect the physical structure of the film which resulted in the change of parameter $n$. 
Figure 6.12. Release of benzoate in DI water at 21°C as release fraction of $M_t/M_\infty$ from PHBV films with various concentrations of modified LDH with 12.3% benzoate (LDH-SB1). $M_t$ and $M_\infty$ are amounts of benzoate released at time $t$ and equilibrium, respectively.

Figure 6.13. Release of benzoate in DI water at 21°C as release fraction of $M_t/M_\infty$ from PHBV films with various concentrations of modified LDH with 20.9% benzoate (LDH-SB2). $M_t$ and $M_\infty$ are amounts of benzoate released at time $t$ and equilibrium, respectively.
Figure 6.14. Release of benzoate in DI water at 21°C as release fraction of $M_t/M_\infty$ from PHBV films with various concentrations of modified LDH with 34.9% benzoate (LDH-SB3). $M_t$ and $M_\infty$ are amounts of benzoate released at time $t$ and equilibrium, respectively.

Table 6.7. Parameter n calculated from power law model for benzoate release in DI water at 21°C from PHBV films with different concentrations of LDH modified by sodium benzoate, LDH-SB1 with 12.3% benzoate, LDH-SB2 with 20.9% benzoate, or LDH-SB3 with 34.9% benzoate

<table>
<thead>
<tr>
<th>LDH concentration</th>
<th>Power Law Model Parameter n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LDH-SB1</td>
</tr>
<tr>
<td>2%</td>
<td>0.249 ± 0.008$^a$</td>
</tr>
<tr>
<td>5%</td>
<td>0.280 ± 0.019$^{a,b}$</td>
</tr>
<tr>
<td>10%</td>
<td>0.400 ± 0.018$^c$</td>
</tr>
</tbody>
</table>

Values are the mean ± one standard deviation. Means followed by the same letter are not significantly different ($P > 0.05$).
6.3.1.3.1. Benzoate Effective Diffusivity (D<sub>eff</sub>) Calculation

The diffusivities for all PHBV films were calculated using the same method as described in section 6.3.1.2.1. From two-way ANOVA table (Table B.2 in Appendix B), both concentration and type of LDH-SB had significant effects on the effective diffusivity of benzoate release (P < 0.05). Furthermore, the interaction between these two factors had significant effect as well. As Table 6.8 shown, PHBV films with LDH-SB2 always had the slowest release rate regardless of the level of concentration. As mentioned previously, two factors from modified nanoparticles may slow down the benzoate release, one was barrier effect provided by the exfoliated nanolayers, and the other was the interaction between the nanolayers and benzoate. It is also shown that the PHBV films with 5% nanoparticles had lower effective diffusivity than with 2% or 10% nanoparticles. The lowest effective diffusivity was reached by sample PHBV-LDH_SB2-5%, which was 3.41 × 10⁻¹⁶ m²/s.

Table 6.8. Effect of type (modified LDH with 12.3% benzoate (LDH-SB1), 20.9% benzoate (LDH-SB2), and 34.9% benzoate (LDH-SB3)) and concentration of on effective diffusivity of benzoate release in DI water at 21°C

<table>
<thead>
<tr>
<th>LDH concentration</th>
<th>Effective diffusivity (m²/s) × 10⁻¹⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LDH-SB1</td>
</tr>
<tr>
<td>2%</td>
<td>6.81 ± 1.30&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>5%</td>
<td>6.53 ± 0.91&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>10%</td>
<td>7.07 ± 0.60&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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

6.3.1.3.2. Benzoate Release: Weibull Modeling

Successful fittings were obtained when Weibull model (Eq. 8) was fitted to the entire release curve. One representative release profile for each sample was shown in Figure 6.15. The coefficient of determination (R²) was 0.98 and above (Figure 6.15).
From Table 6.9, all films had the Weibull shape factor ($\beta$) in the range of 0.35-0.69, which indicated Fickian diffusion (Paradopoulou and others 2006) and the type and concentration of nanoparticles did not change the overall diffusion mechanism. However, two-way analysis of variance (Table B.4 in Appendix B) showed that the effects of nanoparticle type, concentration, and interaction between type and concentration on parameter $\beta$ were significant ($P < 0.05$). The results agreed with these in the power law model fitting relative to the parameter $n$ (Section 6.3.1.3). The change of $\beta$ may be due to the change of physical structure of the film with different type and concentration of nanoparticles, however, the difference of physical structure did not change the overall diffusion mechanism of the film. It is shown that LDH-SB1 and LDH-SB3 have the opposite effect on $\beta$ in term of concentration and $\beta$ is significantly decreased with 10% of LDH-SB3, which may be due to the change of physical structure of the film caused by the benzoate absorbed on the surface of nanoparticle of LDH-SB3 and the aggregation of nanoparticle at high concentration. On the other hand, the scale parameter, $\alpha$, defines the rate and represents the time needed to accomplish 63% of the process (Marabi and others 2003), was influenced by the concentrations and types of LDH. Two-way analysis of variance (Table B.3 in Appendix B) showed that the effects of nanoparticle type and concentration on parameter $\alpha$ were significant ($P < 0.05$), but the interaction between type and concentration were not significant ($P = 0.10$). The statistical analysis showed that $\alpha$ was significantly higher for LDH-SB2 than for LDH-SB1 and LDH-SB3, and $\alpha$ was higher with 5% of nanoparticle than 2% or 10% of nanoparticles. For LDH-SB2 and LDH-SB3, films with 5% nanoparticles had the slowest release rate and films with LDH-SB2 took the longest time to accomplish 63% of the release regardless of concentration levels comparing to films with LDH-SB1 and LDH-SB3. These results were in agreement with these from the calculated effective diffusivity. The time to accomplish 63% of the release varied from 76 hours for PHBV-LDH_Sb2-5% to 11 hours for PHBV-LDH_Sb1-2%.
A. PHBV-LDH_SB1-2%

\[
\frac{M_t}{M_\infty} = 1 - \exp\left(\frac{-t}{11.4}\right)
\]

\[R^2 = 0.984\]

