

KELEN CRISTINA DOS REIS

**CHARACTERIZATION OF POLYHYDROXYBUTYRATE-
HYDROXYVALERATE (PHB-HV)/ CORN STARCH BLEND FILMS**

Tese apresentada à Universidade Federal de Lavras como parte das exigências do Programa de Pós-Graduação Stricto Sensu em Ciência dos Alimentos, para obtenção do título de “Doutor”.

Orientadora

Profa. Dra. Joelma Pereira

**LAVRAS
MINAS GERAIS - BRASIL
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APROVADA em 06 de julho de 2007.

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**LAVRAS
MINAS GERAIS - BRASIL
2007**

À Deus

... que incomparável e inconfundível na sua infinita bondade,
compreendeu os meus anseios e me deu a necessária coragem para atingir
o meu objetivo.

Pedi forças e saúde para sempre agir com eficiência em meu trabalho e
acerto em minhas decisões.

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A vocês, que iluminaram o meu caminho com afeto e dedicação, para
que eu trilhasse sem medo e cheia de esperança.

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Inha, minha irmã e amiga tão querida,
Dani, razão da minha alegria,
Pedro Henrique, alegria em minha vida...

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Eu sei que vou te amar,
por toda a minha vida, eu vou te amar,
em cada despedida, eu vou te amar.
Desesperada mente, eu sei que vou te amar,
e cada verso meu será para te dizer,
que eu sei que vou te amar por toda a minha vida.
Eu sei que vou chorar,
a cada ausência tua vou chorar,
mas cada volta tua a de apagar,
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eu sei que vou sofrer,
a eterna desventura de viver,
a espera de viver ao teu lado, por toda a minha vida....

Tom Jobim

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*“Se um dia tiver que escolher
entre o mundo e o amor...
Lembre-se
Se escolher o mundo, ficará sem o amor, mas
se escolher o amor, com ele conquistará o mundo...”*

Albert Einstein

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RESUMO

REIS, Kelen Cristina dos. **Caracterização de filmes de blenda de polihidroxibutirato-hidroxivalerato (PHB-HV)/ amido de milho**. 2007, 57p. Tese (Doutorado em Ciência dos Alimentos) – Universidade Federal de Lavras, Lavras, MG.*

Blendas de polihidroxibutirato-hidroxivalerato (PHB-HV) com amido de milho em diferentes concentrações foram preparadas pelo processo “casting” com clorofórmio. As propriedades mecânica, térmica, morfológica e físico-química das blendas foram investigadas usando as análises de textura, calorimetria diferencial de varredura, espectroscopia no infra-vermelho com transformada de Fourier, difração de raio-X, microscopia ótica, microscopia eletrônica de varredura e colorimetria. As propriedades mecânicas, módulo de elasticidade, resistência à tração, alongamento na ruptura e punctura diminuíram com a adição de amido na blenda. As análises em FTIR indicaram que não houve interação molecular entre os dois polímeros, sendo as blendas imiscíveis. As blendas apresentaram baixa cristalinidade (<4%). Blendas de PHB-HV/ amido de milho obtidas pelo processo “casting” foram imiscíveis; entretanto, os dois componentes da blenda foram miscíveis na fase amorfa. Microscopia ótica dos filmes de PHB-HV/ amido de milho mostraram que as blendas não foram fundidas completamente durante a mistura. As blendas mostraram uma pobre adesão interfacial entre amido de milho e o PHB-HV. Uma dispersão heterogênea dos grânulos de amido na matriz rica em PHB-HV foi verificada nas superfícies dos filmes pela microscopia ótica e microscopia eletrônica de varredura. A diferença de cor e opacidade dos filmes apresentou pequena variação com o aumento do conteúdo de amido na blenda.

*Comitê Orientador: Dra. Joelma Pereira - UFLA (Orientadora), Dr. Carlos Wanderlei Piler de Carvalho – Embrapa Agroindústria de Alimentos, Dr. Andrew Charles Smith – IFR – UK.

ABSTRACT

REIS, Kelen Cristina dos. **Characterization of polyhydroxybutyrate-hydroxyvalerate (PHB-HV)/ corn starch blend films**, 2007, 57p. Thesis (Doctor in Food Science) – Universidade Federal de Lavras, Lavras, MG.*

Blends of polyhydroxybutyrate-hydroxyvalerate (PHB-HV) with corn starch were prepared by solution casting from chloroform solutions at different compositions. The mechanical, thermal, morphological and physical-chemical properties were investigated using texture analyzer, differential scanning calorimetry, Fourier transform infrared spectrometry, X-ray diffractometry, optical microscopy, scanning electron microscopy and colorimetry. The Young's Modulus, stress, strain and puncture decreased with increase in starch content in blend with PHB-HV. FTIR measurements indicated that no intermolecular interactions existed between the two polymers as no shift in the absorption peaks of the PHB-HV or starch in the blends was observed which suggests that PHB-HV and starch are immiscible. The blend showed low crystallinity (<4%). PHB-HV/ starch blends obtained by casting process were immiscible; however, the two components were completely miscible in the amorphous phase. Optical micrographs of PHB-HV/ starch blends films did not melt completely during blending. The blends showed a lack of interfacial adhesion between starch and PHB-HV and heterogeneous dispersion of starch granules over PHB-HV-rich matrix, as evidenced by SEM and optical micrographs of the surface. The colour difference and opacity showed small variation with increase of starch content.

*Guidance Committee: Dra. Joelma Pereira - UFLA (Adviser), Dr. Carlos Wanderlei Piler de Carvalho – Embrapa Agroindústria de Alimentos – RJ, Dr. Andrew Charles Smith – IFR – UK.

INTRODUÇÃO

O uso de embalagens plásticas tende a crescer na vida diária da população, pois novas aplicações surgem com frequência. Os filmes plásticos, devido às suas características, são amplamente utilizados nas mais diversas aplicações, inclusive para embalagens de alimentos. No entanto, devido à sua dificuldade de degradação e à exaustão das reservas mundiais de petróleo, o uso de polímeros constitui um problema ambiental significativo. As pesquisas, até pouco tempo atrás, eram no sentido de dar durabilidade, tornar mais estáveis e melhorar as propriedades mecânicas dos plásticos, no entanto, preocupações ambientais associadas à grande disposição de materiais plásticos têm proporcionado a investigação de alternativas solucionadoras em substituição ao plástico comum, principalmente no tocante à biodegradabilidade destes materiais.

As pesquisas na busca de materiais biodegradáveis sejam eles plásticos, materiais de consumo e outros, estão avançando em ritmo acelerado. Novas tecnologias com polímeros adicionados de produtos que tornam plásticos convencionais e outros materiais em produtos biodegradáveis para a preservação do nosso ambiente já estão disponíveis no mercado.

Polihidroxialcanoatos (PHA's) são poliésteres naturais produzidos por uma larga variedade de bactérias a partir de materiais de reserva intracelular. Esses polímeros termoplásticos podem ser obtidos a partir de fontes renováveis. Eles são biocompatíveis e têm sido reconhecidos como os substitutos potenciais dos termoplásticos derivados do petróleo, podem ser depositados em aterros sanitários sem prejudicar a degradação dos outros materiais constituintes do lixo. No caso da existência de coleta seletiva podem ser misturados com a matéria

orgânica e utilizados como fertilizantes, após compostagem existindo também a possibilidade de serem reciclados.

O polihidroxibutirato-co-valerato (PHB-HV) é um polímero 100% biodegradável e pode ser produzido a partir de fontes renováveis, no entanto apesar da vantagem no critério ambiental, não é largamente usado devido ao seu alto custo e, por serem menos flexíveis, têm aplicações mais limitadas que os sintéticos. O amido é obtido a partir de fontes renováveis, sendo biossintetizado e estocado pelas plantas na forma de grânulos de tamanhos variáveis, que dependem da fonte.

O presente trabalho teve como objetivo principal avaliar a miscibilidade de filmes de blendas de polihidroxibutirato-hidroxivalerato e amido de milho regular preparados pelo processo “casting”, com o intuito de produzir uma mistura polimérica de baixo custo e 100% biodegradável. O objetivo específico foi estudar as características mecânicas, térmicas, morfológicas e físico-químicas de filmes de blendas de polihidroxibutirato – hidroxivalerato (PHB-HV) e amido de milho regular .

CAPÍTULO I

LITERATURE REVIEW

1 Polyhydroxyalkanoates (PHAs)

The family of microbial polyesters known as polyhydroxyalkanoates (PHAs) has been receiving considerable attention due to their potential use as environmentally friendly thermoplastics. These polymers undergo hydrolytic and enzymatic degradation. Poly (3-hydroxybutyrate), P(3HB), is a well known example of the PHAs.

Poly (3-hydroxybutyrate), P(3HB), was first isolated and characterized in 1926 by Lemoigne at the Pasteur Institute in Paris (Lemoigne, 1926). Since then, this and other poly(3-hydroxyalkanoates) (PHAs) have been studied extensively by scientists who have come to the general conclusion that bacteria store P(3HB) as an energy reserve in much the same way that mammals accumulate fat. Although P(3HB) is the most common type of PHA, many different polymers and copolymers of this class are produced by a variety of organisms (Nonato et al., 2001). Beyond their biological curiosity, many PHAs have functional properties that are quite suitable for commercial applications (Camargo, 2003; Innocentine-Mei, 2003).

PHB is a rather brittle material which is somewhat difficult to process since it decomposes at temperatures roughly 10°C above its 177°C melting point. But adding HV (hydroxyvalerate) to the polymer leads to several improvements, including a drop in melting point, reduction in average crystallinity, and increased flexibility and toughness. Some of the properties of the PHB-HV range span those of polypropylene to polyethylene. But PHB-HV properties can also be enhanced by adding normal polymer additives such as natural plasticizers, fillers, colorants, etc. (Luzier, 1992).

Some typical properties of PHB (Poly 3-hydroxybutyrate) homopolymer and PHB-HV (Polyhydroxybutyrate-hydroxyvalerate) copolymer are shown in Table 1.

TABLE 1 Typical Properties of PHB-HV*

Property	HV content, mol %		
	0	10	20
Melting point, 0°C	177	140	130
Crystallinity, %	80	60	35
Tensile strength, MPa	40	25	20
Flexural modulus, GPa	3.5	1.2	0.8
Extension at break, %	8	20	50
Impact strength, J/ m	60	110	350

*According to Luzier (1992)

1.1 Technological Process for Obtaining a Sugar-based Plastic (PHB)

The polyhydroxyalkanoates (PHAs) have very interesting properties such as being biocompatible and totally and rapidly biodegraded to carbon dioxide and water by a large number of micro-organisms (Ramsay et al., 1993). They can be compounded to thermoplastic resins that have physicochemical and mechanical properties similar to petrochemical-based polymer, e.g., polyethylene and polypropylene, and standard plastic-engineering molding procedures can be applied to them. The chemical structure of PHB and PHB-HV are shown in the figure 1.

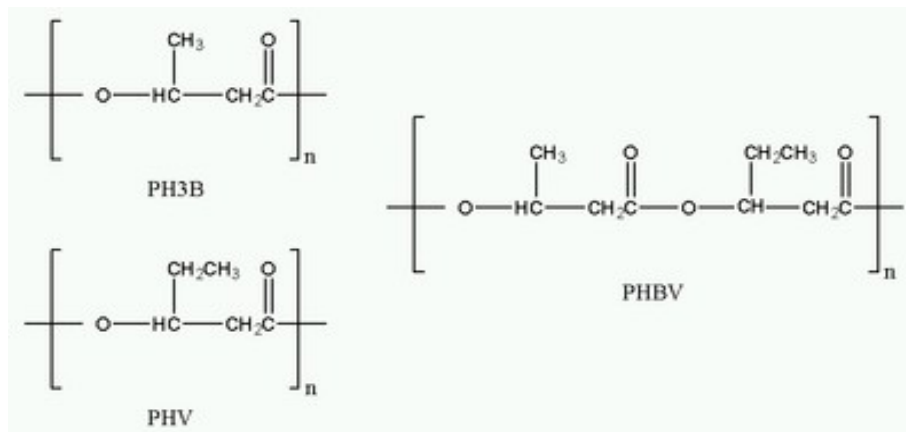


FIGURE 1 Chemical structure of PHB and PHB-HV

At present, though, PHB is the material of choice due to economic and environmental reasons: it is produced directly from sugar by naturally occurring microorganisms and without any petrochemical-derived substrates. Sugar is, for this model, the cheapest, most appropriate substrate available (Lai et al, 2005).

