EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES

http://www.ejbps.com

ISSN 2349-8870
Volume: 3
Issue: 3
07-22
Year: 2016

STUDY OF THE SYNTHESIS AND CHARACTERIZATION OF A POLY (ETHYLENE SUCCINATE) BIODEGRADABLE POLYESTER WITH POSSIBLE APPLICATION AS ENCAPSULATING MATRIX OF ESSENTIAL OIL OF LEMONGRASS.

Carlos A. L. M.[1], Diré G. F.[2,3*], Nascimento C. C. H. C.[2], Nascimento S. F.[2,3], Vasconcelos S.D. D. de [2,5], Camacho A. C. L. F.[4], Oliveira J. F. F.[3], Nogueira R. I.[7], Barreto A. S.[2,6], Pinto P. R.[1]

[1]Laboratory Materials Technology - LTM, UEZO (State University Center of West Zone), Avenida Manuel Caldeira de Alvarenga, 1203. Campo Grande, Rio de Janeiro (RJ), CEP23.070 200, Brazil
[2]Laboratory of Chemical and Biological Analysis/ LAQB, Sector Center of Biological and Health Sciences (CSBS), UEZO (State University Center of West Zone), Rio de Janeiro, Brazil.
[3]Estácio de Sá University, Health Sciences Center, Rio de Janeiro, Brazil.
[4]Department of Nursing and Administration Fundamentals (MFE/UFF), Aurora de Afonso Costa School of Nursing of the Fluminense Federal University (UFF), Niterói, Rio de Janeiro, Brazil.
[5]Federal Center of Technological Education Celso Suckow da Fonseca -CEFET-NI.
[6]Brazilian Association of University Education Abeu/UNIABEU, Rio de Janeiro, Brazil.
[7]Brazilian Agricultural Research Corporation (Embrapa), Rio de Janeiro, Brazil.

***Author for Correspondence: Diré G. F.**
Estácio de Sá University, Health Sciences Center, Rio de Janeiro, Brazil.

Article Received on 19/12/2015 **Article Revised on 09/01/2016** **Article Accepted on 02/02/2016**

ABSTRACT

In recent years, interest in biomaterials grew in the biotechnology, pharmaceutical and food industries. Within these areas, studies on natural and synthetic biopolymers for encapsulating bioactive matrices are greatly developed. To study the possibility for use as bioactive encapsulating matrix by the techniques of spray-drying and lyophilization, was obtained by the polycondensation of polyester biodegradable poly(ethylene succinate) (PES). The obtained polymer was characterized as the microstructure by Infrared Spectroscopy Fourier transform (FTIR), and thermal stability by thermogravimetric analysis (TGA) Thermogravimetric analysis and derivative (DTG), as the characteristic crystalline by optical microscopy (MO) and as to the toxicity by preliminary microbiological disk diffusion testing for bacterial strain of *Staphylococcus aureus* (*S. aureus* ATCC 8096). The FTIR profile of the curve showed an absorption around 3000 cm -1 assigned to terminal OH PES grouping. Absorptions around 1750 cm^{-1} and 1190 cm^{-1} confirmed the presence of ester carbonyl at 2450cm and vibration^{-1}, 2200cm^{-1} and 2000cm^{-1} were assigned to be the grouping CH absorptions. The thermogravimetric curves showed a loss of 1.3% volatile at 100 °C, attributable to residual water generated as a byproduct of the reaction the PES also demonstrating its hydrophobic character. A maximum peak at 419.03°C. Degradation was also observed, and a total residual content of only 1.8% for the synthetic route employed. The micrographs obtained in optical microscopy confirmed the crystalline feature of PES obtained, as evidenced by spherulitic morphology. Preliminary in vitro microbiological tests showed no toxicity of poly(ethylene succinate) at concentrations of 12.5 mg/mL and 50mg/mL. The PES obtained was used as polymer matrix to encapsulate the essential oil of lemon grass by freeze-drying technique and the microparticles were evaluated by scanning electron microscopy (SEM). The images obtained by SEM showed particles with cluster appearance, with a well-defined and varied dimensions morphological structure, but with a tendency to form spherical structures.

KEYWORDS: Poly(ethylene succinate); bioactive; Microencapsulation.

INTRODUCTION

In recent years, interest in biomaterials grew in the biotechnology, pharmaceutical and food industries. Although they have lower mechanical and thermal resistance, biopolymers are generally biodegradable and have almost or no toxicity and is well tolerated in living organisms. Among the classes of biopolymers, natural polymers are preferred for use as wall-forming material in controlled release systems and asset protection materials, due to the structure of their supply chains that allow mobility and control of porosity. Active ingredients and suitable modifiers can be physically or chemically incorporated. However, although natural polymers preferably have encapsulating matrix as active ingredients, many studies have been conducted with other classes of biopolymers in this area, for example,

biodegradable aliphatic polyesters (PLA, PGA, PLGA) homo and copolymers lactate and glycolate, Ɛ poly-caprolactone (PCL), polyhydroxyalkanoates and certain acrylic polymers.

The polyesters derived from lactic and glycolic acids are noteworthy as they are approved by the FDA for use in human clinical and have a long history of safety.

Biodegradable polyesters obtained by the synthetic route, which had already been studied and applied as alternatives to commercial polymers used in packaging, particularly in the issue of waste management associated with non-degradable synthetic plastics, are also being studied as alternatives to natural biopolymers on encapsulating active compounds.

Microencapsulation of unstable compounds which can suffer from external effects such as oxidation and volatilization is particularly useful and necessary to prevent these compounds react with other components in the formulation and may produce undesirable by-products and other disadvantages. Microencapsulation of these assets becomes an alternative to promote increased stability of these compounds, among other benefits. Among these compounds, we highlight the essential oils that are volatile compounds that undergo a lot of times, with the action of light and oxidation, thus compromising its quality and the action of its active principles. Essential oils have a higher demand in perfumery, food and medicines for having, in most cases, phytosanitary, antibacterial and antifungal characteristics industries, such as the essential oil of lemon grass, making it an extremely interesting alternative microencapsulation for these compounds.

This paper proposes the synthesis and characterization of biodegradable polyester poly(ethylene succinate) (PES) as an alternative to encapsulating matrix of essential oil of lemongrass.

OBJECTIVES

General Objective

Synthesize and characterize poly(ethylene succinate) - biodegradable polyester - for use in microencapsulation of essential oil of lemongrass.

Specific Objectives

PES as to characterize the microstructure by Infrared Spectroscopy Fourier transform (FTIR), and thermal stability by thermogravimetric analysis (TGA). Identify the crystal structure of the PES by optical microscopy (OM). Microencapsulate the essential oil of lemon grass in PES matrix by means of the technique of Spray-Drying and Freeze-through and evaluate the morphology of the microparticles by means of Scanning Electron Microscopy (SEM).

LITERATURE REVIEW

The history of essential oils in Brazil began with the work of Theodor Peckolt. The researcher with Polish nationality and pharmaceutical training came to Brazil in 1847. Theodor Peckolt studied on the diversity of flora identifying composition and yield of various essential oils from Brazilian and/or acclimated plants in the country. These studies are registered in more than one hundred publications in which noteworthy periodicals of German nationality (Bizzo et al., 2009).

Essential oils (EO) are compounds extracted from plants consisting mainly of mono-and sesquiterpenes and phenylpropanoids. A variety of plant polyphenols have been reported to have anti-inflammatory, frequently associated with erythema, edema, hyperplasia, skin photoaging and photocarcinogenesis. Cymbopogon citratus (DC). Stapf (Poaceae) is a worldwide known medicinal plant, used in traditional medicine in inflammation-related conditions (Costa et al., 2015).