B. PHBV-LDH_SB1-5%

\[
\frac{M_t}{M_\infty} = 1 - \exp\left(\frac{-t}{27.6}\right)
\]

\[R^2 = 0.993\]

C. PHBV-LDH_SB1-10%

\[
\frac{M_t}{M_\infty} = 1 - \exp\left(\frac{-t}{19.5}\right)
\]

\[R^2 = 0.989\]

D. PHBV-LDH_SB2-2%

\[
\frac{M_t}{M_\infty} = 1 - \exp\left(\frac{-t}{41.6}\right)
\]

\[R^2 = 0.996\]

E. PHBV-SB2-5%

\[
\frac{M_t}{M_\infty} = 1 - \exp\left(\frac{-t}{75.9}\right)
\]

\[R^2 = 0.995\]

F. PHBV-LDH_SB2-10%

\[
\frac{M_t}{M_\infty} = 1 - \exp\left(\frac{-t}{46.8}\right)
\]

\[R^2 = 0.995\]
Figure 6.15. Experimental (symbols) and Weibull predicted (lines) benzoate release in DI water at 21°C from PHBV films with different concentrations of modified LDH with 12.3% benzoate (LDH-SB1) (A-C), 20.9% benzoate (LDH-SB2) (D-F), or 34.9% benzoate (LDH-SB3) (G-I). $M_t$ and $M_\infty$ are amounts of benzoate released at time $t$ and equilibrium, respectively.
Table 6.9. Parameters $\alpha$ and $\beta$ calculated from Weibull model for benzoate release in DI water at 21°C from PHBV films with different concentrations of LDH with 12.3% benzoate (LDH-SB1), 20.9% benzoate (LDH-SB2), or 34.9% benzoate (LDH-SB3).

<table>
<thead>
<tr>
<th>Nanoparticles</th>
<th>Concentration</th>
<th>Scale parameter $\alpha$ (hours) $\pm$</th>
<th>Weibull shape factor $\beta$ $\pm$</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH-SB1</td>
<td>2%</td>
<td>$11.4 \pm 0.4^a$</td>
<td>$0.50 \pm 0.02^b$</td>
</tr>
<tr>
<td></td>
<td>5%</td>
<td>$29.8 \pm 14.9^{a,b}$</td>
<td>$0.51 \pm 0.01^b$</td>
</tr>
<tr>
<td></td>
<td>10%</td>
<td>$28.1 \pm 9.1^{a,b}$</td>
<td>$0.59 \pm 0.02^a$</td>
</tr>
<tr>
<td>LDH-SB2</td>
<td>2%</td>
<td>$42.4 \pm 9.8^b$</td>
<td>$0.54 \pm 0.03^{a,b}$</td>
</tr>
<tr>
<td></td>
<td>5%</td>
<td>$75.9 \pm 1.6^c$</td>
<td>$0.54 \pm 0.02^{a,b}$</td>
</tr>
<tr>
<td></td>
<td>10%</td>
<td>$48.8 \pm 2.8^b$</td>
<td>$0.52 \pm 0.02^b$</td>
</tr>
<tr>
<td>LDH-SB3</td>
<td>2%</td>
<td>$32.2 \pm 8.2^{a,b}$</td>
<td>$0.55 \pm 0.02^{a,b}$</td>
</tr>
<tr>
<td></td>
<td>5%</td>
<td>$40.6 \pm 7.4^{a,b}$</td>
<td>$0.43 \pm 0.02^b$</td>
</tr>
<tr>
<td></td>
<td>10%</td>
<td>$20.1 \pm 2.3^a$</td>
<td>$0.39 \pm 0.02^c$</td>
</tr>
</tbody>
</table>

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

6.3.1.4. Effect of Temperature on Release Kinetics

Figure 6.16 shows the release profile of PHBV film with 2% of LDH-SB2 at different temperatures. The time to reach the equilibrium decreased with the increase of temperature due to the temperature dependence of diffusion. Equilibrium for benzoate release was reached after 650 h and 197 h at 4 °C and 45 °C, respectively. The plot of ln ($M_t/M_\infty$) vs. ln (t) of the first 60% of benzoate release (Figure 6.) was used to calculate the parameter $n$ for the power law model. At 4, 10, 21, and 35 °C, with $n < 0.5$, pseudo-Fickian behavior occurred. At 45 °C, $n = 0.53$, indicated Fickian behavior. In pseudo-Fickian mechanism, sorption curves resembled Fickian curves, but approach to final equilibrium was very slow (Khan and Rousseau 2006).
6.3.1.4.1. Benzoate Effective Diffusivity (D_{eff}) Calculation and Activation Energy Determination

The dependence of effective diffusivity on temperature for PHBV-LDH SB2-2% film was described using Arrhenius activation energy model. The diffusivities were calculated using the same method as described in section 6.3.1.2.1 (Table 6.10). The effective diffusivity increased with the increase of temperature. The plot of ln (D) vs. T^{-1} for benzoate produced a straight line (R^2 = 0.99) without break points, which indicates that no morphological changes occurred within the film in the temperature range studied (4 to 45 °C) (Gennadios and others 1993; Redl and others 1996). The thermodynamic glass transition temperature (T_g) of PHBV in present study was measured in Chapter 4 with the value of -2.6 °C, so the film did not undergo glass transition in the temperature range studied for diffusion. The activation energy (E_a^D) was determined using the Arrhenius equation (Eq. 9) and calculated as 66.4 kJ/mol. The value was in the reasonable range compared to other polymer/antimicrobial agent systems. Teerakarn et al. (2002) reported the E_a ranged from 44 to 85 kJ/mol for different protein films (cast corn zein film, cast wheat gluten film, heat-pressed corn zein film, and heat-pressed wheat gluten film) with nisin. E_a for sorbic acid diffusivity in edible wheat gluten and lipid based films ranged from 30.0 to 39.8 kJ/mol (Redl and others 1996). E_a for propyl paraben was found to be 88 kJ/mol (Chung and other 2001). The value of benzoate diffusivities at temperatures that were not included in the present study can be estimated using the Arrhenius equation.
Figure 6.16. Benzoate release in DI water from PHBV film with 2% of modified LDH with 20.9% benzoate (PHBV-LDH_SB2-2%) at different temperatures. \( M_t \) and \( M_\infty \) are amounts of benzoate released at time \( t \) and equilibrium, respectively.