The integration of the PHB production plant into a sugar mill in the Brazilian context offers unique advantages not only for cost savings but also for an environmentally sound process. The most significant advantage is the availability of low price and large quantities of sugar. Brazil is not only the largest, but also the lowest cost sugar producing country in the world. Secondly, sugar mills have available of energy -both thermal and electric- from low cost and renewable sources (bagasse); have effective residue and waste disposal management both from the production process and purification and separation process; know-how and facilities for large-scale fermentation process; availability of biodegradable and natural solvents (by-products of ethanolic fermentation) produced inside the sugar mill and used to obtain PHB with a high degree of purity (Nonato et al, 2001).

The production process of PHB developed in by this case is described by Nonato et al. (2001): “The process comprises a fermentation step, in which strains of *Ralstonia eutropha* [...] are aerobically grown to a high cell density in a well-balanced medium consisting of cane sugar and inorganic nutrients. Cell growth is then shifted to PHB synthesis by limiting nutrients others than the carbon source, which is continually fed as high-concentration sugar syrup. After 45-50 hours, the fed-batch fermentation process is stopped, with a final dry cell mass of 125- 150 kg/m³, containing nearly 65-70% PHB.

The fermented medium is thermally inactivated in a heat exchanger, diluted with water, and flocculated. Separation and concentration procedures yield a cell sludge containing 20-30% solids which is then submitted to a multi-stage extraction process with medium chain- length alcohols in continuous-stirred tank reactors. The extract is purified for cell debris removal and then cooled down to recover a PHB gel. Solvent from the gel is removed by mechanical and thermal concentration. The resulting PHB paste is mixed with water and distilled to remove the remaining solvent. PHB granules are then collected by a sieve, vacuum dried, compounded and extruded as pellets.”

This procedure yields a highly pure polymer by solvent extraction, avoiding the negative environmental impacts of other processes.

1.2 Application areas to PHB-HV

PHB-HV's key properties are its biodegradability, apparent biocompatibility, and its manufacture from renewable resources. Primary application areas in which these features meet some market needs are (i) disposable personal hygiene: PHB-HV could be used as the sole structural material or as part of a degradable composite; (ii) packaging: PHB-HV could be used for films, blow- molded bottles, and as a coating on paper; and (iii) medical: PHB-HV's biocompatibility coupled with its slow hydrolytic

degradation lead to potential in reconstructive surgery and controlled release fields (Thire et al, 2006)

2 Starch

Starch is the most interesting raw material for the development and production of bioplastics. It is not only available everywhere, but also offers a particularly good cost-performance ratio. It is stored in numerous plants in the form of microscopic granules. Whereas maize, wheat and potatoes are the most important supplies of starch in Europe, America and South Africa, cassava is the main source in Asia. Industrial processes separate by-products such as proteins, oils and vegetable fibre so that only highly purified starch remains. Starch-bearing flours are also well suited for the production of bioplastics and biodegradable products.

Native starch is the main energy storage supply in botanical sources. Starch is a naturally occurring carbohydrate of green plant cells and microorganisms. Like other naturally occurring carbohydrates, starch is a polysaccharide consisting of glucosidic units only.

Starch consists of two molecules, amylose and amylopectin, which both contain alpha-D glucose units only. Amylose is a linear or sparsely branched polymer with a molecular mass in the range of 10^5 to 10^6 g/mol primarily by 1-4 linked glucose units. These chains form a spiral shaped single or double helix (Soest, 1996). Figure 2 shows the molecular structure and helical shape of amylose.

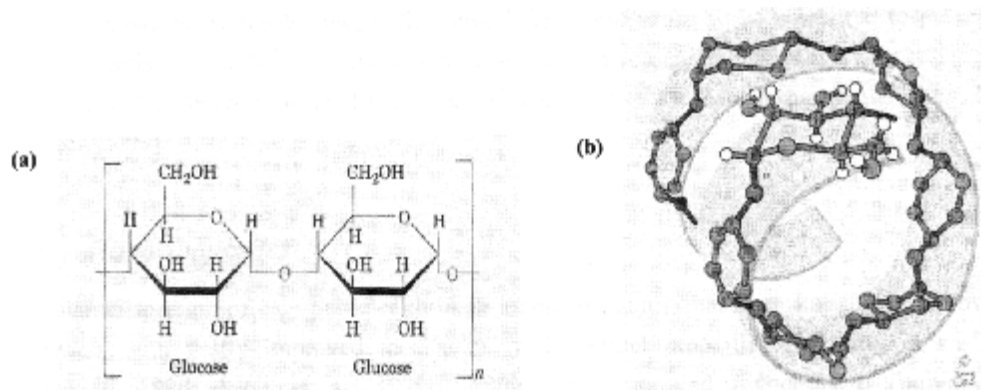


FIGURE 2 (a) The molecular structure of amylose (b) Helical shape of amylose polymer (Voet and Voet, 1999).

Amylopectin is a highly branched molecule with a molecular mass between 107 and 109 g/mol. Amylopectin also contains 1-4 linked glucose units, but has in addition 1-6 glucosidic branching points occurring every 25-30 glucose units. Figure 3 shows the molecular structure and crystal microstructure of amylopectin.

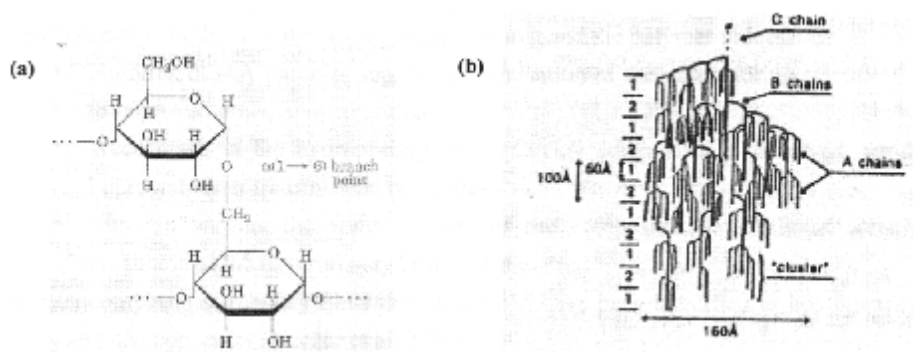


FIGURE 3 (a) The molecular structure (Voet and Voet, 1999) (b) microstructure of amylopectin (Soest, 1996).

The amylose content of most starches varies between 20-30%, which leads to the crystallinity of native starches ranging from 20-45% (Soest, 1996). The branching amylopectin molecules dominate the crystallinity and are responsible for the amorphous regions in the starch (Figure 4).

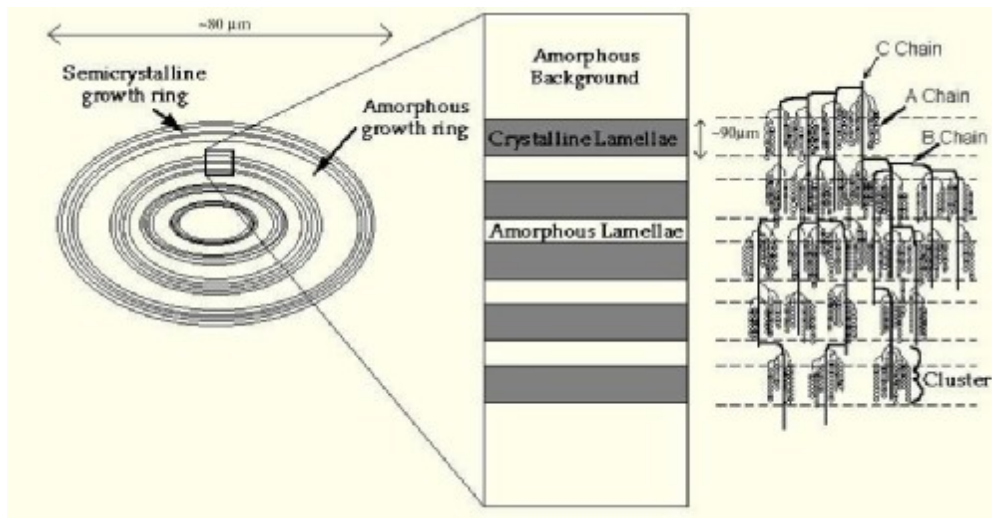


FIGURE 4 Schematic view of the structure of a starch granule, with alternating amorphous and semi-crystalline zones constituting the growth rings (Waigh et al., 1997).

3 Blend development

Under the term polymer blends, the physical characteristics for two compatible or incompatible polymer components will be explained. It is the aim to develop a new material with better properties, which cannot be achieved with homo- or copolymer alone. For incompatible polymer blends the material passes a phase separation with bad adhesion at the boundary surfaces and two glass transition temperatures. For compatible polymer blends the material has only one glass transition temperature.

Most polymer blends consist of thermodynamically immiscible components. The physical properties of a blend are determined by its morphology. It is well known that the morphology of a heterogeneous polymer system is influenced by the properties of the blend components, e.g. rheological and interfacial properties, the blend composition, and by the blending

conditions. The evolution of the morphology during the processing of immiscible blends is controlled by two counteracting processes: (I) particle deformation and break-up and (II) coalescence. However, the understanding of morphology changes during the blending process is still limited (Pionteck et al., 2003).

The use of the terms compatibility and miscibility is very arbitrary in the literature. It might be necessary to distinguish these two terms. Miscibility is a property of the amorphous phase. Crystalline/ crystalline polymer blends are normally immiscible. Nevertheless, they might be miscible in the amorphous region, as in the case of the PHB/PVA80 blend, and give rise to the compatibility of the blends. Those blends are often referred to as “compatible” polymer blends (Huang et al., 2005)

4 Mechanical properties

Tensile testing is a common technique used to determine the mechanical properties of materials. Typical parameters that characterize materials are ultimate tensile stress, strain at break and Young's modulus of elasticity. Most research on biodegradable plastics ultimately seeks to improve the mechanical properties to a level that satisfies a particular application. So quantification of mechanical properties directly, through tensile testing is a valuable tool that can allow detection of improvements quickly (Gormal, 2002).

In solid mechanics, Young's modulus (E) is a measure of the stiffness of a given material. It is also known as the *Young modulus*, modulus of elasticity, elastic modulus or tensile modulus (the bulk modulus and shear modulus are different types of elastic modulus). It is defined as the ratio, for small strains, of the rate of change of stress with strain (IUPAC, 2007). This can be experimentally determined from the slope of a stress-strain curve created during

tensile tests conducted on a sample of the material (Figure 5). Young's modulus, E , can be calculated by dividing the tensile stress by the tensile strain:

$$E \equiv \frac{\text{tensile stress}}{\text{tensile strain}} = \frac{\sigma}{\varepsilon} = \frac{F/A_0}{\Delta L/L_0} = \frac{FL_0}{A_0\Delta L}$$

Where,

E is the Young's modulus (modulus of elasticity) measured in pascals;

F is the force applied to the object;

A_0 is the original cross-sectional area through which the force is applied;

ΔL is the amount by which the length of the object changes;

L_0 is the original length of the object.

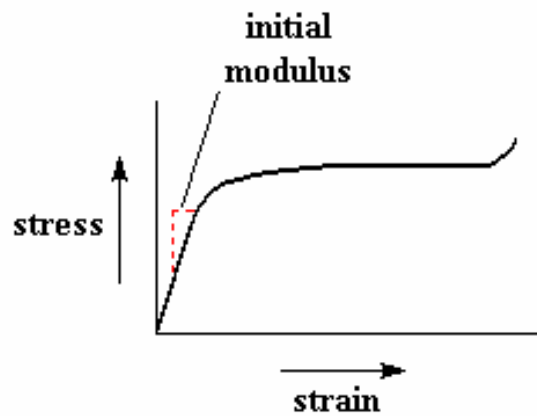


FIGURE 5 Tensile graph (After, 1991)

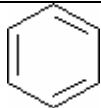
5 Infrared spectroscopy (FT-IR)

Spectroscopy is the study of the interaction of electromagnetic radiation with a chemical substance. The nature of the interaction depends upon the

properties of the substance (Moghaddam, 2006). Typically, when infra-red (IR) radiation passes through a sample (solid, liquid or gas), certain specific frequencies of the radiation are absorbed by the molecules of the substance leading to the molecular vibrations. The frequencies which are absorbed are dependent upon the functional groups within the molecule and the symmetry of the molecule (Table 2). IR radiation can only be absorbed by bonds within a molecule if the radiation has exactly the right energy to induce a vibration of the bond. This is the reason only the frequencies of absorbed radiation are unique for each molecule which provides the characteristics of a substance (Lau, 1999).