They can be obtained through the technique of steam distillation or by pressing the pericarp, in the case of citrus fruits (Bizzo et al., 2009). They are mainly used as flavors, fragrances, fragrance fixatives, pharmaceutical and oral compositions. Can be sold in its raw form or benefit. When their isolated components provide purified substances of important economic value such as limonene, citral, citronellal, eugenol, menthol and safrole. (Silva-Santos, 2006). Essential oils have a higher demand in perfumery, food and medicines by having industries, much of the time, plant characteristics, antibacterial and antifungal.

Chaisripipat et al. (2015) described that the hair tonic formulation with 10% of lemongrass oil seems to be the most effective preparation as an Anti-dandruff Hair Tonic.

The global OE market is around $ 15 million / year, with growth of approximately 11% per year (ITC, 2005; COMTRADE, 2005). According to the American base COMTRADE database (United Nations Commodity Trade Statistics Database), the biggest consumers of OE in the world are the United States, with about 40%, followed by the European Union, which has 30% of consumption, with a highlight of leading importing country for France, and Japan (7%), alongside the United Kingdom, Germany, Switzerland, Ireland, China, Singapore and Spain to.

In 2004 the Brazil appeared among the top five countries to provide citrus essential oils to the EU, with 5% of imported oil, and are among one of the major international exporters. According to Eurostat, Brazil exported to Europe 12,526 tons of orange oil in 2006, and 43% for the Netherlands, 20% in Germany and 19% for the UK. According to the American body DO, Brazil is the leading exporter of OE orange to the U.S. from

January 2005 to October 2008, 38.024 tons of orange oil were exported.

ESSENTIAL OIL OF LEMON GRASS

Lemongrass (*Cymbopogon citratus* (DC) Stapf), belonging to the family Poaceae, is an aromatic plant cultivated for commercial production of essential oil that usually presents as components in kind: citral, myrcene, limonene, nonanal, nerol, geraniol, decanal, linalool, terpineol and the geranyl acetate (Abegaz Yohannes, 1983; Carriconde et al, 1996). Being the major constituents citral and the monoterpenes myrcene. The increased use of essential oil of lemongrass has been in the pharmaceutical industry, serving as the starting material for the synthesis of important compounds such as ionones, methyl ionones and vitamin A.

Citral is a mixture of isomers, known as A or E isomer citral (geranial) and citral B or Z isomer (neral), with a characteristic lemon aroma. Presents antioxidant and inhibitory activities of mycelial growth of microorganisms fungitoxic being also employed in enteric treatments (Bakkali et al., 2008). Cheel and colleagues (2005) evaluated the antioxidant effect of extracts of lemon grass for the purpose of scavenging free radicals by three methods. The first method was evaluated by the level of whitening of 1,1-diphenyl-2-picryl-hydrazyl (DPPH). The second method was evaluated for the elimination of superoxide radical and the third method was evaluated by lipid peroxidation in human erythrocytes. The extracts showed effect on the assay DPPH and superoxide anion with inhibition values of free radicals ranging from 40 to 68%. Lipid peroxidation in erythrocytes showed a content of free radicals inhibition of 19-71% at a concentration of 500 g of extract/ mL.

Myrcene now it is a triolefínico compound having characteristic odor that can vary fresh, spicy, terpene, and aromatic balsamic (ANDRADE et al., 2000). While myrcene is one of the major components of lemongrass oil him, individually, has antioxidant and fungicidal activity, but has extreme economic importance because it is used as an important syntheses such as Vitamin A and E (FOCA et al., 2014) and Dhanalakshmi Vaultier, 2003). Furthermore, myrcene was identified as the active principle responsible for the analgesic effect of lemon grass tea in rodents (FIOCRUZ, 2014). Essential oils are highly volatile compounds and to ensure their chemical stability against external effects is desirable to be encapsulated.

Microencapsulation AND POLYMER MATRIX

Microencapsulation has been defined as "the technology include solid, liquid and gaseous materials in small capsules that release their content in a controlled manner for extended periods of time" (GOUIN, 2004). Or, according to Gibbs et al (1999), as a technology of packaging material into thin polymer layers, which release their content at controlled rates, or protect the external environment. The covered or encapsulated material is known as active, filling material, internal phase, or fill payload and the shell can be called capsule wall material, membrane, charger or peel.

Microencapsulation of unstable compounds which can suffer from external effects such as oxidation and volatilization is particularly useful and necessary to prevent these compounds react with other ingredients in formulations of drugs, cosmetics, for example, may produce undesirable by-products and other disadvantages. Microencapsulation of these assets becomes an alternative to promote increased stability of these compounds, among other benefits.

Studies by Shu et al (2006) show the difference in the effect of chemical degradation of the encapsulated and unencapsulated lycopene. The stability of lycopene was evaluated for degradation by the action of light, heat and oxygen, micro encapsulating this asset by spray-drying technique using a conjugate of gelatin and sucrose to the encapsulating agent. The polymers are good candidates for use as encapsulating matrix, because When removing the solvent from the polymer solution, films with good film forming properties. The most widely used polymers for this application are the polysaccharides. These polymers are chosen by structures of their chains, which allow for mobility and having little or no toxicity. The most commonly used materials include encapsulating the polysaccharides (gum arabic, agar, alginates, carrageenans, starches, chitosan, and derivatives of cellulose), lipids and proteins (Jacobs and Mason, 1993).

Other classes of polymers are used Also the encapsulating bioactive matrices, such as aliphatic biodegradable polyesters (PLA, PGA, PLGA) homo and copolymers of glycolate and lactate, Ɛ poly-caprolactone (PCL), polyhydroxyalkanoates and some acrylic polymers.

These materials are the most widely used in the development of nanostructured for encapsulation and controlled release of bioactive compounds systems. The polyesters derived from lactic and glycolic acids are noteworthy as they are approved by the FDA for use in human clinical and have a long history of safety (Duran, 2006; SILVA, et al, 2003).

Several studies microencapsulation of proteins and peptides in arrays of polylactic acid (PLA) have been studied in recent decades. Ma et al (2001) micro encapsulated insulin PLA matrix by solvent evaporation technique. In this study, PLA microcapsules containing insulin were named PLA-MCI. The morphology of the microcapsules was observed by scanning (SEM) electron microscopy. The release profile of insulin was determined in two ways: in-vitro by measuring insulin by UV spectrophotometry techniques and in vivo by measuring blood glucose in 34 diabetic rats.

The *in vitro* release profile of insulin from microcapsules was determined under the following conditions: MCI-PLA was dissolved in 10 mmol/L phosphate buffer solution (PBS) (pH 7.4) at 37 ± 1 °C. The concentration of insulin released was determined at intervals using the technique of UV at a wavelength of 477 nm. The peak release was found between 6-10 hrs. After 36h, the release rate became very slow, reaching a plateau. The release persisted for 120h. The rate of insulin release from MCI PLA-2, 6, 8 and 10 hours were 38, 58, 65 and 74%, respectively.

The *in vivo* release profile of insulin from microcapsules PLA-MCI (containing 2.5 mg of insulin) was given by oral administration in 34 diabetic rats. It was found that the rate of blood glucose decreased by about 68.5% in twenty-three rats, approximately 39.7% in seven rats and no change was observed in four rats. On average BG decreased from 57 ± 21% (n = 34). PLA-MCI led to a decrease in blood glucose 1-3 hours after oral administration.

Liu et al (2005) microcapsules prepared with uniform sizes of PLA by combining the techniques of emulsification membrane in a membrane Shirasu porous glass (SPG) with the technique of emulsion-solvent evaporation to microencapsulate multiple lysozyme. An aqueous phase containing lysozyme was used as internal water phase (W1). PLA and sorbitan sesquioleate emulsifier (Arlacel 83) was dissolved in a mixed solvent of dichloromethane (DCM) and toluene that was used as the oil phase (O). These two solutions were emulsified by a homogenizer to form a primary emulsion (w1/ O).