Figure 6.17. Plot of \( \ln (M_t/M_\infty) \) vs \( \ln (t) \) for the early portion release of benzoate from PHBV film with 2% of modified LDH with 20.9% benzoate (PHBV-LDH_SB2-2%) at different temperatures. Symbols represent the experimental data and lines show the trend. \( M_t \) and \( M_\infty \) are amounts of benzoate released at time \( t \) and equilibrium, respectively.
Table 6.10. Effective diffusivity of benzoate released in DI water from PHBV film with 2\% of modified LDH with 20.9\% benzoate (PHBV-LDH_{SB2-2\%}) at different temperatures

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Effective diffusivity (m^2/s) × 10^{-16}</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 °C</td>
<td>0.89 ± 0.23</td>
</tr>
<tr>
<td>10 °C</td>
<td>1.30 ± 0.29</td>
</tr>
<tr>
<td>21 °C</td>
<td>4.60 ± 1.48</td>
</tr>
<tr>
<td>35 °C</td>
<td>11.99 ± 1.21</td>
</tr>
<tr>
<td>45 °C</td>
<td>38.03 ± 10.59</td>
</tr>
</tbody>
</table>

Figure 6.18. Arrhenius plot of ln (effective diffusivity) versus 1/T for benzoate release in DI water at temperature range of 4 to 35 °C from PHBV film with 2\% of modified LDH with 20.9\% benzoate.
6.3.1.4.2. Weibull Modeling and Activation Energy Determination

A. 4 °C

\[ \frac{M_t}{M_m} = 1 - \exp\left(\frac{-t}{130.2}\right)^{0.69} \]
\[ R^2 = 0.950 \]

B. 10 °C

\[ \frac{M_t}{M_m} = 1 - \exp\left(\frac{-t}{150.2}\right)^{0.65} \]
\[ R^2 = 0.999 \]

C. 21 °C

\[ \frac{M_t}{M_m} = 1 - \exp\left(\frac{-t}{41.5}\right)^{0.54} \]
\[ R^2 = 0.996 \]

D. 35 °C

\[ \frac{M_t}{M_m} = 1 - \exp\left(\frac{-t}{21.9}\right)^{0.56} \]
\[ R^2 = 0.996 \]

E. 45 °C

\[ \frac{M_t}{M_m} = 1 - \exp\left(\frac{-t}{20.4}\right)^{0.52} \]
\[ R^2 = 0.992 \]

Figure 6.19. Experimental (symbols) and Weibull predicted (lines) benzoate release in DI water from PHBV film with 2% of modified LDH with 20.9% benzoate (PHBV-LDH_SB2-2%) at different temperatures.
Figure 6.19 illustrates the experimental and predicted (Weibull model) benzoate release from PHBV-LDH_SB2-2% film at different temperatures plotted as a function of time. Weibull model gave an excellent prediction of experimental data with a coefficient of determination (R^2) 0.95 and above. The parameters α and β are shown in Table 6.11.

Table 6.11. Parameters α and β calculated from Weibull model for the benzoate release in DI water from PHBV film with 2% of modified LDH with 20.9% benzoate (PHBV-LDH_SB2-2%) at different temperatures

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Scale parameter α (hours)</th>
<th>Weibull shape factor β</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>130.2</td>
<td>0.60</td>
</tr>
<tr>
<td>10</td>
<td>159.2</td>
<td>0.55</td>
</tr>
<tr>
<td>21</td>
<td>41.6</td>
<td>0.54</td>
</tr>
<tr>
<td>35</td>
<td>21.9</td>
<td>0.56</td>
</tr>
<tr>
<td>45</td>
<td>20.4</td>
<td>0.52</td>
</tr>
</tbody>
</table>

As seen in Table 6.11, the parameter α increased with the decrease of temperature, which means that longer time to complete 63% of the release process was required at lower temperature. The parameter β at all temperatures was lower than 0.69, which indicated Fickian diffusion and temperature did not change the diffusion mechanism in the studied range.

The plot of ln (k_a) vs. T^-1 for benzoate produced a straight line (R^2 = 0.90) without break points, which is similar to the plot of ln (D) vs. T^-1. The activation energy (E_a k_a) was determined using the Arrhenius equation (Eq. 10) and calculated as 39.6 kJ/mol.
Figure 6.20. Arrhenius plot of ln (kinetic constant from Weibull model) versus 1/T for benzoate release in DI water at temperature range of 4 to 35 °C from PHBV film with 2% of modified LDH with 20.9% benzoate.