Infrared absorption spectra are obtained using infrared spectrometers. In general, a spectrometer consists of a source of radiation, a means of separating radiation into its distinct frequencies, and a method to detect the radiation that passes through a sample. Simply put, if all frequencies of infrared radiation are passed through a sample, and some of these frequencies are absorbed by the sample in going from the ground state to the first excited vibrational state, then the "spectrum" will show a plot of the frequencies that are absorbed by the sample. That is, an infrared spectrum is a plot of the intensity of light absorbed as a function of the frequency of vibration. Reading the frequencies where light is absorbed gives the vibrational frequencies of the molecules in the sample. This method can be applied for solid, liquid (or solution), and gaseous samples (Griffiths & Haseth, 1986).

TABLE 2 Characteristic Infrared absorption frequencies

Functional Group	Absorption Band Position (cm⁻¹)
C-H	2850-2960
=C-H	3020-3100
C=C	1650-1670
≡C-H	3300
C≡C	2100-2260
C-Cl	600-800
C-Br	500-600
C-I	500
O-H	3400-3640
C-OH	1050-1150
>C-H	3030
	1600-1500
N-H	3310-3500
C-N	1030-1230
C=O	1670-1780
COOH	2500-3100
C≡N	2210-2260
NO₂	1540

A Fourier transform spectrometer consists of two mirrors located at a right angle to each other and oriented perpendicularly, with a beamsplitter

placed at the vertex of the right angle and oriented at a 45° angle relative to the two mirrors. Radiation incident on the beamsplitter from one of the two "ports" is then divided into two parts, each of which propagates down one of the two arms and is reflected off one of the mirrors. The two beams are then recombined and transmitted out the other port. When the position of one mirror is continuously varied along the axis of the corresponding arm, an interference pattern is swept out as the two phase-shifted beams interfere with each other. The laser He-Ne is used to calculate the Fourier transform which it is a mathematical operation used to translate a complex curve into its component curves (Bell, 1972).

6 X-ray diffraction

X-ray diffraction (XRD) is a versatile, non-destructive technique that reveals detailed information about the chemical composition and crystallographic structure of natural and manufactured materials. X-rays are electromagnetic radiation of wavelength about 1 Å (10^{-10} m), which is about the same size as an atom. They occur in that portion of the electromagnetic spectrum between gamma-rays and the ultraviolet. The discovery of X-rays in 1895 enabled scientists to probe crystalline structure at the atomic level. X-ray diffraction has been in use in two main areas, for the fingerprint characterization of crystalline materials and the determination of their structure. Each crystalline solid has its unique characteristic X-ray powder pattern which may be used as a "fingerprint" for its identification. Once the material has been identified, X-ray crystallography may be used to determine its structure, i.e. how the atoms pack together in the crystalline state and what the interatomic distance and angle are etc. X-ray diffraction is one of the most important characterization tools used in solid state chemistry and materials science. We can determine the size and the shape of the unit cell for any compound most easily using the diffraction of x-

rays (Bowen & Tanner, 1998; Cullity, 1978). When certain geometric requirements are met, X-rays scattered from a crystalline solid can constructively interfere, producing a diffracted beam (Figure 6). The path difference between two waves:

$$2x = 2d \sin(\theta)$$

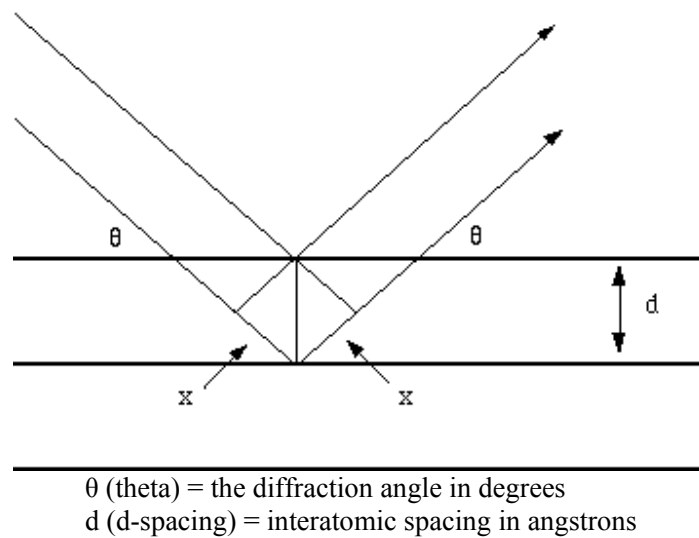


FIGURE 6 Reflection of x-rays from two planes of atoms in a solid.

A polymer can be considered partly crystalline and partly amorphous. The crystalline domains act as a reinforcing grid, like the iron framework in concrete, and improves the performance over a wide range of temperature. However, too much crystallinity causes brittleness. The crystallinity parts give sharp narrow diffraction peaks and the amorphous component gives a very broad peak (halo). The ratio between these intensities can be used to calculate the amount of crystallinity in the material (Bower & Tanner, 1998).

7 Thermal properties

Differential scanning calorimetry or DSC is a thermoanalytical technique in which the difference in the amount of heat required to increase the temperature of a sample and reference are measured as a function of temperature. Both the sample and reference are maintained at very nearly the same temperature throughout the experiment. Generally, the temperature program for a DSC analysis is designed such that the sample holder temperature increases linearly as a function of time. The reference sample should have a well-defined heat capacity over the range of temperatures to be scanned (Lai, 1991).

The basic principle underlying this technique is that, when the sample undergoes a physical transformation such as phase transitions, more or less heat will need to flow to it than the reference to maintain both at the same temperature. Whether more or less heat must flow to the sample depends on whether the process is exothermic or endothermic. For example, as a solid sample melts to a liquid it will require more heat flowing to the sample to increase its temperature at the same rate as the reference. This is due to the absorption of heat by the sample as it undergoes the endothermic phase transition from solid to liquid. Likewise, as the sample undergoes exothermic processes, such as crystallization, less heat is required to raise the sample temperature. By observing the difference in heat flow between the sample and reference, differential scanning calorimeters are able to measure the amount of heat absorbed or released during such transitions. DSC may also be used to observe more subtle phase changes, such as glass transitions. DSC is widely used in industrial settings as a quality control instrument due to its applicability in evaluating sample purity and for studying polymer curing (Dean, 1995; Pungor, 1995; Skoog et al., 1998).

Using this technique it is possible to observe fusion and crystallization events as well as glass transition temperatures (T_g) (Figure 7). DSC can also be used to study oxidation, as well as other chemical reactions (Dean, 1995).

Glass transitions (T_g) may occur as the temperature of an amorphous solid is increased. These transitions appear as a step in the baseline of the recorded DSC signal. This is due to the sample undergoing a change in heat capacity; no formal phase change occurs (Dean, 1995; Skoog et al., 1998).

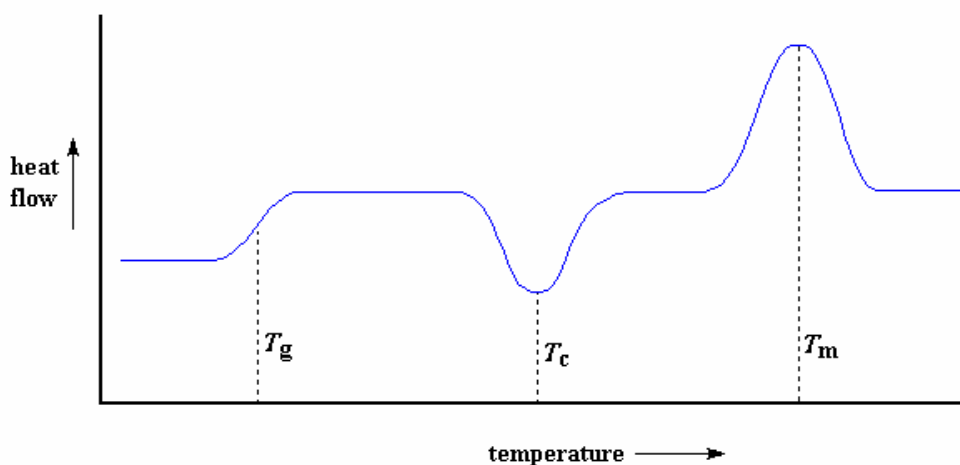


FIGURE 7 A schematic DSC curve (T_g , glass transition temperature; T_c , crystallization temperature; T_m , melting temperature).

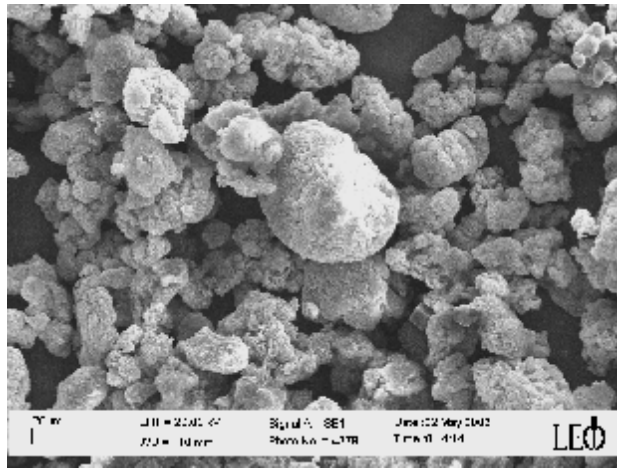
As the temperature increases, an amorphous solid will become less viscous. At some point the molecules may obtain enough freedom of motion to spontaneously arrange themselves into a crystalline form. This is known as the crystallization temperature (T_c). This transition from amorphous solid to crystalline solid is an exothermic process, and results in a peak in the DSC signal. As the temperature increases the sample eventually reaches its melting temperature (T_m). The melting process results in an endothermic peak in the

DSC curve. The ability to determine transition temperatures and enthalpies makes DSC an invaluable tool in producing phase diagrams for various chemical systems (Dean, 1995).

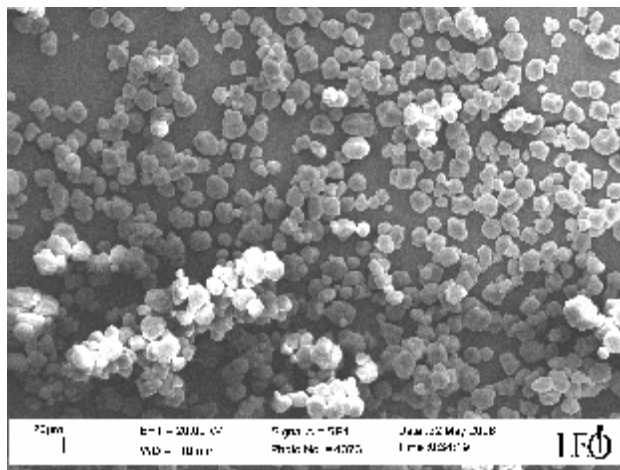
8 Morphological properties

Morphology is an important part of blend analysis. The analysis of morphology is necessary since most of the blends properties, especially mechanical properties depend on it.

Microscopy is the most common form of analysing blend morphology. Several types of microscopy are available including optical microscopy (OM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Each of this technique has their own specific advantages and disadvantages. SEM was used to analyze blend morphology in the case of Thiré et al. (2006) for polyhydroxybutyrate/ starch blends. However, in the case of Mani & Bhattacharya (2000) starch/ polyester blends were studied successfully through the use of the simple optical microscope. Figure 8 illustrates the scanning micrographs of polyhydroxybutyrate-hydroxyvalerate and regular corn starch granules.



(a)



(b)

FIGURE 8 Scanning electron microscopy images of granules (a) polyhydroxybutyrate-hydroxyvalerate (PHB-HV), (b) regular corn starch.

9 Optical properties

The optical properties of plastics, such as gloss, colour, opacity and transparency, are very important when these materials are used, for example, for food packaging and consumer electronics. These properties can be a criteria for miscibility. Films made from two mutually miscible or compatible polymer are optically clear whereas those made from incompatible polymers are usually translucent or opaque (Paul & Newman, 1978).