The primary emulsion was permeated through the uniform pores of 5.25 mm in a membrane of GSP to the external aqueous phase with pressure nitrogen gas to form droplets w1/o/w2 uniforms. Then, the solid polymeric microcapsules were obtained by simple solvent evaporation. Among other things, the effect of the molecular weight of the PLA in the encapsulation efficiency of the technique used to TECNIA and stirring was evaluated. PLAs were obtained with different molecular weights 10 kDa, 20 kDa, 100 kDa and 300 kDa, which were used to prepare the lysozyme-loaded microcapsules SPG membrane emulsification technique and method of agitation, respectively.

For both microcapsules prepared by the emulsification technique in SPG membrane as for the method of stirring, the drug encapsulation efficiency increases with increasing molecular weight of the PLA more evident in the second case. When the microcapsules were prepared using PLA 300 kDa, most encapsulation efficiency (71.63%) was obtained.

For the microcapsules prepared by the method of stirring, the drug encapsulation efficiency increased gradually as the increase of the molecular weight of the PLA. When higher molecular weight PLA were used, a primary emulsion had improved stability, which was considered an important factor in increasing the efficiency of drug encapsulation. Furthermore, the double emulsion was not easily broken in the case of the method of agitation, and the drug was more difficult to diffuse in the external aqueous phase through the oil phase, while the increased viscosity of the oil phase, resulting in less chance of leakage of the drug. It was evident then that the PLA is a good candidate for matrix encapsulation in various molecular weights.

Microencapsulation techniques

The selection of the microencapsulation method depends on some aspects such as the physicochemical properties of both the material to be encapsulated as the encapsulating agent (mainly solubility); the application or purpose of microparticles; the size, shape and texture of the microparticle and release mechanism of the material to be encapsulated (Bansode et al., 2010).

Each microencapsulation process depends on several aspects, but the basic principle is common to all. In general, this corresponds to the deposition of the encapsulating agent on the agent to be encapsulated, following a series of steps. Initially, the encapsulating agent is dissolved or melted, lying in the liquid state. In turn, the encapsulating agent may be present in the form of small particles (if solid nature) or droplets (if liquid in nature) or even in the form of gas.

The material to be encapsulated is placed in a suitable medium and then, on this is deposited the encapsulating agent. Finally, the encapsulating agent undergoes solidification forming microparticles (Venkatesan et al., 2009).

The literature classifies microencapsulation methods into three types, namely physical, chemical and physico-chemical methods. Physical methods are those that take into account the physical transformation of materials by physical process without any chemical interaction, being subclassified into: spray drying, spray chilling, spray coating, extrusion, co-crystallization, lyophilization (freeze drying); Chemical methods are those made by polymerization reactions have been necessary chemical reaction being subclassified into: in situ polymerization, interfacial polymerization, molecular inclusion.

Physicochemical methods are those that do not depend only on physical processes, chemical reactions but are also necessary during the process, being subclassified into: coacervation (which can be simple or complex), organic phase separation, solvent evaporation, spray forming the crosslinking agent (Thies 2003; Jackson and Lee, 1991). Chemical and physico-chemical methods are not treated in this work.

Microencapsulation by Spray-Drying Technique

In this process the active ingredient to be encapsulated is mixed or homogenized in a solution of polymeric

encapsulating agent to form a stable emulsion. The emulsion is sprayed droplets are formed and immediately dried, usually by hot air, which turns them into a powder with particles of active material inside. This process is relatively fast drying up a volume of 20 mL /min. (GHARSALLAOUI et al. 2007).

Spray drying is a technique commonly used for the microencapsulation of food ingredients (DESOBRY et al. 1997). It is a simple and cheap method wherein the polymeric matrices can be used to create the membrane encapsulation. However, this technique has some disadvantages such as low yields, difficult to control the particle size, inadequate to encapsulate compounds that degrade below 200 ° C. Adding to that this technique is not commonly used for water-insoluble matrices (GHAESALLAOUI et al, 2007;. JOHANSEN et al, 2005.).

The limitation of this technique predominantly favor the use of water-soluble polymeric matrices such as polysaccharides.

Barbosa et al (2005) compared mixtures of polysaccharides in the microencapsulation of natural pigment annatto, using the technique of spray drying in order to protect the compound from the action of light. Formulations of 95/5 acacia / sucrose (formulation) 80/20 maltodextrin/sucrose (formulation B), 100% maltodextrin (formulation C) 99.8/0.2 maltodextrin / emulsifier (formulation were prepared d). The pigment encapsulated in nature and each of the formulations were exposed in aqueous solution, a light source of 700 lux at 21 ° C. The retention efficiency (final concentration / initial concentration) of pigment versus time of light exposure in hours.

The unencapsulated pigment showed loss of nearly 80% in its concentration before reaching 50 hours of exposure to light. Have the microcapsules showed a high retention rate of pigment, especially the formulation composed of 95% gum arabic + 5% sucrose (formulation), which retained the pigment almost in full, even after 400 hours of exposure light. Microencapsulation is a viable polysaccharides protect the natural pigment annatto, which allows the pigment to be used in foods without any loss by the action of light environment (BARBOSA et al.2005).

Microencapsulation by Spray-Coating Technique

The technique of spray-coating has similar conditions and process variables of the spray drying technique. However, spray-coating the mixed agent is encapsulated or dispersed in a molten encapsulating agent and not a solution. Subsequently the hot air drying, the dispersion is cooled in a stream of cold air forming the particles by the solidification of the encapsulating matrix. In spray-coating has the advantage of not using water or organic solvents resulting in lower energy use and less time to process, and the great advantage over the spray-drying technique (Bansode, et al., 2010).

Microencapsulation by Spray-Chilling Technique

Spray-chilling is the most commonly used technology for the encapsulation of several organic salts, inorganic as well as for textured ingredients, enzymes, flavorings, and other functional ingredients to improve the heat stability, slow release in humid environments, and / converting liquids or powders hydrophilic ingredients (solids). It is the least expensive technology of encapsulation. The particles produced by spray-chilling generally released all their content within minutes after being incorporated into the application material, which becomes a drawback of the technique where a long release time of the encapsulated active principle is required (GOUIN, 2004).

Microencapsulation by Extrusion Technique

The technique of microencapsulation by extrusion this is the dispersion of the material to be encapsulated in the melt of the encapsulating agent process. The dispersion is pushed by the screw throughout the extruder barrel with a multiple output array holes toward a cold bath of liquid desiccant. Along the way, the movement of air breaks the core material in spherical droplets, being completely coated with the encapsulating agent. The solidification of the encapsulant by cooling or by contact with the desiccant liquid, depending on the properties of the encapsulant (Azeredo, 2005). The filaments of extruded materials are broken into smaller fragments and dried by anti-caking agents (Shahidi and Han, 1993).

One advantage of this technique is that it is the most suitable for the encapsulation of flavors and volatile and unstable oils in carbohydrate matrices, which are very common in mothers microencapsulation techniques. One of the drawbacks of this technology is the formation of large particles which restrict the use of flavorings in applications where taste is paramount (GOUIN, 2004).