6.3.2. Sodium Gallate

6.3.2.1. Effect of Incorporation Methods on Mechanical Properties

Sodium gallate was incorporated into PHBV films using three different methods, including directly incorporated into the PHBV matrix, directly incorporated into the PHBV matrix with unmodified LDH nanoparticle, and modified onto LDH nanolayers. Mechanical properties of PBHV films prepared by these three methods with two concentrations of sodium gallate, 0.88% and 2.2% are shown in Table 6.12 and 6.13, respectively. Tensile strength and elastics modulus showed no significant change with sodium gallate directly incorporated into PHBV, but elongation at break increased with sodium gallate, which may be due to the plasticizing effect of the gallate in the polymer. However, both tensile strength and elongation at break were increased when the equivalent amount of sodium gallate was first modified in LDH nanoparticles and then incorporated into the matrix compared to virgin PHBV film and the elongation at break was even higher than the film with sodium gallate directly incorporated into PHBV.
Table 6.12. Mechanical properties of PHBV films with 0.88% sodium gallate using different incorporation methods

<table>
<thead>
<tr>
<th>Sample</th>
<th>UTS (MPa)</th>
<th>EM (GPa)</th>
<th>EL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHBV</td>
<td>16.79±0.96a</td>
<td>1.25±0.02a</td>
<td>2.42±0.61a</td>
</tr>
<tr>
<td>PHBV-SG0.88%</td>
<td>19.28 ± 1.61a,b</td>
<td>1.06 ± 0.20a</td>
<td>4.12 ± 0.28b</td>
</tr>
<tr>
<td>PHBV-SG0.88%&amp;LDH1.12%</td>
<td>18.00 ± 0.95a</td>
<td>1.04 ± 0.12a</td>
<td>3.09 ± 0.10a,b</td>
</tr>
<tr>
<td>PHBV-LDH_SG-2%</td>
<td>21.07 ± 0.28b</td>
<td>0.97 ± 0.14a</td>
<td>7.52 ± 0.98c</td>
</tr>
</tbody>
</table>

Three incorporation methods: sodium gallate either directly incorporated into PHBV without LDH or with LDH or modified onto LDH nanolayers first and then incorporated into PHBV. Values are the mean ± one standard deviation. Means in the same column followed by the same letter are not significantly different \((P > 0.05)\). UTS: ultimate tensile strength; EM: elastic modulus; EL: elongation at break.

Table 6.13. Mechanical properties of PHBV films with 2.2% sodium gallate using different incorporation methods

<table>
<thead>
<tr>
<th>Sample</th>
<th>UTS (MPa)</th>
<th>EM (GPa)</th>
<th>EL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHBV</td>
<td>16.79±0.96a</td>
<td>1.25±0.02a</td>
<td>2.42±0.61a</td>
</tr>
<tr>
<td>PHBV-SG2.2%</td>
<td>15.33 ± 2.53a</td>
<td>0.89 ± 0.10b</td>
<td>3.35 ± 0.65a</td>
</tr>
<tr>
<td>PHBV-SG2.2%&amp;LDH2.8%</td>
<td>16.35 ± 1.57a</td>
<td>1.02 ± 0.18a,b</td>
<td>2.81 ± 0.18a</td>
</tr>
<tr>
<td>PHBV-LDH_SG-5%</td>
<td>17.02 ± 1.24a</td>
<td>0.99 ± 0.15a,b</td>
<td>5.06 ± 0.10b</td>
</tr>
</tbody>
</table>

Three incorporation methods: sodium gallate either directly incorporated into PHBV without LDH or with LDH or modified onto LDH nanolayers first and then incorporated into PHBV. Values are the mean ± one standard deviation. Means in the same column followed by the same letter are not significantly different \((P > 0.05)\). UTS: ultimate tensile strength; EM: elastic modulus; EL: elongation at break.

6.3.2.2. Effect of Incorporation Methods on Release Kinetics

Figure 6.21 shows the release profiles of gallate from the PHBV films prepared by the three methods mentioned above with 0.88% of gallate and Figure 6.22 shows the release profiles with 2.2% of gallate. \(M_t\) and \(M_\infty\) are amount of gallate released at time \(t\) and equilibrium, respectively. It was shown that unmodified LDH had no effect on the release profile. However, the release of gallate from PHBV film with modified LDH by
gallate was slower than the release of gallate simply blended to PHBV. It is also worth mentioning that the release of gallate was much faster than the release of benzoate from PHBV films. The reason for faster release of gallate could be due to higher polarity of gallate resulting in weaker interaction with PHBV polymer chain compared to benzoate. There was more than 70% of gallate released from PHBV films with gallate simply blended within 5 minutes; therefore, the power law model cannot be used to analyze the release profiles of these films since it can only be used to fit the first 60% of release curve. However, it took more than 15 minutes to have 60% gallate released for the films with modified LDH by gallate. The analysis using power law model and effective diffusivity calculation will be conducted for films with different concentrations of LDH-SG in the section 6.3.2.3. Successful fittings were obtained when the Weibull model was fitted to the entire release curve of benzoate; however, the Weibull model was not applicable for the release curve of gallate.

![Figure 6.21. Release of gallate in DI water at 21°C as release fraction of $M_t/M_\infty$ from PHBV films with 0.88% sodium gallate using different incorporation methods of gallate either directly incorporated into PHBV without LDH or with LDH or modified onto LDH nanolayers first and then incorporated in PHBV. $M_t$ and $M_\infty$ are amounts of gallate released at time t and equilibrium, respectively.](image)
Figure 6.22. Release of gallate in DI water at 21°C as release fraction of \( M_t/M_\infty \) from PHBV films with 2.2% sodium gallate using different incorporation methods of gallate either directly incorporated into PHBV without LDH or with LDH or modified onto LDH nanolayers first and then incorporated in PHBV. \( M_t \) and \( M_\infty \) are amounts of gallate released at time \( t \) and equilibrium, respectively.

6.3.2.3. Effect of Concentrations of Modified Nanoparticles on Release Kinetics

PHBV films were incorporated with varying concentrations of modified LDH nanoparticles with gallate (LDH-SG) in order to study the effect of concentrations of nanoparticles on the release profile. Figures 6.23 illustrates the release profiles of gallate from PHBV films with varying concentrations of (2%, 5%, and 10%) LDH-SG. The values of \( n \) in the power law model were calculated from the plot of \( \ln (M_t/M_\infty) \) vs. \( \ln (t) \) of the first 60% of gallate release shown in Figure 6.24. With \( n < 0.5 \), the diffusion exhibited pseudo-Fickian behavior for gallate.
Figure 6.23. Release of gallate in DI water at 21°C as release fraction of $M_t/M_\infty$ from PHBV films with various concentrations of modified LDH with 43.9% gallate (LDH-SG). $M_t$ and $M_\infty$ are amounts of gallate released at time $t$ and equilibrium, respectively.