Although films of any two amorphous, compatible polymers are always clear, absolute judgment on the compatibility of the two polymers cannot be made on this basis. Under special circumstances, films made from blends of two incompatible or semicompatible polymers can be optically clear. For example, films that are very thin, so that light encounters only one of the two phases in passing through the material, can appear to be clear. In addition, films consisting of two layers as a result of phase separation during casting may be obtained in a heterogeneous polymer system as a result of either the two polymers having equal refractive indices or as a result of the dispersed phase having dimensions smaller than the wavelength of visible light (Brandrup & Immergut, 1989).

10 References

After, O. G. **Principles of polymerization**, 3rd.ed. J.Wiley, New York, 1991.

Bell, R. J. **Introductory fourier transform spectroscopy**. Academic Press., New York, 1992.

Bowen, D.K., Tanner, B.K. **High resolution x-ray diffractometry and topography**, Taylor & Francis, Ltd. 1998.

Brandup, J. Immergut EH. **Polymer handbook**, 3 rd ed. New York: Wiley; 1989.

Camargo, F.A. de. **Obtenção, caracterização e estudo de biodegradação de blendas de poli(3-hidroxi-butirato-co-valerato)/ (PHBV) com lignina de bagaço de cana-de-açúcar**. Campinas, São Paulo: UNICAMP, 2003. 90p. (Dissertação – Mestrado em Ciência e Tecnologia de Materiais).

Cullity, B.D. **Elements of x-ray diffraction**, 2nd Ed., Addison-Wesley, New York, 1978.

Dean, John A. **The analytical chemistry handbook**. New York. McGraw Hill, 1995.

Gormal, S. **Compatibilisation of biodegradable starch/ Bionelle™ blends**. Department of Chemical Engineering, University of Queensland, Brisbane, Australia, 2002.

Griffiths, P. R., Haseth, J. A. de. **Fourier transform infrared spectroscopy**, Wiley Interscience, 2rd.ed., New York, 1986.

Huang, H., Hu, Y., Zhang, J., Sato, H. Zhang, H. Noda, I., Ozaki, Y. Miscibility and hydrogen-bonding interaction in biodegradable polymer blends of poly(3-hydroxybutyrate) and a partially hydrolyzed poly(vinyl alcohol). **Journal Physical Chemistry**, 109: 19175-19183, 2005.

International Union of Pure and Applied Chemistry (IUPAC). Compendium of chemical terminology. Disponível em: <http://goldbook.iupac.org/MO3966>. Acesso em: 12 jun. 2007.

Lai, K. Physicochemical changes and rheological properties of starch during extrusion (A Review). **Biotechnology Progress**, 7: 251-266., 1991.

Lai, S.-M., Don, T.-M., Huang, Y.-C. Preparation and properties of biodegradable thermoplastic starch/ poly (hydroxybutyrate) blends. **Journal of Applied Polymer Science**, 100: 2371-2379, 2005.

Lau, W.S. **Infrared characterization for microelectronics**. World Scientific, Singapore, 1999.

Lemoigne, M. Produit de déshydratation et de polymérisation de l'acide b-oxxybutyrique. **Bull. Soc. Chim. Biol.** 8: 770-782, 1926.

Luzier, W. D. **Materials derived from biomass/ biodegradable materials**. Proc. Natl. Acad. Sci. USA, 89: 839-842, 1992.

Mani, R., Bhattacharya, M. Properties of injection moulded blends of starch and modified biodegradable polyesters. **European Polymer Journal** 37: 515-526, 2000.

Moghaddam, L., Rintoul, L., Halley, P.J., Fredericks, P.M., Infrared microspectroscopy mapping of the homogeneity of extruded blends: application to starch/polyester blends. **Polymer Testing** 25: 16–21, 2006.

Nonato, R.V., Mantelatto, P.E., Rossel, C.E.V. (2001) Integrated production of biodegradable plastic, sugar and ethanol. **Applied Microbiology and Biotechnology**, 57: 1-5.

Paul, D.R., Newman, S. **Polymer blends**, Academic Press, London, 1978.

Pionteck, P., Pötschke, N., Proske, H., Zhao, H., Malz, D., Beyerlein, U., Schulze, B. V. Morphology of reactive PP/PS blends with hyperbranched. **Polymers Macromol. Symp.**198: 209-220, 2003.

Pungor, Erno. **A practical guide to instrumental analysis**. Boca Raton, Florida. pp. 181–191, 1995.

Ramsay, B.A., Langlade, V., Carreau, P.J., Ramsay, J.A. Biodegradability and mechanical properties of poly-(β -hydroxybutyrate-co- β -hydroxyvalerate)/ starch blends. **Applied and environmental microbiology**, 50, 4: 1242-1246, 1993.

Skoog, Douglas A., F. James Holler and Timothy Nieman. **Principles of Instrumental Analysis**. Fifth Edition. New York, pp. 905–908., 1998.

Soest, J.J.G.V. **Starch Plastics: structure-property relationships**. Wageningen, Agrotechnological Research Institute, 1996.

Thiré, R.M.S.M., Ribeiro, T.A.A., Andrade, C.T. Effect of Starch Addition on Compression-Molded Poly (3-hydroxybutyrate)/ starch blends. **Journal of Applied Polymer Science**, 100: 4338-4347, 2006.

Voet, D., Voet, J.G., and Pratt, C.W. **Fundamentals of biochemistry**. Wiley, New York, 1999.

Waigh, T.A, Hopkinson, I., Donald, A.M., Analysis of the native structure of starch granules with X-ray microfocus diffraction, **Macromolecules**, 30, 3813-3820, 1997.

CAPÍTULO II

Characterization of polyhydroxybutyrate-hydroxyvalerate (PHB-HV)/ corn starch blend films

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(Preparado de acordo com as normas da revista “Carbohydrate
Polymers”)

Abstract

Blends of polyhydroxybutyrate-hydroxyvalerate (PHB-HV) with corn starch were prepared by solution casting from chloroform solutions at different compositions. The mechanical, thermal, morphological and physical-chemical properties were investigated by using texture analyzer, differential scanning calorimetry, Fourier transform infrared spectrometry, X-ray diffractometry, optical microscopy, scanning electron microscopy and colorimetry. The Young's Modulus, stress, strain and puncture decreased with increase in starch content in blend with PHB-HV. FTIR measurements indicated that no intermolecular interactions existed between the two polymers as no shift in the absorption peaks of the PHB-HV or starch in the blends was observed which suggests that PHB-HV and starch are immiscible. The blend showed low crystallinity (<4%). PHB-HV/ starch blends obtained by casting process were immiscible; however, the two components were completely miscible in the amorphous phase. Optical micrographs of PHB-HV/ starch blend films did not melt completely during blending. The blends showed a lack of interfacial adhesion between starch and PHB-HV and heterogeneous dispersion of starch granules over PHB-HV-rich matrix, as evidenced by SEM and optical micrographs of the surface. The colour difference and opacity showed small variation with increase of starch content.

Key-words: PHB-HV, Corn starch; Biodegradable polymers; polymer blends, compatibility.

1 Introduction

The amount of plastic waste increases every year and the exact time needed for its biodegradation is unknown. Nowadays plastics and synthetic polymers are mainly produced using petrochemical materials that cannot be decomposed. Therefore they contribute to environmental pollution threatening animals and plants.

In recent years, there has been an increasing concern over the harmful effects of synthetic plastic materials in the environment. This ecological awareness impelled the development of new biodegradable materials, especially for single-use plastic items.

Poly(hydroxyalkanoates) (PHAs) have attracted much attention as biocompatible and biodegradable thermoplastic with potential application in agricultural, marine, and medical fields. These biopolymers are polyesters of various hydrocarboxylic acids, which are accumulated as an energy/ carbon storage or reducing power material by numerous micro-organisms under unfavourable growth conditions in the presence of excess carbon source. PHAs exhibit material properties similar to various synthetic thermoplastic and elastomeres currently in use, from polypropylene to synthetic rubber. Besides, upon disposal, they are completely degraded to water and carbon dioxide (and methane under anaerobic conditions) by micro-organisms in various environments such as soil, sea lakes and sewage (Thiré et al, 2006).

Poly (3-hydroxybutyrate) (PHB) is a homopolymer of 3-hydroxybutyrate, partially crystalline with a high melting temperature and a high degree of crystallinity. PHB is thermoplastic and one of the most

widely investigated members of the family of polyhydroxyalkanoate (PHAs). It has physical and mechanical properties comparable to those of isotactic polypropylene (Thiré et al., 2006). PHB is stiff and brittle, which are not considered good properties for plastic applications (Innocentini-Mei et al., 2003). The degree of brittleness depends on the degree of crystallinity, glass temperature and microstructure. It is not water soluble but is 100% biodegradable. PHB has low permeability for O₂, H₂O and CO₂. But PHB has another disadvantage which is high cost (Lai et al., 2005).

Blending of PHB with other polymers is an interesting approach to overcome these drawbacks: price and brittleness. Numerous studies have been published in this field, including blends with biodegradable polymers such as poly (vinyl alcohol), poly (ϵ -caprolactone), and polysaccharides. As an interesting polysaccharide for blending with PHB, starch is inexpensive, totally biodegradable, and is produced in abundance beyond market available. Therefore, it is expected that use of starch in blends with PHB can result in completely biodegradable material, which can have desired physical properties with concomitant reduction in cost (Thiré et al., 2006).

Some attempts have been made to produce PHB/ starch blends with improved physical properties. Godbole et al. (2003) studied the thermal and mechanical properties of solvent-cast blends of bacterial PHB and starch. They reported that blends containing up to 30% starch presented lower cost and better properties than those with only PHB. Other researchers (Innocentini-Mei et al., 2003) investigated injection-moulded blends of PHB with starch and starch derivatives. They observed

that blends containing natural starches and starch adipate resulted in brittle materials.

The main aim was to investigate the miscibility of polyhydroxybutyrate-hydroxyvalerate (PHB-HV) and corn starch blend films prepared by a casting technique. The specific aim of this study was to investigate the thermal, mechanical, morphological and physical-chemical properties of binary blends of polyhydroxybutyrate-hydroxyvalerate (PHB-HV) with corn starch.

2 Materials and experimental

2.1 Materials

PHB-HV (Polyhydroxybutyrate-hydroxyvalerate) (18% mol% HV), was supplied by PHB Industrial S/A (São Paulo, Brazil) as a white fibrous powder. Regular corn starch was supplied by Corn Products Brazil (Sao Paulo, Brazil).

2.2 Regular corn starch/ PHB-HV blend films

Regular corn starch (contained 27 wt% amylose and 73 wt% amylopectin) and PHB-HV blend films were prepared by conventional solution-casting technique. The blends of PHB-HV/ regular corn starch in different ratios (wt/wt) (100/0, 80/20, 70/30, 60/40 and 50/50), were prepared from 5% (wt/vol) chloroform solutions. Sample solutions were produced at 60°C, which was lower than chloroform boiling point (61.2°C), for 1 h under constant stirring on a magnetic stirrer-hotplate. The blends in the form of thin films (15-20 µm) were obtained by pouring

the solution onto a glass Petri dish (70mm) as a casting surface, allowing the solvent to evaporate on flat surface at room temperature overnight.

2.3 Mechanical properties

The mechanical properties were studied by using Texture Analyzer TA-XT2 (Stable Micro Systems, UK). Ten sample strips (5mm x 70 mm) of each formulation were cut and clamped between two tensile grips. Force (N) and deformation (mm) were recorded during extension at 50 mm.min⁻¹ and with an initial distance between the grips of 50 mm. The Young's modulus, E , the stress σ and strain at break ε , were calculated as average values from stress-strain diagram curves determined with 10 different specimens. Puncture test was carried out to determine puncture strength (N) using the same equipment. Samples with diameters of 70 mm were fixed on the plate of the equipment with a role of 20 mm diameter with help of a tape. A cylindrical probe of 5 mm diameter was moved perpendicularly to the film surface at a constant speed of 1 mm.s⁻¹ until the probe passed through the film. At rupture point force was determined. Five samples were analyzed for each formulation.

2.4 Infrared spectroscopy (FT-IR)

BioRad FTS175c Fourier transforms infrared spectrometer (DigiLab, now Varian, USA). The tests were performed at ambient temperature. A total de 100 scans were taken with a resolution of 2 cm⁻¹ in all cases.

2.5 X-ray diffraction

X-ray diffractograms were recorded for 2θ between 4 and 50 at 0.1° intervals (scanning rate at 6 s^{-1}) using a Bruker D5005 (Bruker AXS, UK) diffractometer equipped with a copper tube operating at 40 kV and 40 mA producing $\text{CuK}\alpha$ radiation of 1.54 \AA wavelength. This analysis was performed at University of Nottingham, UK.