Microencapsulation by the Technical Co-crystallization

Co-crystallization is the process whereby the material to be encapsulated is incorporated within a cluster of crystals of the encapsulating matrix (Labell, 1991). Sucrose is used as a matrix. In this process, the crystal structure of sucrose is modified from a perfect crystal to a conglomerate. This structure has a porous configuration that allows the addition of a second ingredient which is the material to be encapsulated (BERISTAIN, et al. 1994). During the co-crystallization, the sucrose solution is concentrated to supersaturation state and maintained at high temperature (above 120 °C) to avoid recrystallization. A predetermined amount of the material to be encapsulated is then added to the concentrated sucrose solution under vigorous mechanical agitation of the mixture by promoting the nucleation sucrose - active material. The heating is stopped and the mixture reaches the temperature at which crystallization begins, being released a substantial amount of heat due

to the phase transition, which in turn contributes to the drying of the material. Stirring is continued until crystallization to promote the formation of the agglomerated product. These agglomerates consist of single crystals with sizes less than 30μM. The co-crystallized dried (if necessary) and screened to uniform particle size. The encapsulated active materials are incorporated in the interstices between the crystals. The advantages of this technique have a product which has low hygroscopicity and good flow properties and dispersion fully protected against oxidation, since the sensitive compounds are trapped within the agglomerates.

As disadvantages of the technique can highlight the specific matrix and the same need for high working temperatures, and is not suitable for encapsulating materials that degrade below 120°C. (Kim, et al, 2004;. AWAD and CHEN, 1993; BHANDARI, et al, 1998.).

Microencapsulation Technique for Freeze-drying

The technique of lyophilization is a process that consists in drying the emulsion of the encapsulating and encapsulated with a solvent, previously frozen by means of sublimation. (POLAK and PITOMBO, 2011). It's a simple process since it only involves drying the emulsion of the core in the wall material.

The lyophilization process does not alter the structure of the lyophilised component, as is immediately reconstituted in their original morphology when immersed again in the solvent (usually water). The technique ensures good stability of the component as storage, increasing delays shelf of the lyophilised component (Marques et al., 2006).

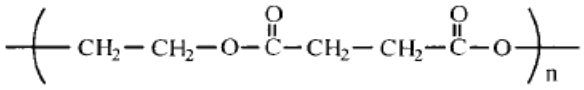
However, has the disadvantage in the high time of the process beyond the high costs involved. The capsules obtained by this technique do not have regular shapes, unlike other techniques. (Ratti, 2001; FANG and BHANDARI, 2010).

Polycondensation of polyesters Condensation reactions are common in organic chemistry. Are reactions that involve the formation of a new chemical bond between two or more molecules of the starting reagents. The most common are the condensation reactions to form new compounds by the loss of small molecules such as water and volatile products thereby generating heteroatoms molecular segments of the initial reactants. When you have bifunctional reagents in the reaction can be generated polymers, styling polycondensation reaction. One of the main features of polycondensation is the consumption of monomers during the reaction that is gradually disappearing in the early stages and gradually forming dimers that react to form larger molecules, unlike polyaddition, where the monomers will gradually fading into the reaction medium. Other features of polycondensations are low molecular weights and low yields due to the high viscosity of the reaction medium which hinders the standardization and thus the degree of polymerization. Thus, polymers obtained by polycondensation have molecular weights of only tens of thousands, whereas the polyaddition can reach hundreds of thousands.

A major advantage of the polymers obtained by condensation which have heteroatoms such as oxygen or nitrogen, is that they permit intermolecular interactions that favor the increase of mechanical strength, making polymers having low molecular weight have a higher mechanical strength than polymers sometimes high molecular weight obtained by polyaddition.

POLY(SUCCINATO DE ETILENO) (PES)

Poly(ethylene succinate) (PES) have recently attracted considerable attention. As a biodegradable polyester, commercially available PES has been considered as the most promising chemosynthetic biodegradable polymers. The PES has a relatively high melting temperature and favorable mechanical properties, which are similar to those of polymers used extensively as low density polyethylene and polypropylene (DOI, 2002). The molecular structure of PES is described in the figure below.



The crystal structure, crystal polymorphism, the kinetics of crystallization, morphology, growth kinetics of spherulites, the melting behavior, enzymatic degradation and thermal degradation of the PES have been studied extensively. (RAY AND KHATHA MA, 2009).

Chen et al (2008) synthesized Poly (Butylene succinate), poly (ethylene succinate) (PES) and copolymers of polybutylene succinate (BS) containing 7, 10 or 48% by mol BS through a reaction of direct polycondensation with titanium tetraisopropoxide as catalyst. These polymers and copolymers were analyzed for molecular weight by measuring the intrinsic viscosity, as regards thermal stability by TGA, DSC, for the compositions and distributions of copolymers sequence of spectra from H and C NMR and as a WAXD crystallinity.

MATERIALS AND METHODS

CHEMICALS

The main reagents and solvents used in preparing this course are listed below:

Ethylene: Provenance: Vetec Fine Chemicals Ltd.; Degree of Purity: P.A; Used as received.

Succinic Acid: Provenance: Vetec Fine Chemicals Ltd.; Degree of Purity: P.A; Used as received.

Titanium tetrabutoxide: Wako Pure Chemical; Purity: 95%; Used as received.

Antimony trioxide: Provenance: Vetec Fine Chemicals Ltd.; Purity: 99.5%; Used as received.

Phosphoric Acid: Provenance: Vetec Fine Chemicals Ltd.; Degree of Purity: PA; Used as received.
Chloroform: Provenance: Vetec Fine Chemicals Ltd.; Degree of Purity: PA; Used as received.
Essential Oil Lemongrass: LASZLO Aromatherapy Ltd,. 100% purity; Extracted by steam distillation of herbs.

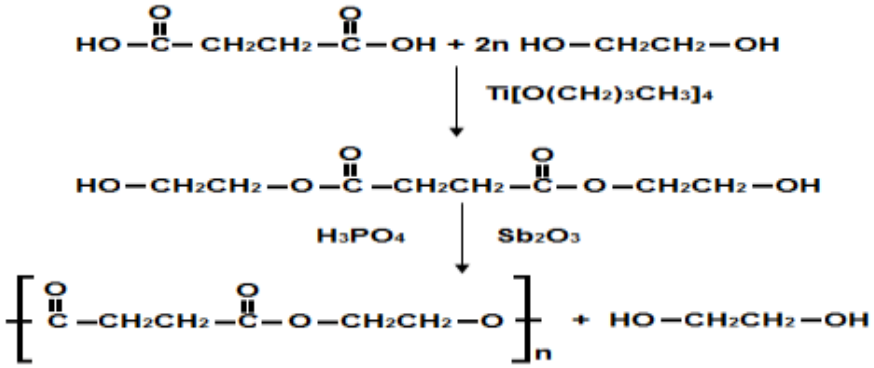
EQUIPMENT

In addition to the apparatus and common to research laboratories glassware, were also used in this course work the following equipment:
Vacuum Pump
Spectrophotometer: Thermo Scientific Nicolet model 6700;
Thermogravimetric Analyzer: Instrument SDT, template-driven Q600;
Optical Microscope: Leica DMSL, model DM / SL IV 98, with ranges of 10x, 40x and 100x;
Magnetic Turrax emulsifier: IKA T25 Ultra-Turrax;
Mini Spray Dryer: Büchi model B-190;
Freeze dryer: Boc Edwards Pirani 501 model;
Scanning Electron Microscope: JEOL / EO JSM-6490.

OBTAINING OF POLY(ETHYLENE succinate) (PES)

Synthesis of poly(ethylene succinate) and yield of the reaction:
Poly(ethylene succinate) PES was synthesized by polycondensation reaction between the monomers ethylene glycol (EG) and succinic acid (SA) with an initial molar ratio of EG/ AS 1.5:1 according to the literature (Fradet and Tessier 2003).

The polycondensation reaction to obtain Poly (ethylene succinate) is described in Figure below.



Reaction of poly condensing poly(ethylene succinate).