Figure 6.24. Plot of $\ln \left( \frac{M_t}{M_\infty} \right)$ vs $\ln (t)$ for the early portion release of gallate in DI water at 21°C as release fraction of $M_t/M_\infty$ from PHBV films with various concentrations of modified LDH with 43.9% gallate (LDH-SG). $M_t$ and $M_\infty$ are amounts of gallate released at time $t$ and equilibrium, respectively. Symbols represent the experimental data and lines show the trend. The slope of the curve represented by $n$ (power law function shown in the inset) indicates the mechanism of release.
The diffusivities for all PHBV films were calculated using the same method as described in section 6.3.1.2.1. One-way ANOVA showed that the concentration did not significantly affect the effective diffusivity at 95% confidence level ($P = 0.099$). The effective diffusivity for gallate was higher than that for benzoate. The reason for faster release of gallate could be due to higher polarity of gallate resulting in weaker interaction with PHBV polymer chain compared to benzoate.

Table 6.14. Effect of concentration of nanoparticles modified by gallate in the films on gallate effective diffusivity

<table>
<thead>
<tr>
<th>Sample</th>
<th>Effective diffusivity (m$^2$/s) × 10$^{-16}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHBV-LDH_SG-2%</td>
<td>99.9 ± 22.7$^{a}$</td>
</tr>
<tr>
<td>PHBV-LDH_SG-5%</td>
<td>61.53 ± 25.4$^{a}$</td>
</tr>
<tr>
<td>PHBV-LDH_SG-10%</td>
<td>118.5 ± 41.9$^{a}$</td>
</tr>
</tbody>
</table>

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

6.4. Conclusions

LDH nanoparticles modified by sodium benzoate and sodium gallate having antimicrobial properties were incorporated into polyhydroxybutyrate-co-valerate, to obtain polymeric nanocomposites able to release the antimicrobial agents at slower release rates. The release of benzoate and gallate into DI water from PHBV composite films with LDH modified by benzoate and gallate followed pseudo-Fickian behavior fitted with power law model. The release of benzoate from PHBV composite films with LDH modified by benzoate was also fitted into Weibull model indicating Fickian behavior. A comparison of mechanical properties and release kinetics of antimicrobial agents directly dispersed in PHBV and loaded in the nanoparticles and then dispersed in PHBV was shown. The results indicated that mechanical properties increased and release rate decreased in the latter case. The concentration of LDH modified by sodium benzoate and the loading of benzoate in the modified LDH showed the significant effect on the release kinetics of benzoate. The diffusivities of sodium benzoate at 21 °C ranged from 3.41 to $14.97 \times 10^{-16}$.
m²/s. The slowest release rate was achieved by the PHBV nanocomposite film containing 5 % w/w of modified LDH with medium loading of benzoate (21 % w/w of benzoate) in nanoparticles. The release of gallate from PHBV was much faster than that of benzoate. The effective diffusivity of benzoate increased with increase of temperature and the activation energy Eₐ for benzoate diffusion was calculated as 66.4 kJ/mol. The results of the present investigation showed that it was possible to prepare biodegradable polymeric composites containing antimicrobial agents that can be released at different release rates and amounts when parameters such as incorporation methods, the nature of the interaction between the intercalated antimicrobial agents and the LDH layers, the amount of filler in the polymer, and the amount of antimicrobial agents in the LDH were taken into consideration. It will thus be possible to design biodegradable polymeric nanocomposites with tunable release rates of active molecules for various applications.

6.5. References


Han, J. H.; Floros, J. D., Simulating migration models and determining the releasing rate of potassium sorbate from antimicrobial plastic film. Food Science Biotechnology 2000, 9, 68-72.


Sanchez-Valdes, S.; Ortega-Ortiz, H.; Valle, L.; Medellin-Rodriguez, F. J.; Guedea-Miranda, R., Mechanical and antimicrobial properties of multilayer films with a


Chapter 7
CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH

The results presented in this study showed that the ability for antimicrobial agents to intercalate into layered double hydroxides depended on the nature of the antimicrobial agents, such as size, spatial structure, and polarity, etc. Benzoate anions can be easily intercalated into LDH and the amount of benzoate intercalated in LDH can be controlled by reaction conditions. Benzoate had the lowest molecular volume, which made it easy to intercalate into LDH. Gallate had higher molecular volume compared to benzoate, but its higher polarity due to the three hydroxide groups made it still able but required harsher conditions to intercalate into LDH compared to benzoate. Sorbate had similar molecular volume with gallate but less polarity, which made it not able to intercalate into LDH at all. Intercalation of benzoate increased the interlayer distance in LDH. The arrangement of the benzoate anion between the layers in LDH was affected by the loading of benzoate. The LDH-SBs in the present study showed the interstratified structure. Besides interstratified structure, benzoate anions were in vertical bilayer arrangements in LDH-SB3, which had the highest loading of benzoate. Gallate anions were only in horizontal arrangements in gallate-intercalated LDH because of the three hydroxide groups.

PHB and PHBV-based LDH composite films with 2% unmodified LDH and LDH modified by sodium benzoate were prepared by the solvent casting technique. Intercalated or partially exfoliated structures were obtained using modified LDH, however, only the phase-separated structure was formed using unmodified LDH. Filtration and the presence of nanoparticles significantly increased the tensile strength and elongation at break of PHBV films, but did not show significant effect on PHB films. Impurities in unfiltered PHB enhanced the nucleating step resulting in an increase of the crystallization temperature compared to the filtered version. LDH nanoparticles enhanced recrystallization during melting, which may due to the nucleation effect by nanoparticles.