2.6 Differential scanning calorimetry (DSC)

The glass transition (T_g), melting point (T_m), crystallization temperatures (T_c), melting enthalpy (ΔH_m) and crystallization enthalpy (ΔH_c) of the different blends were determined by DSC.

Samples, as cast films, 2-3 mg were weighed into hermetic aluminium pans and analyzed in a DSC Q200 (TA Instruments, New Castle, USA) calibrated with indium, according to the methodology described by Zhang et al. (1997).

2.7 Optical microscopy

The surface of the films obtained was observed by optical microscopy, by using a Pro-Plus 4.5 optical microscopy at 4x and 20x amplification.

2.8 Scanning electron microscopy

The films' surface was observed using a scanning electron microscopy. The samples were mounted on aluminium stubs and coated with a gold film (Evaporator SCD 050 Blazers), they were observed in Scanning Microscopy Leo EVO 40.

2.9 Colour and Opacity

Colour was evaluated with a Minolta Chroma Meter CR 300 colorimeter (Minolta, Kyoto, Japan) employing the Hunter and CIE scale. Circular samples of 70 mm diameter were employed for these tests. Determinations were performed in triplicate and the average was reported. Colour differences were calculated as:

$$\Delta C = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{0,5}$$

Where, L, represents lightness; a, redness; b, yellowness, and $\Delta a = a_i - a$, $\Delta b = b_i - b$, $\Delta L = L_i - L$. The index i indicates the values for white standard, for each parameter ($L_i = 92,4$; $a_i = 0,3162$; $b_i = 0,3326$). The color difference (ΔC) is the magnitude of the resultant vector of three component differences: lightness difference, ΔL ; red-green chromaticity difference, Δa ; yellow-blue chromaticity difference, Δb (Valencia Rodriguez, 2001). The opacity was calculated according to Hunter lab method (Paschoalick et al., 2003), using the same equipment. The determinations were performed in triplicate after colorimeter calibration with a white standard and black standard. The opacity was calculated as:

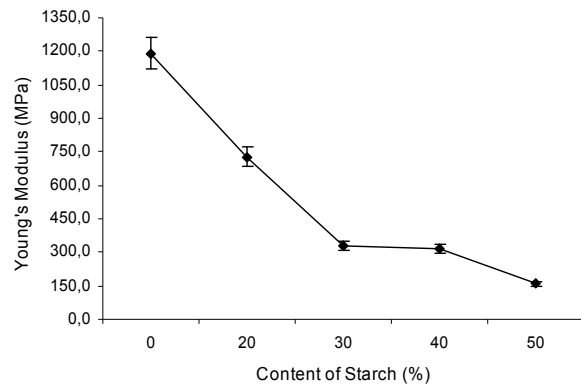
$$Op = (Op_N / Op_B) \cdot 100$$

Where, Op = film opacity (%); Op_N = opacity in a black plate; Op_B = opacity in a white plate.

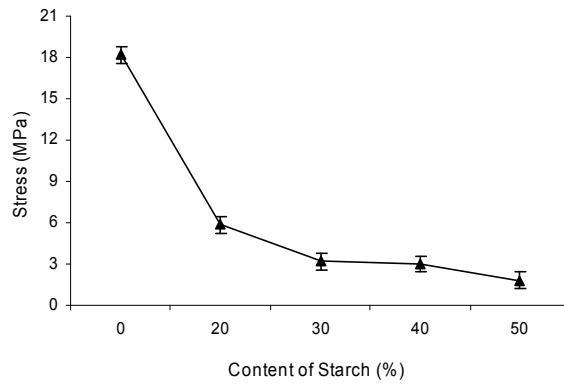
3 Results and discussion

3.1 Mechanical properties

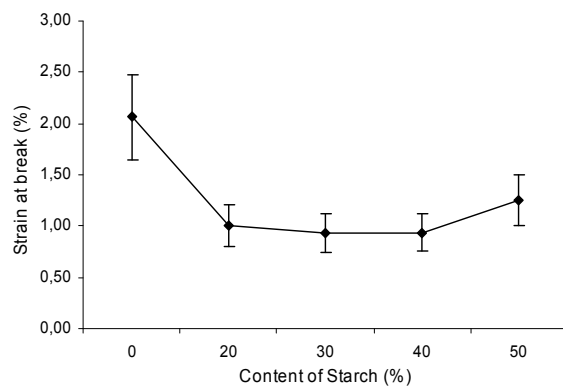
The influence of starch content on the mechanical properties of solvent-casting PHB-HV/ starch blends can be seen in Figure 1.



(a)



(b)



(c)

Fig. 1. Mechanical properties (a) Young's modulus, (b) stress, (c) strain at break of blends PHB-HV/ regular starch films. The points are the mean \pm standard deviation for ten determinations.

The Young's Modulus, stress and strain at break in PHB-HV/starch blends films were affected by starch content (Figure 1).

Pure PHB is brittle and hard (Innocentini-Mei et al., 2003). It can be observed that Young's Modulus decreases with increase in the content starch (Figure 1a). As starch content increased, the modulus rapidly decreased from 1200 MPa, on average, to 726 MPa, resulting in more flexible materials. This result agrees with the findings of Godbole et al. (2003) and Thiré et al. (2006), 1708 Mpa to 578 MPa, 1317 MPa to 366 MPa, respectively.

Stress (MPa) decreased with the increase of content of starch. All blends were less resistant to tensile strength than pure PHB-HV (Figure 1b). A possible explanation for the decrease in stress after the addition of starch to PHB-HV could be the low interfacial interaction between the components of the blend, which would lead to mechanical rupture at the blend interface.

When starch is added to PHB-HV strain at break showed a sharp decrease (Figure 1c). This fact probably indicates a lack of interfacial adhesion between PHB-HV and regular starch, since this property may be related to the interfacial state of material (Thiré et al., 2006).

Mechanical properties are dependent of filler volume, filler particle size and shape as well as degree of adhesion of the filler in the polymer matrix (Willett, 1994). Thus these results are probably because hydrophobic PHB-HV is not compatible with hydrophilic starch. It is a general trend that the better the dispersion of the starch in the thermoplastic matrix, the better the mechanical properties (Thiré et al., 2006).

Seves et al. (1998), who studied the morphology and thermal behaviour of poly(3-hydroxybutyrate-co-hydroxyvalerate)/ starch valerate blends, observed a decrease of Young's modulus and stress at break in all blends studied when increased starch valerate content.

The puncture strength in the solvent-casting PHB-HV/ starch blends can be seen in Figure 2.

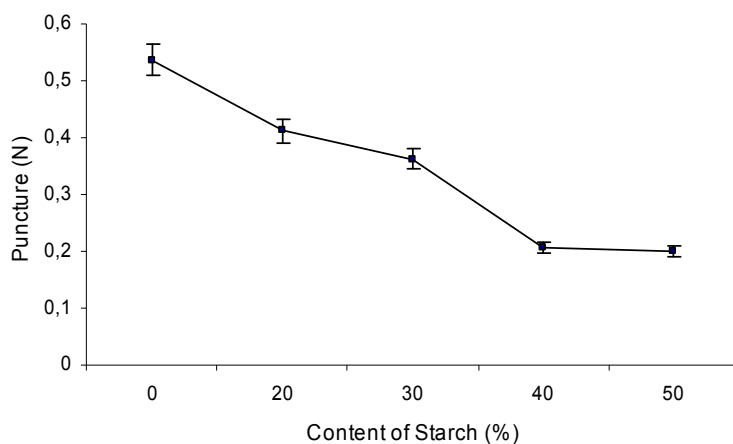


Fig. 2. Puncture strength of blends PHB-HV/ regular starch films. The points are the mean \pm SD for five determinations.

There was a decrease in puncture strength after the addition of starch to PHB-HV. This decrease in puncture strength was associated with the low interfacial interaction between the components of the blend, which would lead to mechanical rupture at the blend interface. Puncture strength varied between 0.20 N and 0.54 N.

3.2 Infrared spectroscopy (FT-IR)

The two components of the polymer blends starch and PHB-HV, presented very different spectra. Starch has hydroxyl groups on the backbone, while PHB-HV, has carbonyl groups, along its main chain.

Specific interaction between blend components is often important to achieve blend miscibility (Zhang et al., 1997). According to Graffar (2002), IR provides information about structures, miscibility and analyses the chemical or physical interactions in the blend. Fourier-transform infrared spectroscopy was used to study the possible interaction between PHB-HV and regular corn starch and the effect of starch content in the blend in terms of molecular structure.

Figures 3, 4 and 5 respectively show FT-IR spectra in the regions of $1500-600\text{ cm}^{-1}$, $1500 - 1000\text{ cm}^{-1}$ and $1850-1650\text{ cm}^{-1}$ of PHB-HV, regular starch and PHB-HV/ starch blends, respectively

There are a number of C-C stretching bands in the spectrum of PHB-HV, located between 1000 and 900 cm^{-1} (Lin-Vien et al., 1991). According to Huang et al. (2005) the crystallinity of this stretching bands between 1000 and 900 cm^{-1} provides useful region to monitor the morphological properties of PHB in the blends. The amorphous state of PHB-HV in the blends is confirmed by the disappearance of these crystalline C-C stretching bands in the region between 1000 to 880 cm^{-1} .

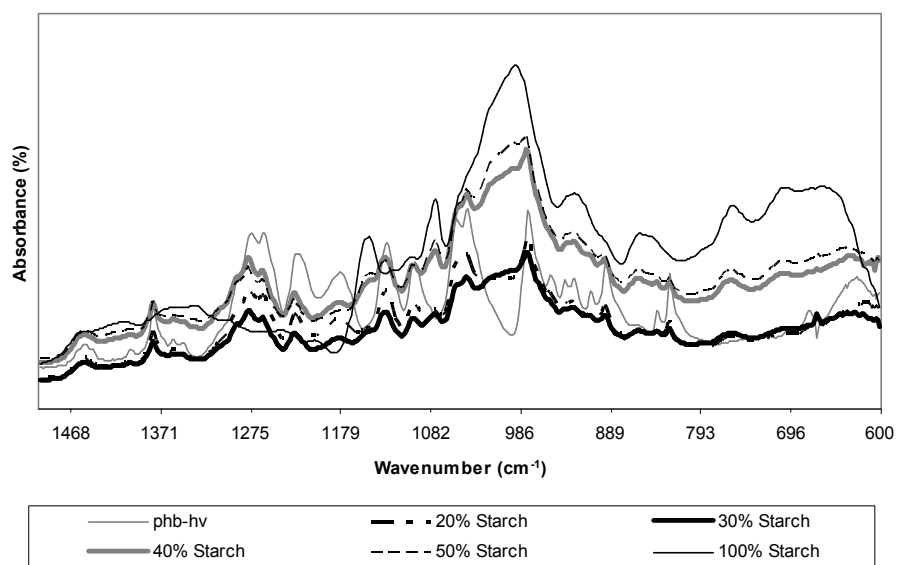


Fig. 3. FT-IR spectrum in the region from 1500-600 cm^{-1} of PHB-HV, regular starch and PHB-HV/ regular starch blends.

The bands at 1228 and 1279 cm^{-1} are crystalline-sensitive bands. These bands are characteristic of C-O-C (Galego et al., 2000). Changes in the degree of crystallinity were observed in the FTIR experiments between the blends studied (Figure 4).

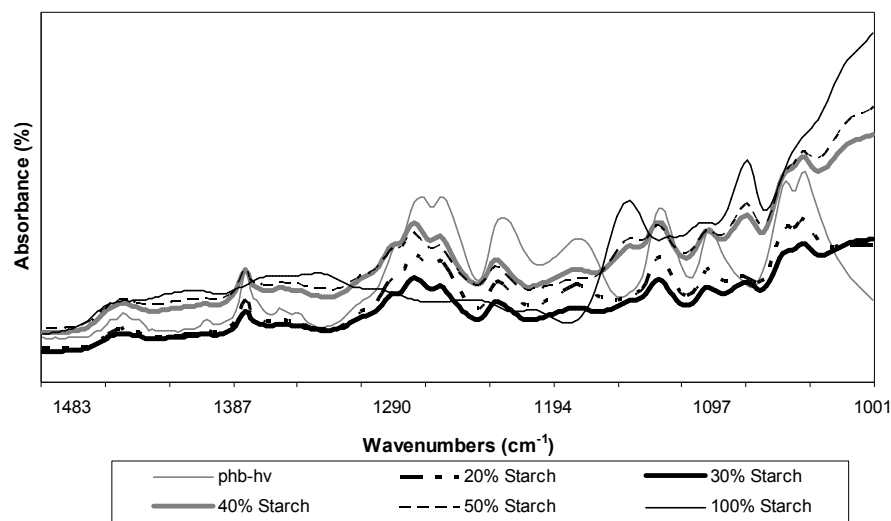


Fig. 4. FT-IR spectrum in the region from 1500-1000 cm^{-1} of PHB-HV, starch and PHB-HV/ Starch blends.