The system was conducted in a 200 mL reactor equipped with magnetic stirring in silicone oil bath. The ethylene glycol and succinic acid monomers and the catalyst the reaction (titanium tetrabutoxide) were taken after bathing system to stabilize at a temperature of 120 °C for 1h. The system was removed from the bath and when it reached room temperature were added 3.1×10^{-4} mol of phosphoric acid and 2.06×10^{-5} mol of antimony trioxide. The bath temperature was raised to 220 ° C and the reaction was continued for another 2h. The vacuum pump was coupled to the system to remove the water formed as a byproduct of the reaction. The system fitted for the reaction is described in Figure below.

Polycondensation reaction of the PES system.

Purification and drying of the reaction product

Poly(ethylene succinate) obtained in Synthesis was purified by dissolution in chloroform and precipitation in ethanol.

The reaction product obtained was dissolved in 200mL of chloroform by vigorous magnetic stirring and mild heating to facilitate solubilization. After being completely soluble PES obtained was precipitated in 400mL of 95% ethanol, cold and under slight agitation to facilitate precipitation. The solution was allowed to precipitate over nigth. The precipitate was filtered and dried in a vacuum oven at 70°C to constant weight.

Characterizations of PES

Characterizations of PES in Infrared Spectrometry (FTIR)

The synthesis product obtained was characterized by atomic absorption spectrometry in the infrared region through the spectrometer Thermo Scientific Nicolet model 6700 in 20scan scanning, the spectral range of 4000-500cm^{-1} in KBr pellet.

Characterization of PES by thermogravimetry (TG) and derivative thermogravimetric (DTG)

The thermal behavior of poly (ethylene succinate) was evaluated by thermogravimetric technique with its

derivative with template-driven equipment Instrument Q600 SDT, under nitrogen atmosphere, in a temperature range from 25°C to 800°C at a rate heating of 5°C/minute. The initial degradation temperature (Td), the temperature of the maximum rate of degradation (Tmax), the final degradation temperature (Tfinal) and the total amount of residue after firing were determined.

Morphological and surface characterization of the PES by optical microscopy (OM):
To analyze the crystalline structure of poly(ethylene succinate) was prepared in a film microscope slide. A mass of 40 mg was melted in PES hotplate at 150°C for 30 seconds beneath the surface of a microscope slide cleaned previously with 95% ethanol and added a coverglass on the film. After cooling at room temperature, superfine PES film was brought to the optical microscope. The characteristic of crystalline poly(ethylene succinate) was then analyzed using a Leica optical DMSL model DM/98 SL IV, with ranges of 10x, 40x and 100x.

Preliminary microbiological testing of in vitro toxicity of PES:
Toxicity tests of the polymer were performed by means of the indirect disk diffusion test and the well plate containing Medium Nutrient Agar gel. The bacterial strain used was the *Staphylococcus aureus* (*S.aureus* ATCC 8096) strain. The bacterial concentration was controlled using a range of Mc Farland 0.5.

The protocols followed were adapted from the protocol of ANVISA (Decree 1480/90) using as positive and negative controls 25µL of the antibiotic Amoxicillin concentration of 50mg/mL and 25µL of saline, saline respectively. The volumes of the controls were placed in absorbing disc, while the concentrations of the suspensions PES 12.5 mg/mL and 50mg/mL Nutrient Agar Nutrient Agar medium in were placed into wells of 25 µL volume.

SPRAY-DRYING
Formulation
A formulation with a ratio of 20 g of PES, 77mL of distilled water and 3 mL of essential oil of lemon grass (acquired by donation from LASZLO Aromatherapy and aromatology Brazilian Ind.) was prepared. This mixture was homogenized in a device IKA Turrax, model T25 at 8,000 rpm at ambient temperature of 25°C for 10 minutes.

Turrax IKA Laboratory Embrapa.

The mixture containing water, PES and the essential oil of lemon grass was taken to dehydration in laboratory spray-dryer Büchi brand, model B-190. The equipment was stabilized within the temperature range of inlet and outlet air of 190°C and 90°C, respectively, using a spray nozzle 0.3 mm. The mixture was maintained under mechanical stirring throughout the process. The following figure shows the spray-dryer equipment used in this work.

Mini Spray Dryer Büchi, Embrapa Laboratory.

Lyophilization
The same formulation containing mixture of water, PES and the essential oil of lemon grass was prepared for

lyophilization and were frozen for 24 hours in the freezing chamber at -17 °C.

The process was carried out at Boc Lyophilizer Edwards, model 501 Pirani, which was operated at a process time of 32 h, with a ramp thawing from -40°C to 30°C. Vacuum pressure of 1.5 × 10 1 atm. The next figure shows the lyophilization equipment used in this work.

Boc Edwards lyophilizer, Embrapa Laboratory.

Morphological and surface analysis of the PES film and microcapsules by scanning electron microscopy (SEM)

The morphology and the surface of the PES film obtained by pressing at 180°C and a pressure of 5ton for 1min, and the surface morphology and the microcapsules obtained in the lyophilization process, have been characterized by Scanning Electron Microscopy JEOL in equipment / EO model JSM-6490, after being covered in gold. Images in ranges of 30, 130, 430, 750x and 15000x, with accelerating voltage of 30kV beam were performed.

RESULTS AND DISCUSSION

The poly (ethylene succinate) (PES) was obtained by synthetic route, evaluated for the reaction yield and characterized by IR spectroscopy in the region by TGA and derivative thermogravimetric and Optical Microscopy. The PES was evaluated for toxicity by preliminary biological testing disk diffusion for bacterial strain of *Staphylococcus aureus* (*S. aureus* ATCC 8096). The synthesis product was used as polymer matrix to encapsulate the essential oil of lemon grass by freeze-drying technique and the microparticles were evaluated by scanning electron microscopy. The results of these analyzes are described below.

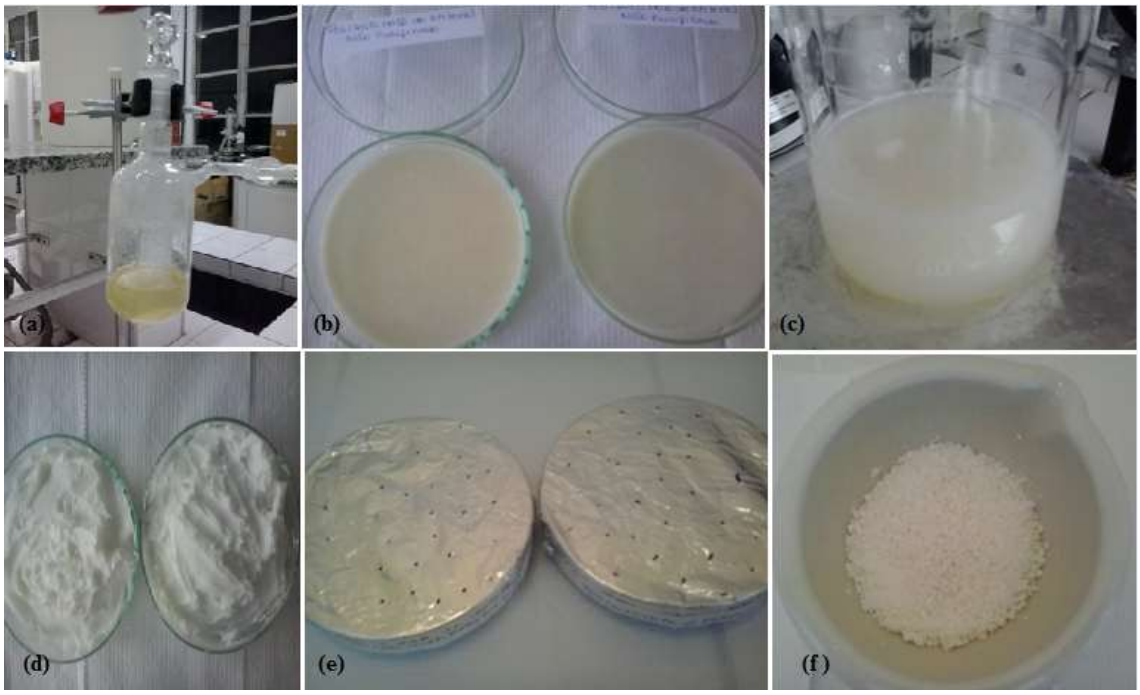
Boiling point in the alternative modular reduced pressure.