The effects of type and concentration of LDH nanoparticles (unmodified LDH and modified LDH with sodium benzoate and sodium gallate) on structure and properties of
PHBV films were then studied. It appeared that the arrangement of LDH in the bio-nanocomposites matrix ranged from exfoliated to phase-separated depending on the type and concentration of LDH nanoparticles. Intercalated or partial exfoliated structures were obtained using modified LDH, however, only phase-separated structures were formed using unmodified LDH. The mechanical (tensile strength and elongation at break) and thermo-mechanical (storage modulus) properties were improved significantly with low concentrations of nanoparticles incorporated into the polymer. The incorporation of LDH modified by sodium benzoate further improved the mechanical properties in comparison with unmodified LDH, which may be due to the increased compatibility between PHBV and nanoparticles and the larger basal distance between nanolayers after modification. The concentration of benzoate anions in LDH nanoparticles was another factor which affected the properties of PHBV composite films. The PHBV film with 2% modified LDH with 20.9 % w/w of benzoate anions in LDH had the best mechanical and thermo-mechanical properties. Apparent glass transition temperature increased with the addition of modified LDH but did not change with the addition of unmodified LDH. Moreover, the effect of nanoparticles on thermal properties as well as crystallization of PHBV composites was dependent on the type of nanoparticles. Unmodified LDH and LDH modified by sodium gallate had limited influence on thermal properties. However, LDH nanoparticles modified by sodium benzoate increased the crystallization temperature and enhanced recrystallization during melting, which may due to the nucleation effect by nanoparticles. The temperature at 10 % weight loss was decreased with the addition of nanoparticles, which indicated the reduction of thermal stability, but the residue left from the polymer at 300 °C was increased and the mass loss rate was decreased. Water vapor permeability was reduced with the increase of unmodified LDH due to the barrier effect of nanolayers providing a tortuous pathway for small molecules to diffuse out of the film. Due to the hydrophilic nature of the modifiers, the water vapor permeability increased with the addition of modified LDH.

A comparison of mechanical properties and release kinetics of antimicrobial agents directly dispersed in PHBV and loaded in the nanoparticles and then dispersed in PHBV was made. The results indicated that mechanical properties increased and release rate
decreased in the latter case. The release of benzoate and gallate into DI water from PHBV composite films with LDH modified by benzoate and gallate followed pseudo-Fickian behavior fitted with power law model. The release of benzoate from PHBV composite films with LDH modified by benzoate was also fitted into Weibull model indicating Fickian behavior in fractal substrate morphologically similar to the percolation cluster. The concentration of modified LDH and the loading of benzoate in the modified LDH showed a significant effect on the release kinetics of benzoate. The diffusivities of sodium benzoate at 21 °C ranged from $3.41 \times 10^{-16}$ to $14.97 \times 10^{-16}$ m$^2$/s. The slowest release rate was achieved by the PHBV nanocomposite film containing 5 % w/w of modified LDH with medium loading of benzoate (21 % w/w of benzoate) in nanoparticles. The release of gallate from PHBV was much faster than that of benzoate. The diffusivity of benzoate increased with increase of temperature and the activation energy $E_a$ for benzoate diffusion was calculated as 66.4 kJ/mol. It will be thus possible to design biodegradable polymeric nanocomposites with a tunable release of active molecules for various applications.

All in all, the results obtained in this work showed substantial improvement of mechanical, thermal, and barrier properties of PHBV were achieved with very low concentration (2%) of unmodified and LDH modified by antimicrobial agents, which extends the application of this bio-based polymer. Moreover, these PHBV/m-LDH nanocomposite films containing antimicrobial agents can release different amounts of antimicrobial agents at different release rates when parameters such as incorporation methods, the nature of the interaction between the intercalated antimicrobial agents and the LDH layers, the amount of filler in the polymer, and the amount of antimicrobial agents in the LDH are taken into consideration.

This study suggested the potential use of these new hybrid materials with antimicrobial agents as antimicrobial food packaging. However, further study will be necessary to verify the antimicrobial activity of the PHBV/m-LDH nanocomposite films with antimicrobial agents.
Suggestions for future research include: a) to verify the antimicrobial activity of antimicrobial agents releasing films; b) to design antimicrobial films for specific food applications (such as cheese, meat, etc.) with proper release profiles and physical properties; c) the single layer of PHBV film may not meet all requirements for a food packaging system. Under this scenario, additional layers or composite films could be necessary combine with antimicrobial PHBV film to be qualified as a food packaging system. Therefore, the properties, such as mechanical, optical, and barrier properties, of the new composite film, should be measured and antimicrobial activity against several microorganisms should be tested as well.
Appendix A

CHAPTER 5 – SUPPLEMENTAL DATA

A.1. Oxygen Permeability

Oxygen transmission rates (OTR) of PHBV films were measured by Dr. Bruce Welt’s group at the University of Florida. The oxygen permeability was then calculated by:

\[ \text{Oxygen permeability} = \text{OTR (ml/m}^2/\text{day)} \times \text{Thickness (m)} \quad (1) \]

A.1.1. Results and Discussion of Oxygen Permeability

Oxygen permeability of the PHBV-LDH composite films is shown in Table A.1. The deviation of the measurement was fairly high, which may be due to the poor uniformity in thickness of the film or errors during testing due to the limitations of the instrument. Because of the high deviation, statistical conclusion was not able to be made from the current data set. However, by briefly reviewing the data, oxygen permeability did not change with the addition of unmodified LDH, but reduced with the low concentration of modified LDH. The decrease of oxygen permeability was possibly due to the barrier effect of the nanolayers, which provided a torturous pathway for the oxygen molecule to diffuse through the film. However, oxygen permeability increased with the high concentration of modified LDH (10%), especially for modified LDH with high modifier loading (LDH-SB3 and LDH-SG). Films with even thickness and better instrument to measure the oxygen permeability are required in order to further study the effect of nanoparticles on the oxygen permeability of PHBV films.
Table A.1. Effect of type and concentration of nanoparticles on oxygen permeability (O2P) of PHBV composite films