Figure 5 shows an IR spectrum of PHB-HV, regular starch and blends at room temperature in the region 1850-1650 cm^{-1} , where bands due to the C=O stretching vibrations are observed. It is noted that the intensity of bands is dependent of starch content. The band at 1720 cm^{-1} is intense at room temperature, but according to Sato et al. (2005) its intensity decreases with temperature and eventually disappears at high temperature. There is no doubt that this band is due to C=O stretching mode of the crystalline part (Sato et al., 2004). According to Sato et al. (2005) there is a broad band centred near 1740 cm^{-1} which is assigned to C=O stretching mode of the amorphous state and are due to different conformations of the main chain.

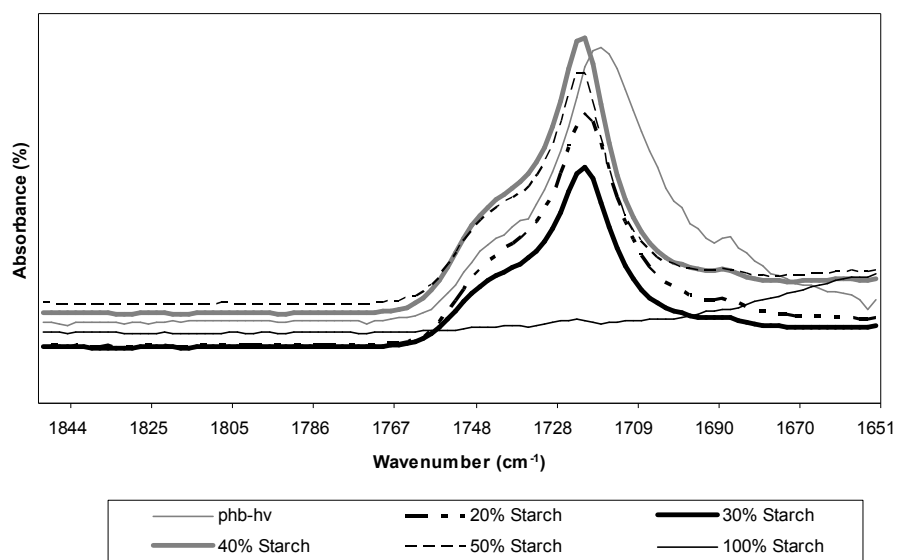


Fig. 5. FT-IR spectrum in the region from 1850-1650 cm^{-1} of PHB-HV, regular starch and PHB-HV/ regular starch blends.

Of note is a weak band near at 1690 cm^{-1} in the PHB-HV spectra. This band is very much characteristic of the crystalline state. Based on its weak intensity and band position, it is speculated that this band is related to the crystal defect that is caused by the interaction of an OH end group and a C=O group of PHB (Zhang et al., 1997).

Absorption of hydroxyl groups in both PHB-HV and starch is indicative of intermolecular hydrogen bonding (Zhang et al., 1997). In the OH stretching region ($3700\text{-}3000 \text{ cm}^{-1}$), the line shapes are almost the same but the intensity are dependent of content of starch (Figure 6). These results show that no specific interaction occurred between the two components.

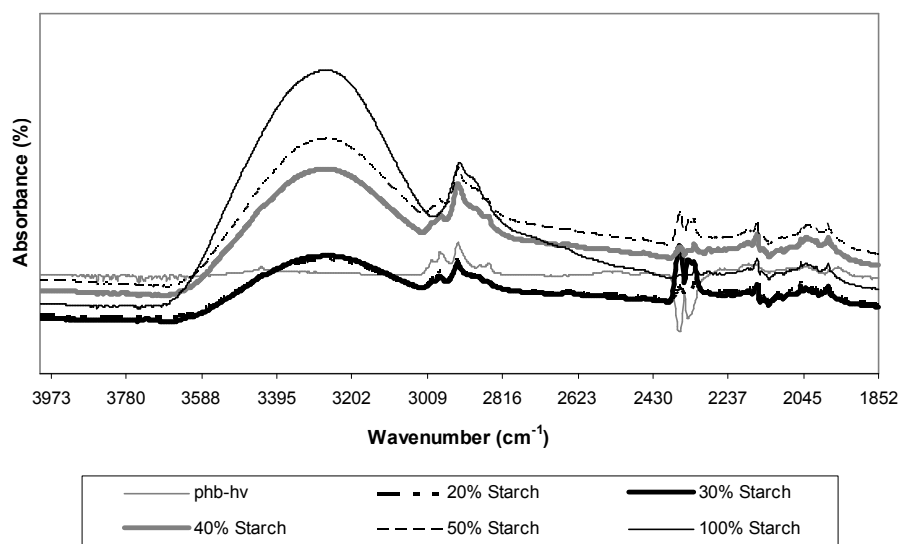


Fig. 6. FT-IR spectrum in the region from 4000-1850 cm^{-1} of PHB-HV, regular starch and PHB-HV/ regular starch blends.

3.3 X-ray diffraction

Solid polymer can exist in a crystalline or amorphous state. However, because of their size and complexity they are often semi crystalline, then the polymer having both amorphous and crystalline regions. Any chain disorder or misalignment will result in an amorphous region (Callister, 2003).

Figure 7 shows the X-ray diffraction patterns for films with 0, 20, 30, 40 and 50% of regular corn starch. The diffraction profile of the raw PHB-HV sample exhibits peaks (2θ) at 13.5° and 16.9° .

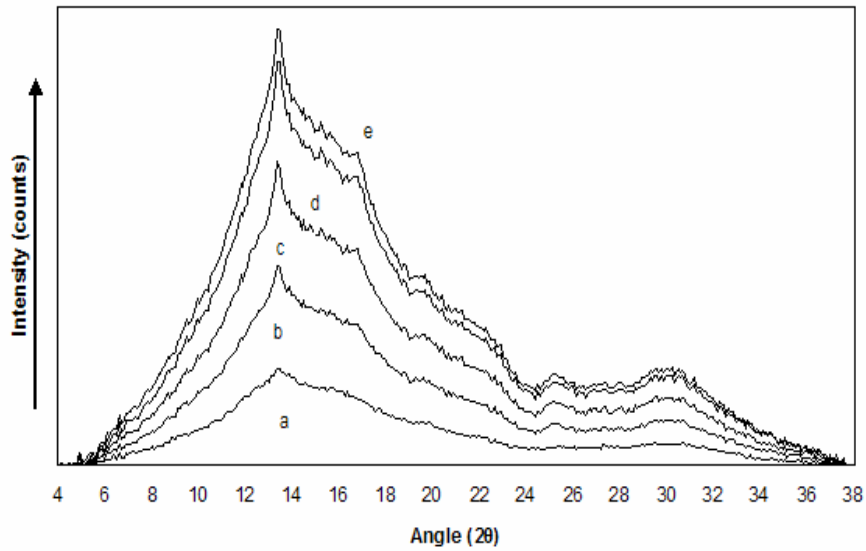


Fig. 7. X-ray diffraction pattern of different content of starch in blends (a) 0% starch (PHB-HV only); (b) 20% starch; (c) 30% starch; (d) 40% starch; (e) 50 % starch.

The diffractograms of the solution cast PHB-HV/ regular starch blends show no crystallinity value from the broad hump and its estimated crystallinity value lower than 4%. The calculated percent crystallinities for blends samples are shown in Table 1. Jayasekara et al. (2004) observed that the diffractogram of cast starch solution shows no crystallinity from de broad hump and its estimated crystallinity value was lower than 5%.

Table 1 Crystallinity (%) of different content (%) of regular starch in the PHB-HV / corn starch blend films

<i>Sample content of starch (%)</i>	<i>Crystallinity (%)</i>
0	12.00
20	2.35
30	2.09
40	2.24
50	3.60

Thiré et al. (2006) observed X-ray diffractograms of raw PHB, native maize starch and compression-molded PHB/ starch blends with varied contents (wt%) of 0, 20, 30, 100 starch after conditioning at 68% RH for 15 days. It was found to be diffraction profile samples that exhibited well-defined peaks (2θ) at 13.6, 17.1, 21.7, 22.7, and 25.6°.

The total of crystallinity of raw PHB is reduced with an increase of starch content in the blends since the degree of crystallinity for pure PHB-HV was 50-60%. The decrease in crystallinity of PHB-HV/ starch blends observed in the present work, in relation of pure PHB-HV film, agrees with the results obtained by Thiré et al. (2006).

3.4 Differential scanning calorimetry (DSC)

Figure 8 shows the DSC scans of pure PHB-HV and its blends with regular corn starch. The transitions are attributed to PHB-HV. However, the positions and intensity of the transitions are affected by corn starch to different degrees.

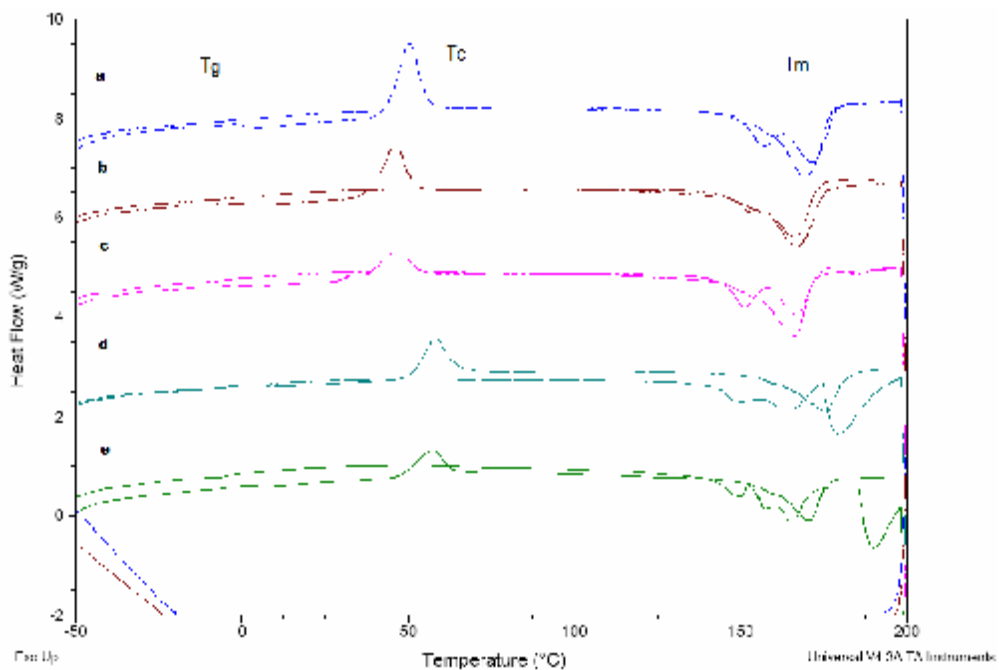


Fig. 8. DSC thermograms of PHB-HV and PHB-HV/ regular corn starch blends as obtained by casting with varied starch contents (wt %): (a) 0, (b) 20, (c) 30, (d) 40, and (e) 50. Tg: glass transition temperature; Tc: crystallization temperature;; Tm: melting temperature.

The data of DSC are summarized in Table 2. Both materials in the blend are semicrystalline, so the amorphous phase constitutes only a fraction of the polymer in the blend. Based on this, the detection of the glass transition temperature of starch by DSC can be difficult due to its low concentration and the presence of crystalline phase that changes the transition (Antunes & Felisberti, 2005).

The glasses transition temperature of PHB-HV in the blends was affected by corn starch content, because it decreases as starch content increases. The Tm of PHB in the blend is lower than that of pure PHB-

HV in some cases (20% and 30% of starch). The melting enthalpy of the blends, ΔH_m , decreased with the increase of starch. The melting point of the blends depends on many factors such as morphology, kinetics of crystallization and blend composition (Ghaffar, 2002). Both blends exhibited a single, composition-dependent T_g value, indicating that a single homogeneous amorphous phase was present in these two mixtures (i.e. the two components were completely miscible in the amorphous phase).