Pressure in mmHg	Water	Ethylene
760mmHg	100°C	197.6°C
500mmHg	85°C	180°C
600mmHg	90°C	185°C

The reaction product was purified by re-dissolution in chloroform and re-precipitation in ethanol and dried to constant weight. The reaction yield was 22%.

The polycondensation have low yields due to the byproducts generated reactions that prevent the system remains equimolar blocking points of chain growth and hindering the collision of reactive groups from one molecule to the other (Mano, 2004.).

The figure below shows the steps of obtaining the PES from the synthesis to the final appearance after drying.

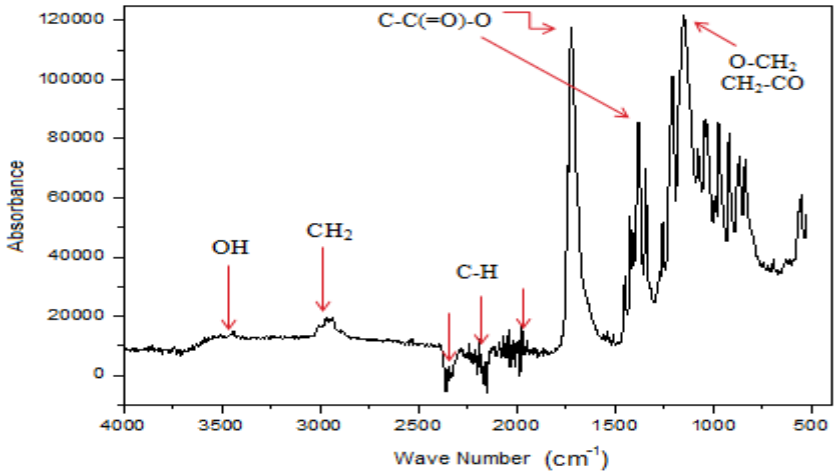


Steps in the process of obtaining the PES: synthesis (a); drying of the reaction (b) mass; Purification (c); purified product (d); post-drying purification (e) the final aspect (f).

Characterizations of PES

Characterization of PES infrared spectrometry (FTIR)

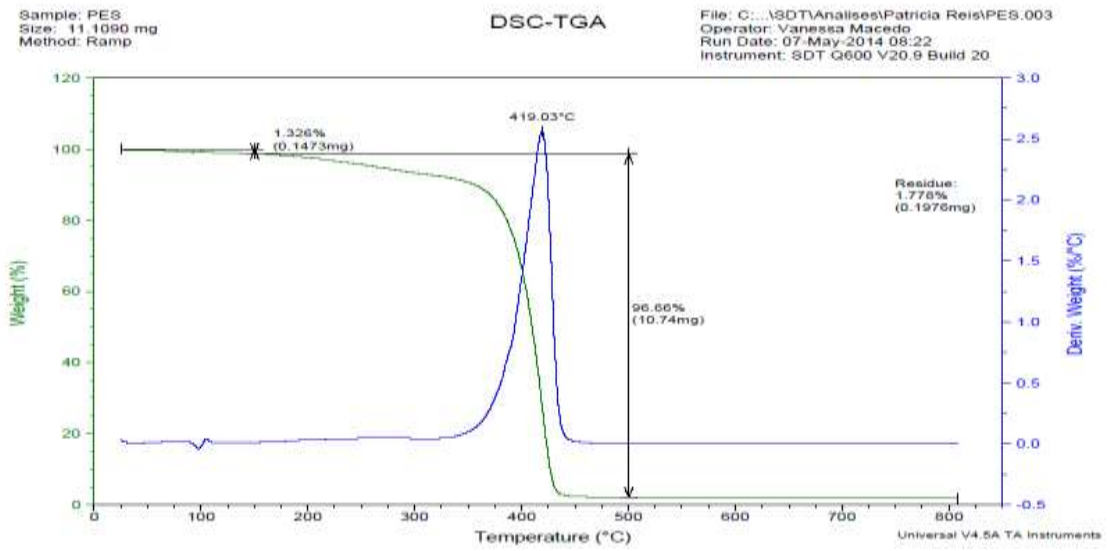
The spectral analysis in the infrared region is useful to identify functional groups present in the poly(ethylene succinate) form. Figure 15 shows the FTIR spectrum for the PES. It can be seen from the curve profile of absorption around 3000 cm^{-1} assigned to terminal OH PES grouping. The absorption around 1750 cm^{-1} and 1190 cm^{-1} confirmed the presence of ester carbonyl. The vibrations at 2450cm^{-1}, 2200cm-1 and 2000cm^{-1} may be attributed to the grouping CH absorptions. The FTIR results provide evidence that for obtaining PES synthesis route was successful.



FTIR spectra obtained for PES.

Characterization of PES by thermogravimetry (TG) and derivative thermogravimetric (DTG)

The thermogram of poly (ethylene succinate) (PES) is shown in Figure, where the values of initial degradation The PES degradation occurred in one step with the degradation temperature of the maximum rate of degradation around 419°C. Observing the TG curve there is a continuous weight loss from 200°C to a temperature of 480°C, with a significant early degradation around 350°C. temperature (Td) can be seen, the temperature of the maximum rate of degradation (Tmax), the final degradation temperature (Tfinal) and the total amount of residue after burning.



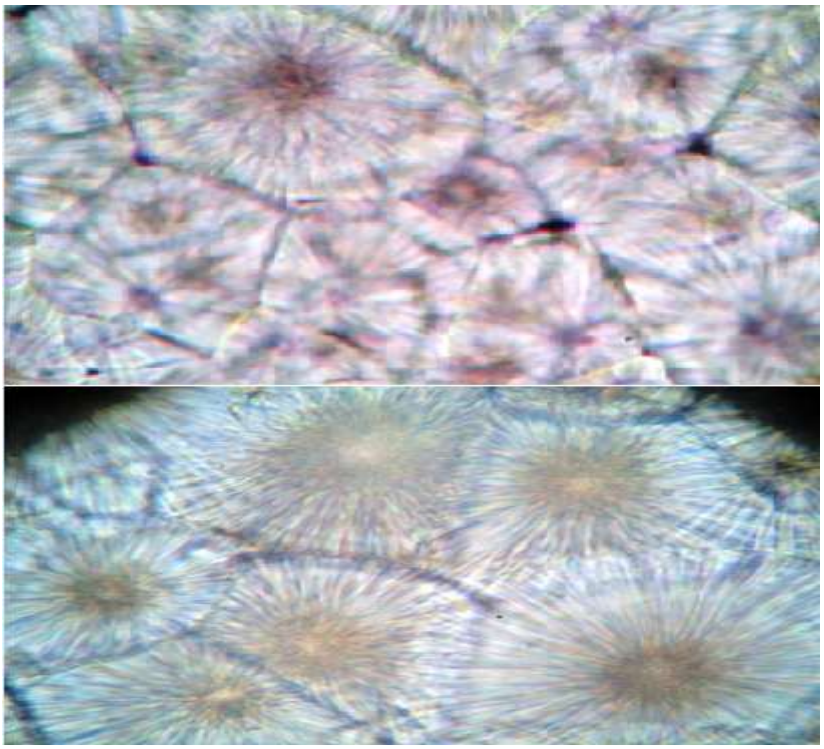
Curves TG / DTG PES.