<table>
<thead>
<tr>
<th>LDH content</th>
<th>Oxygen Permeability (ml·m/m²/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LDH</td>
</tr>
<tr>
<td>0%</td>
<td>0.56±0.62</td>
</tr>
<tr>
<td>2%</td>
<td>0.55±0.81</td>
</tr>
<tr>
<td>5%</td>
<td>0.39±0.20</td>
</tr>
<tr>
<td>10%</td>
<td>0.91±1.18</td>
</tr>
</tbody>
</table>

Table A.2. Two-way ANOVA table for ultimate tensile strength

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Seq SS</th>
<th>Adj SS</th>
<th>Adj MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type*</td>
<td>4</td>
<td>73.377</td>
<td>73.377</td>
<td>18.344</td>
<td>12.82</td>
<td>0.000</td>
</tr>
<tr>
<td>Concentrations**</td>
<td>3</td>
<td>225.924</td>
<td>225.924</td>
<td>75.308</td>
<td>52.65</td>
<td>0.000</td>
</tr>
<tr>
<td>Types*Concentrations</td>
<td>12</td>
<td>269.219</td>
<td>269.219</td>
<td>22.435</td>
<td>15.68</td>
<td>0.000</td>
</tr>
<tr>
<td>Error</td>
<td>40</td>
<td>57.214</td>
<td>57.214</td>
<td>1.430</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>59</td>
<td>625.734</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Types of nanoparticles: unmodified LDH, LDH modified by sodium benzoate: LDH-SB1 with 12.3% benzoate; LDH-SB2 with 20.9% benzoate; and LDH-SB3 with 34.9% benzoate.
** Concentrations of nanoparticles: 0%, 2%, 5%, and 10%.

Table A.3. Two-way ANOVA table for elastic modulus

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Seq SS</th>
<th>Adj SS</th>
<th>Adj MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type*</td>
<td>4</td>
<td>0.26751</td>
<td>0.26751</td>
<td>0.06688</td>
<td>6.50</td>
<td>0.000</td>
</tr>
<tr>
<td>Concentrations**</td>
<td>3</td>
<td>0.21097</td>
<td>0.21097</td>
<td>0.07032</td>
<td>6.83</td>
<td>0.001</td>
</tr>
<tr>
<td>Types*Concentrations</td>
<td>12</td>
<td>0.39581</td>
<td>0.39581</td>
<td>0.03298</td>
<td>3.20</td>
<td>0.003</td>
</tr>
<tr>
<td>Error</td>
<td>40</td>
<td>0.41180</td>
<td>0.41180</td>
<td>0.01030</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>59</td>
<td>1.28609</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Types of nanoparticles: unmodified LDH, LDH modified by sodium benzoate: LDH-SB1 with 12.3% benzoate; LDH-SB2 with 20.9% benzoate; and LDH-SB3 with 34.9% benzoate.
** Concentrations of nanoparticles: 0%, 2%, 5%, and 10%.
Table A.4. Two-way ANOVA table for elongation at break

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Seq SS</th>
<th>Adj SS</th>
<th>Adj MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type*</td>
<td>4</td>
<td>10.9459</td>
<td>10.9459</td>
<td>2.7365</td>
<td>7.69</td>
<td>0.000</td>
</tr>
<tr>
<td>Concentrations**</td>
<td>3</td>
<td>98.0092</td>
<td>98.0092</td>
<td>32.6697</td>
<td>78.61</td>
<td>0.000</td>
</tr>
<tr>
<td>Types*Concentrations</td>
<td>12</td>
<td>38.3702</td>
<td>38.3702</td>
<td>3.1975</td>
<td>7.69</td>
<td>0.000</td>
</tr>
<tr>
<td>Error</td>
<td>40</td>
<td>16.6227</td>
<td>16.6227</td>
<td>0.4156</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>59</td>
<td>163.9780</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Types of nanoparticles: unmodified LDH, LDH modified by sodium benzoate: LDH-SB1 with 12.3% benzoate; LDH-SB2 with 20.9% benzoate; and LDH-SB3 with 34.9% benzoate.

** Concentrations of nanoparticles: 0%, 2%, 5%, and 10%.

Appendix B

CHAPTER 6 – SUPPLEMENTAL DATA

Figure B.1. Plot of ln (M_t/M_∞) vs ln (t) for the early portion release of benzoate in DI water at 21°C as release fraction of M_t/M_∞ from PHBV films with various concentrations of modified LDH with 12.3% benzoate (LDH-SB1). M_t and M_∞ are amounts of benzoate released at time t and equilibrium, respectively. Symbols represent the experimental data and lines show the trend. The slope of the curve represented by n (power law function shown in the inset) indicates the mechanism of release.
Figure B.2. Plot of $\ln \left( \frac{M_t}{M_\infty} \right) \text{ vs } \ln (t)$ for the early portion release of benzoate in DI water at 21°C as release fraction of $M_t/M_\infty$ from PHBV films with various concentrations of modified LDH with 20.9% benzoate (LDH-SB2). $M_t$ and $M_\infty$ are amounts of benzoate released at time $t$ and equilibrium, respectively. Symbols represent the experimental data and lines show the trend. The slope of the curve represented by $n$ (power law function shown in the inset) indicates the mechanism of release.