According to Matzinos et al. (2002) the small decrease in the melting temperature indicates that a phase separation, generally found in non-miscible polymers, has occurred. This can be clearly seen in the micrographs in the blend morphology section.

The crystallization enthalpy, ΔH_c , decreased with an increase of starch in the blend and crystallization temperature decreased in 80/20 and 70/30 blends and increased in 60/40 and 50/50 blends (Table 2). ΔH_c values were lower compared to pure PHB-HV, indicating that blends had a lower degree of crystallinity than pure PHB-HV. As shown by FTIR spectrum and X-Ray diffractograms, the increase of starch content in the blends reduced its crystallinity.

Table 2 Thermal properties of blends of PHB-HV and corn starch in different contents

PHB-HV/ starch ratio (wt/wt)	T _g (°C)	ΔH _c (J g ⁻¹)	T _c (°C)	ΔH _m (J g ⁻¹)	T _m (°C)
100/0	3.57	23.65	51.96	42.08	169.42
80/20	0.45	20.04	45.34	43.04	167.33
70/30	0.75	10.99	46.91	35.00	167.26
60/40	-	16.72	57.72	27.04	174.47
50/50	-	11.06	57.78	19.82	170.96

n.a. - , Not available

T_g: glass transition temperature; ΔH_c: crystallization enthalpy; T_c: crystallization temperature; ΔH_m: melting enthalpy; T_m: melting temperature.

3.5 Optical microscopy

An optical microscopy was used to study the distribution of starch in the blends. The photomicrographs in Figures 9 and 10 show the morphology of blends in different concentrations of regular corn starch by optical microscopy.

These photomicrographs showed the presence of two distinct phases (regular starch and PHB-HV), with little dispersion of starch in PHB-HV matrix. It is a general trend that the better the dispersion of the starch in the thermoplastic matrix, the better the mechanical properties (Thiré et al, 2006). The presence of discrete dark particles indicates unmelted and heterogeneously dispersed starch throughout the PHB-HV matrix. In the Figure 10, it can be seen that the distribution of starch granules is not homogeneously dispersed in the PHB-HV matrix. These results are in agreement with SEM micrographs (Figure 11).

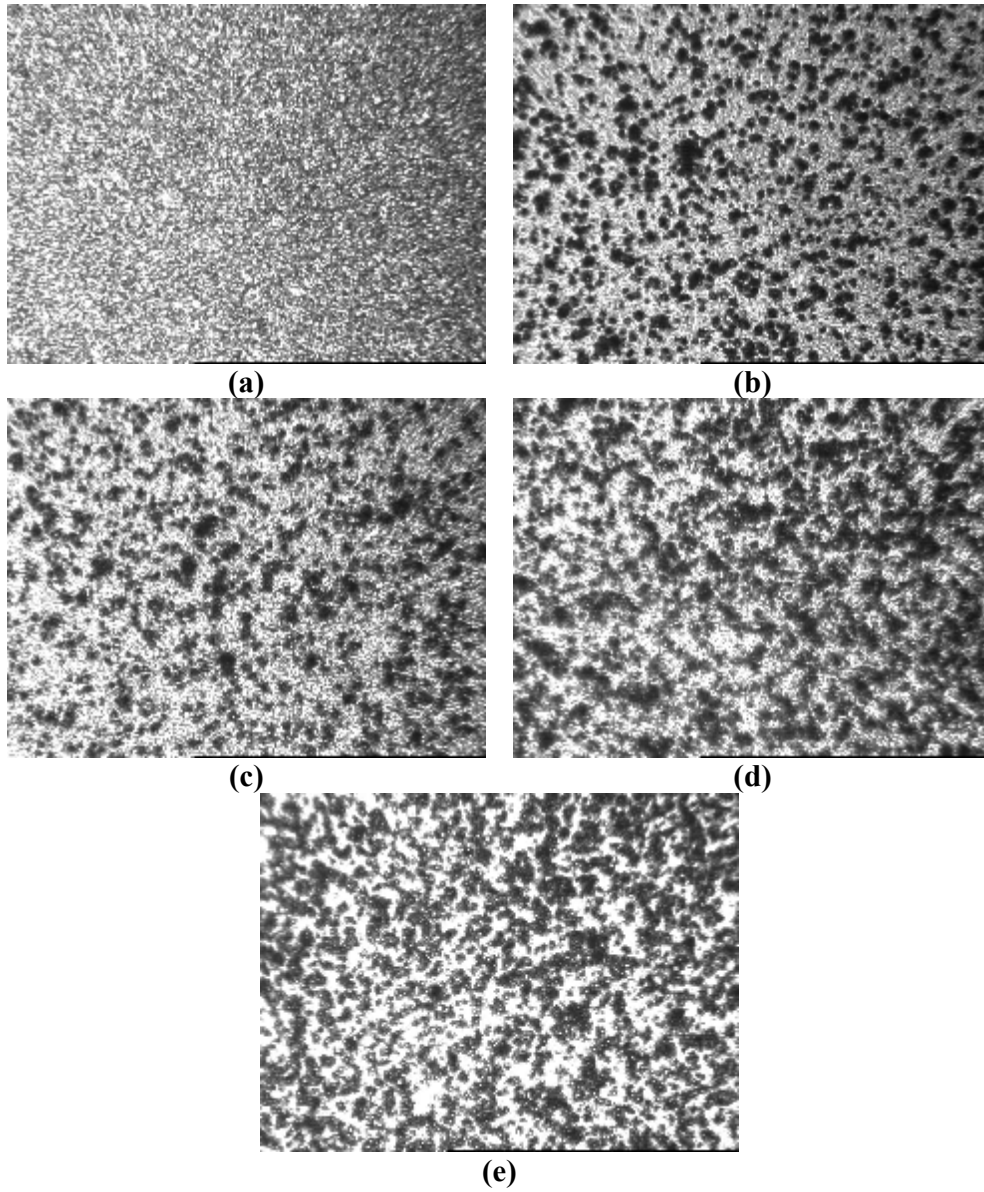


Fig. 9. Optical photomicrographs of the external surface of PHB-HV / regular corn starch blend films in different concentrations of regular corn starch, obtained by casting process, at 4x amplification and ordinary light; (a) 0% starch, (b) 20% starch, (c) 30% starch, (d) 40% starch, (e) 50% starch.

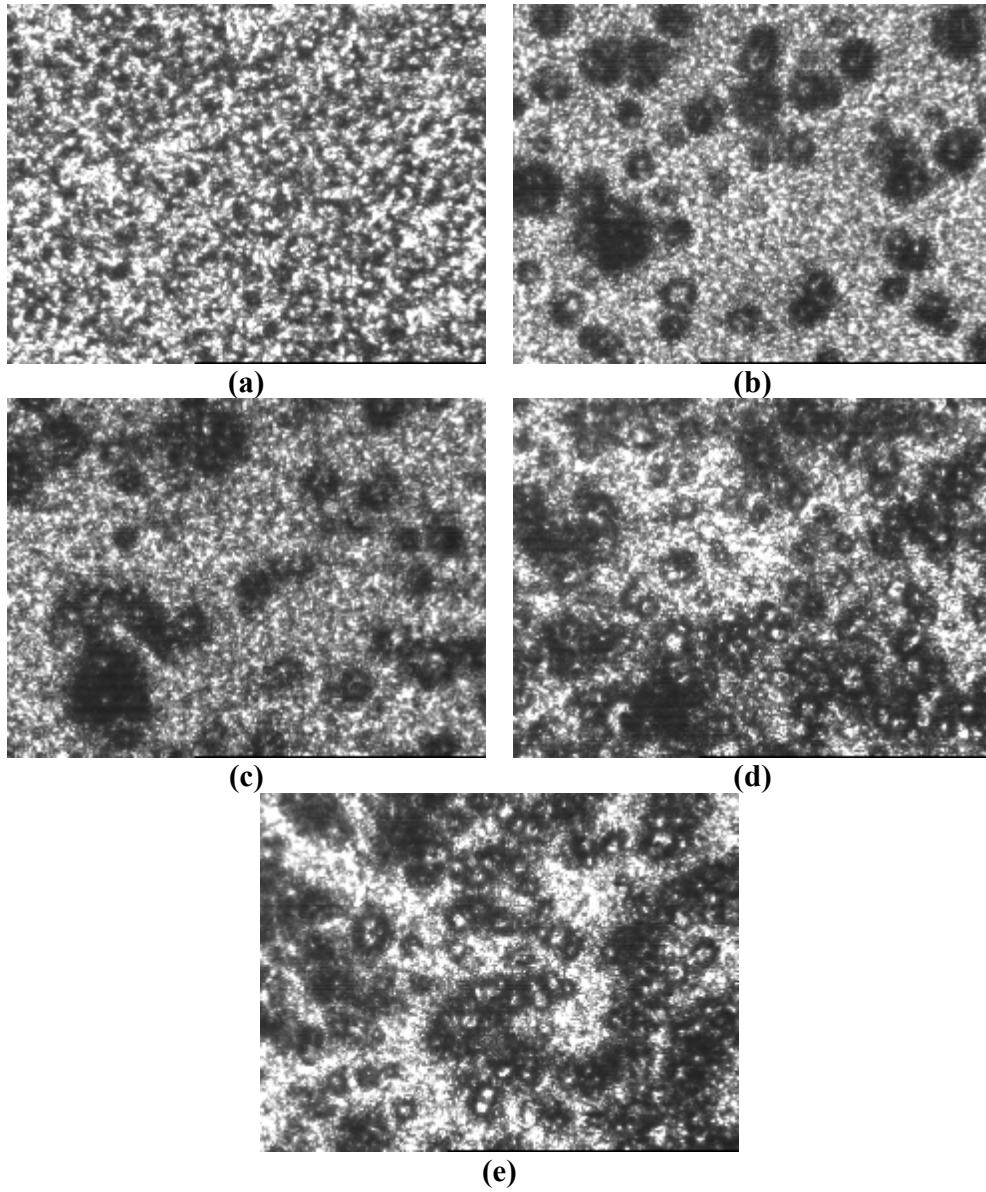


Fig. 10. Optical photomicrographs of the external surface of PHB-HV/ regular corn starch blend films in different concentrations of regular corn starch, obtained by casting process, at 20x amplification and ordinary light; (a) 0% starch, (b) 20% starch, (c) 30% starch, (d) 40% starch, (e) 50% starch.

3.6 Scanning electron microscopy

The morphology of PHB-HV/ starch blends also was investigated by scanning electron microscopy. The scanning electron micrographs are shown in Figure 11. It was found that the dispersion of starch component in the blends was not uniform, indicating phase separation between PHB-HV and starch.