The curve shows the derivative, around 100°C, the low mass loss with subsequent instability of the base line of the curve of DTG. This phenomenon may be associated with loss of moisture and volatile that when leaving the interior of the analyte generates a change in the balance recorded by the equipment. It is noteworthy that the synthesis of PES occurs with the formation of water as a byproduct and part of it may be in the interstices of the polymer matrix. In addition, the hydrophobicity of PES can be demonstrated by the low moisture content and volatile in the material, only 1.3% of the total mass.

At 427°C there is degradation of about 90% of the initial weight of the poly(ethylene succinate). Also observed a low residue, less than 1.8% for the poly(ethylene succinate) for the synthesis route employed. From 500°C in both curves (TGA/DTG) do not occur more significant changes in thermal behavior of the polymer.

Morphological and surface characterization of the PES by optical microscopy (OM)

According to the micrographs described in figure can be seen in crystal structures and individual radial growth cores, where one can clearly see the limits growth of these crystals characterized spherulitic crystalline formation.



Optical micrograph of the films of PES (100x).

Preliminary microbiological testing of in vitro toxicity of PES

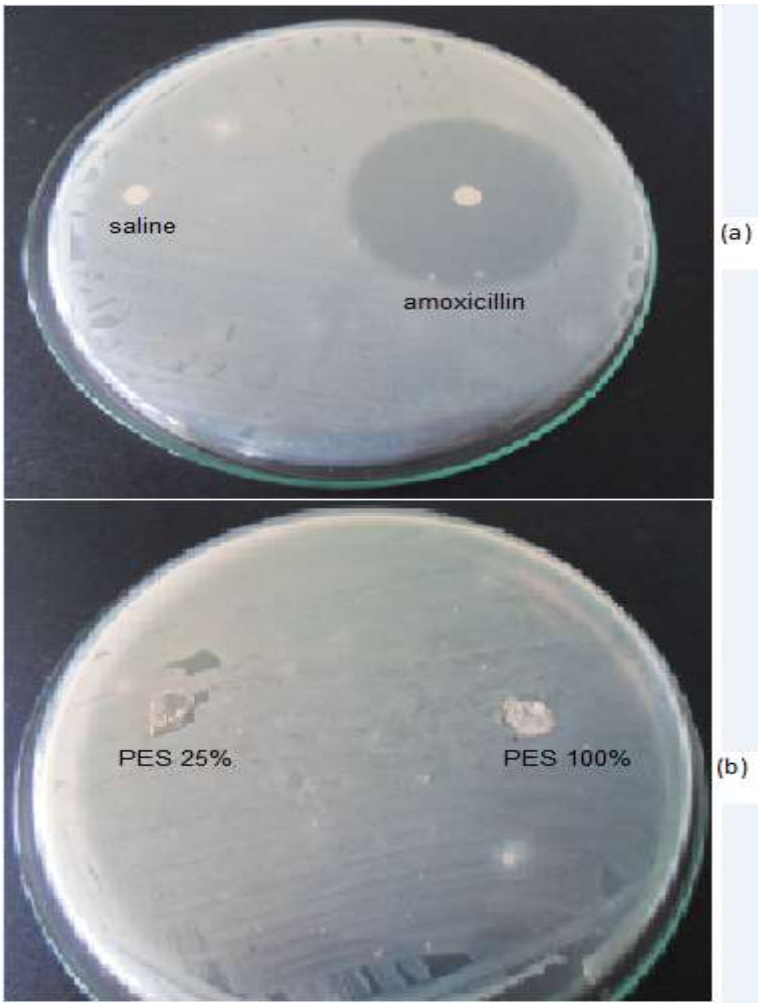
Biomaterials have recently received considerable attention in biomedical and food industries, among which the most studied have been the biopolymers. Materials can be characterized as a bio be of natural origin, being biocompatible, bioabsorbable or yet to be susceptible to action of microorganisms, such as poly(ethylene succinate) (Chen et al., 2008).

However, for use in food wraps and medication toxicity is not crucial to these wall materials. ANVISA advocates toxicological knowledge of each ingredient used in the manufacture of medicines, food or cosmetology as well as its features. The adoption of these measures avoids problems during development of the final product and even after their placing on the market. (ANVISA, 2003). Thus Preliminary bioassays to evaluate the toxicity of poly(ethylene succinate) were performed.

The tests were made using as positive control antibiotic amoxicillin concentration in vitro tests of 50mg/mL, as pre-specified in Protocol ANVISA (Decree 1480/90), and saline as a negative control. Figure 18a shows the results of controls used in the test, where a zone of inhibition of bacterial growth with a radius of 50mm for the disk containing amoxicillin can be observed, which was expected since this is the function of the antibiotic.

As for the saline is not observed the presence of halo because it is a physiological solution. In Figure 18b presents the board with the two concentrations studied PES: PES 25% (containing 12.5 mg of PES suspended in 1mL of medium Nutrient Agar) and 100% PES (PES containing 50mg in 1ml medium Nutrient Agar). The percentage concentrations are based on the PES concentration of amoxicillin studied, using as the maximum percentage concentration of 50mg/mL. It can be seen that for both concentrations studied, there were no zones of inhibition of bacterial growth of the strain of *Staphylococcus aureus* (ATCC 8096), indicating that the PES was not toxic in the concentrations studied.

The tests did not take into account the combination of the polymer with the antibiotic studied, and assessed only the action of the polymer separately and only for the concentrations studied. It is suggested that, for a more illuminating result, the action of the polymer with the antibiotic and at various concentrations is studied.



***S. aureus* culture plates: Controls (a) and PES concentrations 25% and 100% (b).**

SPRAY-DRYING

The aspect of material prepared in Turrax can be seen in Figure below.

Mixed appearance in Turrax.

It can be seen in the figure above, the accumulation of polymer particles on the equipment, even a vigorous agitation of 8000 rpm was not sufficient to obtain a stable emulsion.

Nevertheless, the mixture was taken to spray-dryer in an attempt to verify the possibility of obtaining a powdered product. But the process had to be stopped because of a blockage in the spout of the atomized particles (spray nozzle), as shown in the following figure.

Material accumulated in the nozzle sprinkling of the Spray-dryer.

The particles of polymeric material were retained in the spray nozzle of the equipment and, therefore, the safety of the hose conducting the solution into the spray severed, interrupting the process.

Soluble polymeric materials in water are recommended for this technique is difficult to control particle size (GHAESALLAOUI et al, 2007;. JOHANSEN et al, 2005).

Poly(ethylene succinate) synthesized in this study showed no solubility in water due to its high degree of crystallinity, as evidenced in the analyzes of optical microscopy, although a polymer with some degree of polarity.

After the atomization process is discontinued, and the mixture was collected, again, it became apparent insolubility of PES in water by phase separation, which can be seen in the following figure.

Phase separation of the PES in water.

Lyophilization

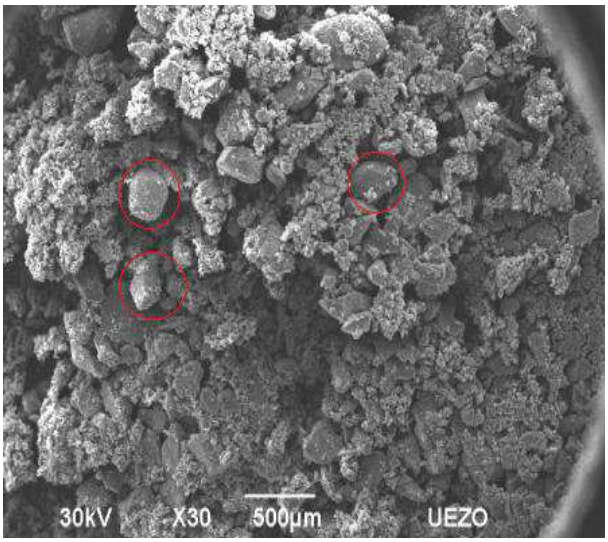
Through this method was obtained a final product powder with characteristic odor of the essential oil of lemongrass. The morphology of the obtained microparticles was evaluated by scanning (SEM) electron microscopy. The process yield was 70%.