Figure B.3. Plot of $\ln \left( \frac{M_t}{M_\infty} \right) \text{ vs } \ln (t)$ for the early portion release of benzoate in DI water at 21°C as release fraction of $M_t/M_\infty$ from PHBV films with various concentrations of modified LDH with 34.9% benzoate (LDH-SB3). $M_t$ and $M_\infty$ are amounts of benzoate released at time $t$ and equilibrium, respectively. Symbols represent the experimental data and lines show the trend. The slope of the curve represented by $n$ (power law function shown in the inset) indicates the mechanism of release.
Table B.1. Two-way ANOVA table for Power Law Model Parameter \( n \)

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Seq SS</th>
<th>Adj SS</th>
<th>Adj MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Types*</td>
<td>2</td>
<td>0.03638</td>
<td>0.03638</td>
<td>0.01819</td>
<td>62.52</td>
<td>0.000</td>
</tr>
<tr>
<td>Concentrations**</td>
<td>2</td>
<td>0.01113</td>
<td>0.01113</td>
<td>0.00556</td>
<td>18.82</td>
<td>0.000</td>
</tr>
<tr>
<td>Types*Concentrations</td>
<td>4</td>
<td>0.03245</td>
<td>0.03245</td>
<td>0.00811</td>
<td>27.43</td>
<td>0.000</td>
</tr>
<tr>
<td>Error</td>
<td>18</td>
<td>0.00532</td>
<td>0.00532</td>
<td>0.00030</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td>0.08528</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Types of nanoparticles: LDH modified by sodium benzoate: LDH-SB1 with 12.3% benzoate; LDH-SB2 with 20.9% benzoate; and LDH-SB3 with 34.9% benzoate.

** Concentrations of nanoparticles: 2%, 5%, and 10%.

Table B.2. Two-way ANOVA table for effective diffusivity

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Seq SS</th>
<th>Adj SS</th>
<th>Adj MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Types*</td>
<td>2</td>
<td>18.541</td>
<td>18.541</td>
<td>9.270</td>
<td>7.67</td>
<td>0.004</td>
</tr>
<tr>
<td>Concentrations**</td>
<td>2</td>
<td>72.500</td>
<td>72.500</td>
<td>36.250</td>
<td>30.01</td>
<td>0.000</td>
</tr>
<tr>
<td>Types*Concentrations</td>
<td>4</td>
<td>41.622</td>
<td>41.622</td>
<td>10.406</td>
<td>8.61</td>
<td>0.000</td>
</tr>
<tr>
<td>Error</td>
<td>18</td>
<td>21.743</td>
<td>21.743</td>
<td>1.208</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td>157.406</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Types of nanoparticles: LDH modified by sodium benzoate: LDH-SB1 with 12.3% benzoate; LDH-SB2 with 20.9% benzoate; and LDH-SB3 with 34.9% benzoate.

** Concentrations of nanoparticles: 2%, 5%, and 10%.

Table B.3. Two-way ANOVA table for Weibull shape factor \( \alpha \)

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Seq SS</th>
<th>Adj SS</th>
<th>Adj MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Types*</td>
<td>2</td>
<td>5214.1</td>
<td>5214.1</td>
<td>2607.0</td>
<td>43.65</td>
<td>0.000</td>
</tr>
<tr>
<td>Concentrations**</td>
<td>2</td>
<td>2058.5</td>
<td>2058.5</td>
<td>1029.3</td>
<td>17.23</td>
<td>0.000</td>
</tr>
<tr>
<td>Types*Concentrations</td>
<td>4</td>
<td>1092.6</td>
<td>1092.6</td>
<td>273.1</td>
<td>4.57</td>
<td>0.010</td>
</tr>
<tr>
<td>Error</td>
<td>18</td>
<td>1075.2</td>
<td>1075.2</td>
<td>59.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td>9440.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Types of nanoparticles: LDH modified by sodium benzoate: LDH-SB1 with 12.3% benzoate; LDH-SB2 with 20.9% benzoate; and LDH-SB3 with 34.9% benzoate.

** Concentrations of nanoparticles: 2%, 5%, and 10%. 
Table B.4. Two-way ANOVA table for Weibull shape factor $\beta$

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Seq SS</th>
<th>Adj SS</th>
<th>Adj MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Types*</td>
<td>2</td>
<td>0.03529</td>
<td>0.03529</td>
<td>0.01764</td>
<td>50.15</td>
<td>0.000</td>
</tr>
<tr>
<td>Concentrations**</td>
<td>2</td>
<td>0.00780</td>
<td>0.00780</td>
<td>0.00390</td>
<td>11.08</td>
<td>0.001</td>
</tr>
<tr>
<td>Types*Concentrations</td>
<td>4</td>
<td>0.04744</td>
<td>0.04744</td>
<td>0.01186</td>
<td>33.71</td>
<td>0.000</td>
</tr>
<tr>
<td>Error</td>
<td>18</td>
<td>0.00633</td>
<td>0.00633</td>
<td>0.00035</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td>0.09687</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Types of nanoparticles: LDH modified by sodium benzoate: LDH-SB1 with 12.3% benzoate; LDH-SB2 with 20.9% benzoate; and LDH-SB3 with 34.9% benzoate.

** Concentrations of nanoparticles: 2%, 5%, and 10%.
VITA

Min Liu DeGruson

Education:

2014 Ph.D. in Food Science. The Pennsylvania State University, PA, USA.
2008 M.S. in Plastics Engineering Technology. Pittsburg State University, KS, USA.
2006 B.S. in Food Science & Technology. Shanghai Jiao Tong University, Shanghai, China.

Experience:

2009-2013 Graduate Assistant in Dept of Food Science at Penn State University
2008-2009 Research Assistant in Kansas Polymer Research Center
2006-2007 Graduate Assistant in Food Science at South China University of Technology
2005-2006 Intern in Nestle, China
2005-2006 Intern in Siemens A&D, China
2004-2006 Laboratory Assistant in Food Laboratory at Shanghai Jiao Tong University

Publications and presentations:

- M. Liu, & J.D. Floros. Functionalized Bio-nanocomposite Films Have Improved Mechanical Properties and Lower Sodium Benzoate Diffusivity. Institute of Food Technologist 2012 Annual Meeting & Food Expo (Poster)

Selected awards:

2012-2013 Janet G. and Frank J. Dudek Graduate Scholarship in Food Science, PSU
Ira W. Minter Memorial Award, PSU
2012 Graduate Student Oral Presentation Competition, 2nd Prize, 11th Conference of Food Engineering
2011-2012 Feeding Tomorrow Scholarship, Institute of Food Technologist in Food Packaging Division