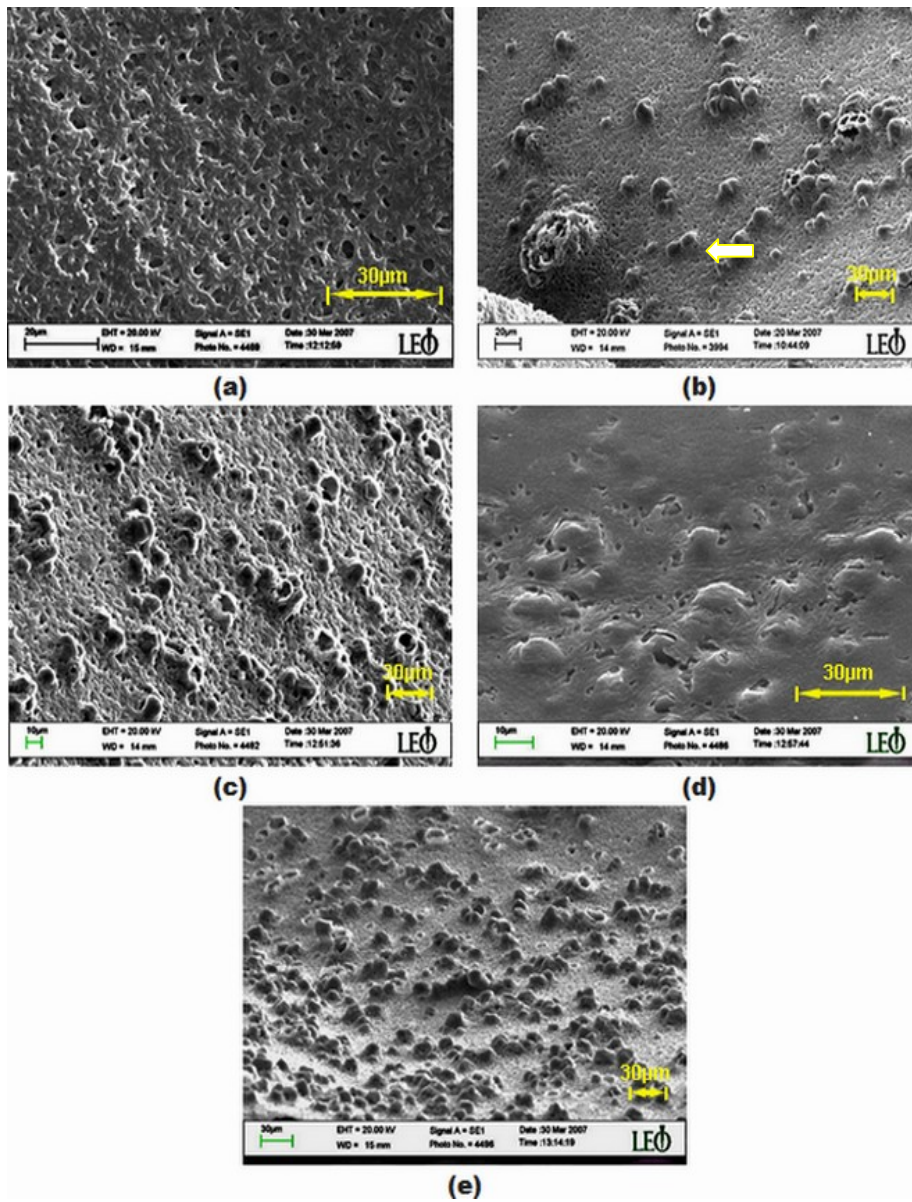


Fig. 11. Scanning electron microscopy of PHB-HV/ regular corn starch blend films with varied starch content (wt %): (a) 0 (pure PHB-HV), (b) 20, (c) 30, (d) 40, (e) 50.

Figure 11 indicates clear heterogeneous morphology of the blends. The surfaces of the blends consisted of a continuous matrix with several features. According to Thiré et al. (2006) that studied the effect of starch addition in compression-molded PHB/ starch blends, it seems reasonable to conclude that the surface features visualized in the matrix of blend consist of starch granules that were not disrupted during process. These granules are dispersed in the continuous PHB-HV rich matrix and are loosely adhering to it. In the figure (11b), the blend with 10 wt% starch, a void (indicated by an arrow) can be observed, which probably resulted from the poor interfacial adhesion of the starch in the PHB matrix. These observations agree with Seves et al. (1998) who studied the morphology of poly(3-hydroxybutyrate-co-hydroxyvalerate)/ starch valerate blends prepared in the form of sheets by mixing the molten polymers in a microextruder.

The starch granules are not well dispersed throughout the matrix, and they are present as agglomerates, grouped together in clearly demarcated domains. According to Thiré et al. (2006) the formation of these agglomerates contributes to poor mechanical properties. It seems clearly that starch did not melt during blending that is similar to the findings of Ramsay et al. (1993) and Thiré et al. (2006).

3.7 Colour and Opacity

Figure 12 shows the colour difference of different blends films with starch concentration variation between 0 and 50%. The films did not show transparency; it can be seen that they showed a little variation in colour difference with increasing of starch content.

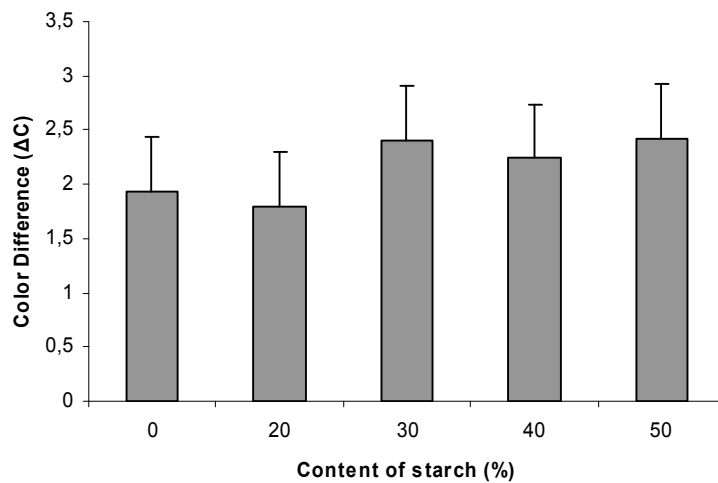


Fig. 12. Colour Differences of PHB-HV/ regular corn starch blend films with varied starch content (wt %).

The opacity showed the variation with different starch content (Figure 13). Opacity in immiscible polymer blends originates from two possible sources: different refractivity due to interfacial voids or the formation of microvoids at the interface resulting from poor surface adhesion and the different thermal expansion coefficients of matrix and dispersed phase (Rodriguez-Gonzalez et al., 2003).

The other potential cause of opacity in immiscible polymer blends is the formation of micro voids in the interface resulting from poor surface adhesion and the different thermal expansion coefficients the matrix and dispersed phase. The films did not show good interfacial adhesion, which was demonstrated by SEM (Figure 12). It is apparent

that poor interfacial contact is responsible for high levels of opacity demonstrated by this immiscible polymer blend.

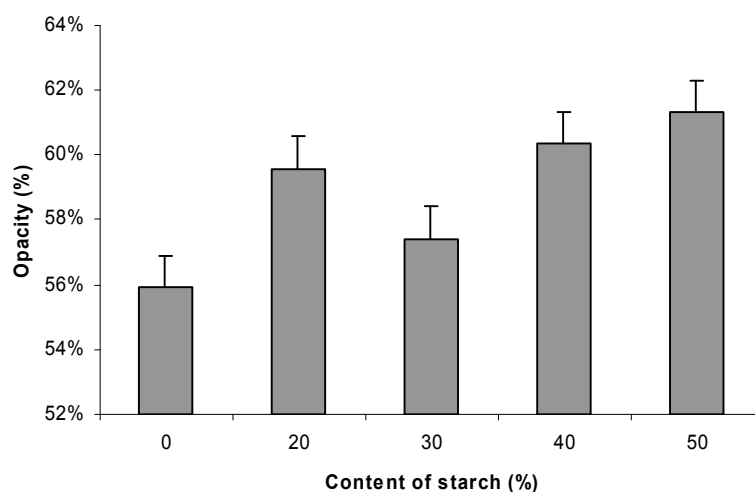


Fig. 13. Opacity of PHB-HV/ regular corn starch blend films with varied starch content (wt %).

According to Paul & Newman (1978) films or molded objects made from two mutually miscible or compatible polymers are optically clear and have good mechanical integrity, whereas those made from incompatible polymers are usually translucent or opaque and weak. Films prepared from two incompatible polymers can be transparent if the two component polymers have equal refractive indices.

4 Conclusions

The Young's Modulus, stress, strain and puncture decreased with an increase in starch content in the blend with PHB-HV. FTIR

measurements indicated that no intermolecular interactions existed between the two polymers as no shift in the absorption peaks of the PHB-HV or starch in the blends was observed, which suggests that PHB-HV and starch are immiscible. The blend showed low crystallinity (<4%).

PHB-HV/ starch blends obtained by casting process are immiscible; however, the two components were completely miscible in the amorphous phase.

Optical micrographs of PHB-HV/ starch blends films showed incomplete starch melting during blending. The blends showed a lack of interfacial adhesion between starch and PHB-HV and heterogeneous dispersion of starch granules over PHB-HV-rich matrix, as evidenced by SEM and optical micrographs of the surface. The colour difference and opacity had a small variation with increase of starch content.

5. References

Antunes, M.C.M., Felisberti, M. I. Blends of poly(hydroxybutyrate) and poly(ϵ -caprolactone) obtained from melting mixture. **Polímeros: Ciência e Tecnologia**, 15:2, 134-138, 2005.

Callister, W.D. Jr. **Materials Science and Engineering: an Introduction**, J. Wiley and Sons, Inc., 2003.

Galego, N., Rozsa, C., Sanchez, R., Fung, J. Vazquez, A., Tomas, J. S. Characterization and application of poly(β -hydroxyalkanoates) family as composite biomaterials. **Polymer Testing**, 19: 485-492, 2000.

Ghaffar, A. **Development material based on poly(3-hydroxybutyrate) PHB**. Dissertation Zur Erlangung des akademischen grades. Doctor rerum naturalium (Dr. rer. Not.) der Martin-Luther Universität Halle – Wittenberg, Halle, Saale, 2002.

Godbole, S., Gote, S., Latkar, M., Chakrabarti, T. Preparation and characterization of biodegradable poly-3-hydroxybutyrate-starch blend films. **Bioresource Technology**, 86: 33-37, 2003.

Huang, H., Hu, Y., Zhang, J., Sato, H. Zhang, H. Noda, I., Ozaki, Y. Miscibility and Hydrogen-Bonding Interaction in Biodegradable Polymer Blends of Poly(3-hydroxybutyrate) and a Partially Hydrolyzed Poly(vinyl alcohol). **Journal Physical Chemistry**, 109: 19175-19183, 2005.

Innocentini-Mei, L. H., Bartoli, J.R., Rodrigo, C. B. Mechanical and thermal properties of Poly(3-Hydroxybutyrate) blends with starch and starch derivates. **Macromolecular Symposium**, 197: 77-87, 2003.

Jayasekara, R., Harding, I., Bowater, I., Christie, G.B.V., Lonergan, G.T. Preparation, surface modification and characterization of solution cast starch PVA blended films. **Polymer Testing**, 23: 17-27, 2004.

Lai, S.-M., Don, T.-M., Huang, Y.-C. Preparation and Properties of Biodegradable Thermoplastic Starch/ Poly (hydroxybutyrate) blends. **Journal of Applied Polymer Science**, 100: 2371-2379, 2005.

Lin-Vien, D., Colthup, N. B., Fateley, W.G., Grasselli, J.G. **The Handbook of Infrared and Raman Characteristic Frequencies of Organic Molecules**; Academic Press: New York, 1991.

Matzinos, P., Tsuki, V., Kontoyiannis, A., Panayiotou, C. Processing and characterization of starch/ polycaprolactone products. **Polymer Degradation and Stability**, 77: 17-24, 2002.

Paul, D.R., Newman, S. **Polymer blends**, Academic Press, London, 1978.

Ramsay, B.A., Langlade, V., Carreau, P.J., Ramsay, J.A. Biodegradability and mechanical properties of poly-(β -hydroxybutyrate-co- β -hydroxyvalerate)/ starch blends. **Applied and environmental microbiology**, 50, 4: 1242-1246, 1993.

Rodriguez-Gonzalez, F.J., Ramsay, B. A., Favis, B.D. High performance LDPE/thermoplastic starch blends: a sustainable alternative to pure polyethylene. **Polymer**, 44: 1517-1526, 2003.

Sato, H., Dybal, J., Murakami, R. Noda, I., Ozaki, Y. Infrared and Raman spectroscopy and quantum chemistry calculation studies of C-O \cdots O hydrogen bondings and thermal behaviour of biodegradable polyhydroxyalkanoate. **Journal of Molecular Structure**, 744-747: 35-46, 2005.

Sato, H., Murakami, R., Pardemshoke, A., Yamagushi, H., Terauchi, H., Ekgasit, S., Noda, I., Ozaki, Y. Infrared Spectroscopy Studies of CH \cdots O Hydrogen Bondings and Thermal Behavior of Biodegradable Poly(hydroxyalkanoate) **Macromolecules**, 37: 7203, 2004.

Seves, A. Beltrame, P. L., Selli, E., Bergamasco, L. Morphology and thermal behaviour of poly(3-hydroxybutyrate-co-hydroxyvalerate)/ starch valerate blends. **Die Angewandte Makromolekulare Chemie**, 260: 65-70, 1998.

Thiré, R.M.S.M., Ribeiro, T.A.A., Andrade, C.T. Effect of Starch Addition on Compression-Molded Poly (3-hydroxybutyrate)/ starch blends. **Journal of Applied Polymer Science**, 100: 4338-4347, 2006.

Zhang, L., Deng, X., Zhao, S., Huang, Z. Biodegradable Polymer Blends of Poly (3-hydroxybutyrate) and Starch Acetate. **Polymer International**, 44: 104-110, 1997.