Morphological and surface analysis of the PES film and microcapsules by scanning electron microscopy (SEM)

By means of scanning electron microscopy was possible to examine the samples and achieve high magnification and depth of focus to elucidate the physical aspects of surface and porosity of the particles obtained by encapsulation by lyophilization process.

In the figures below the micrographs of particles of poly (ethylene succinate) were presented in the encapsulation of essential oil of lemon grass by freeze-drying technique. A panoramic image of the material analyzed by SEM, is described in the following figure. The micrographs revealed particles with cluster appearance, with a well-defined and varied dimensions morphological structure. A tendency to form spherical structures for the formation of dimples on the ends of the surfaces of the particles can be observed. Literature data

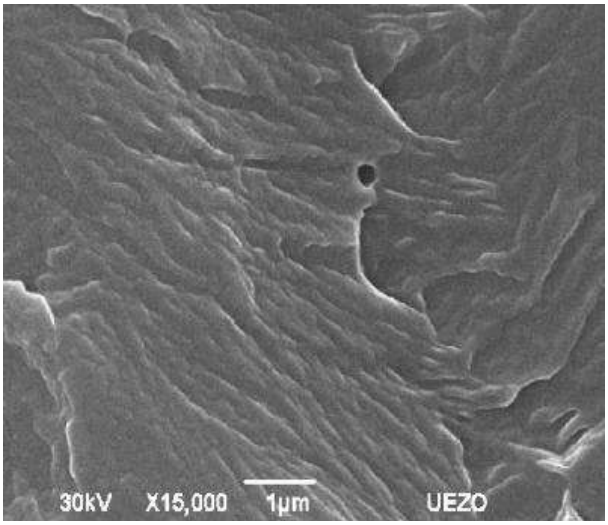
report that the encapsulation technique by lyophilization can lead to irregular morphological structures (Fang and BHANDARI, 2010).



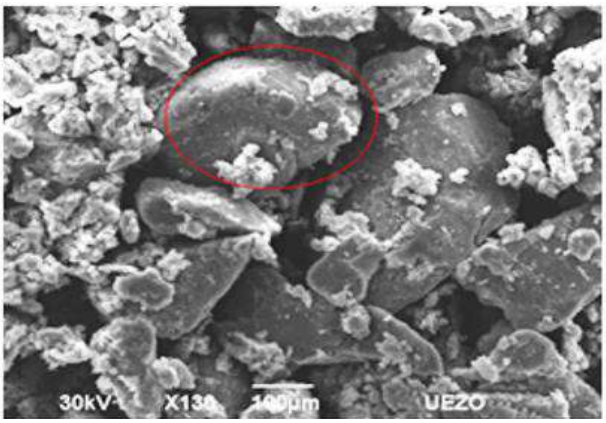
Micrograph of microparticles post-lyophilization PES - panoramic image; 30x increase.

The microparticles showed irregular surface, with slightly rough exterior. The roughness was even observed on the surface of the films of pure PES, prepared as a control. Also observed a compact surface with no pores. Although presenting some superficial erosions, external walls showed no fissures or cracks, which is critical to ensure lower permeability and greater protection for retention of the encapsulated agent.

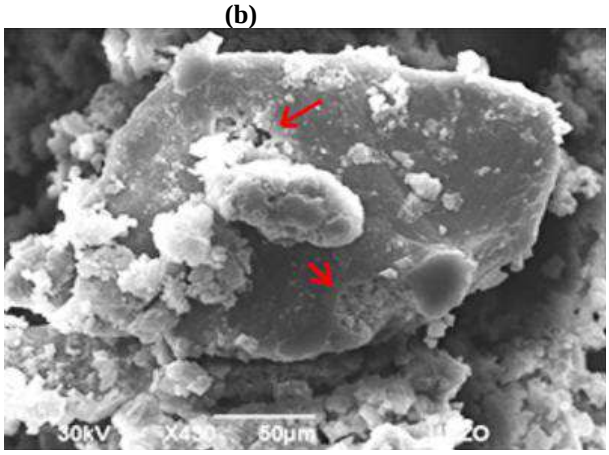
It can be seen material in the absence of free particle surface and in the interstitial spaces of the cluster. It is believed that the essential oil of lemon grass was lost in the system by the use of vacuum during the lyophilization process, although the material obtained after freeze drying process are of significant odor of the essential oil of lemongrass, even for weeks after process.



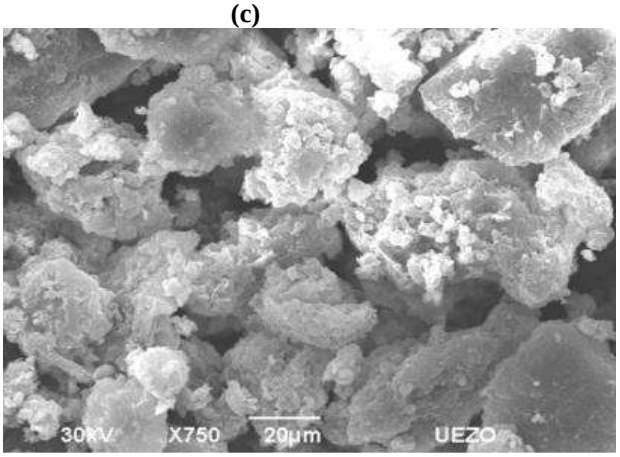
(a)



(b)



(c)



(d)

Morphological pure PES with increased 1500x (a) of the PES particles after lyophilization with increasing 130x (b) of the PES particles after lyophilization with increasing 430x (c) and PES particles after lyophilization with increasing x 750 (d).

CONCLUSIONS

Under the conditions used been possible to obtain poly(ethylene succinate) through the polycondensation reaction of ethylene glycol and succinic acid using titanium tetrabutoxide as a catalyst, in a yield of 22%.

Thermogravimetric (TG) and derivative Thermogravimetric (DTG) analyzes showed that for temperatures below 200°C. Poly (ethylene succinate) can be conveniently employed as the encapsulating matrix.

The micrographs made by optical microscopy (OM) showed a spherulitic crystal structure for poly (ethylene succinate) obtained.

Preliminary tests to determine the toxicity of PES showed that the studied concentrations (12.5 mg/mL and 50mg/mL), without interaction with antibiotics controls, PES was not toxic.

Unable microencapsulate the essential oil of lemon grass by Spray-Drying Technique for the formulation proposed by this work.

Microencapsulation of lemon grass oil in the matrix of poly(ethylene succinate) by means of freeze-drying technique led to the microparticles agglomerated in appearance, with well-defined and with irregular dimensions for the formulation proposed by this work morphological structure.

Poly(ethylene succinate) used with the polymer matrix encapsulating presented compact appearance without the presence of cracks in the process of encapsulation obtained by lyophilization technique.

SUGGESTIONS FOR FUTURE WORK

The author suggests the study of new techniques for microencapsulation of bioactive using PES as encapsulating matrix, new formulations with the use of emulsifiers and study the efficiency of encapsulation. We suggest that further study of the cytotoxicity and genotoxicity of PES and its effect on interaction with the antibiotics used as control.

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