The effect of impurities on the properties of the lactic acid polycondensates

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ZADÁNÍ DIPLOMOVÉ PRÁCE
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Téma práce: **The effect of impurities on the properties of the
                    lactic acid polycondensates**

Zásady pro vypracování:

1) On the basis of literarature survey summarize the state of art of the lactic acid
polycondensation. Beside theoretical aspects, focus on the practical applicability of
such materials.
2) Describe the parameters affecting the purity of the monomer, lactic acid, prepared in
biotechnological way.
3) Determine experimentally the influence of the selected impurities presence on the
resulting characteristics of the prepared lactic acid based polycondensates.
4) Discuss the effect of catalysts on the polycondensation process.

Rozsah diplomové práce:

Rozsah příloh:

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Seznam odborné literatury:

1) WONG, JY., BROZINO, JD. Biomaterials, CRC Press, Bota Raton 2007, ISBN 0-8493-7888-5
2) RAMAKRISHNA, S., HUANG, ZM., KUMAR, GV., BATCHELOR, AW., MAYER, J. An introduction into biocomposites, vol. 1., Imperial Collegue Press, London 2004, ISBN 1-86094-425-6
3) Book and journal databases available through the library of Tomas Bata University in Zlin.

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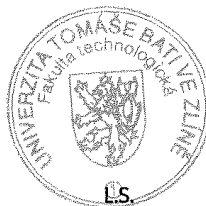
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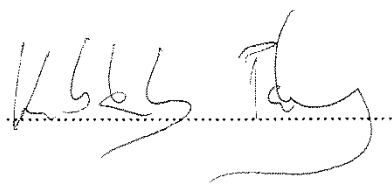Ve Zlíně dne 15. února 2010

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ABSTRAKT

Předkládaná diplomová práce se zabývá zjištěním vlivu přítomnosti zvolených nečistot (kyseliny citrónové, laktózy a bílkovinného koncentrátu ze syrovátky) na výsledné vlastnosti polykondenzátů kyseliny mléčné. Vzorky byly připraveny metodou přímé polykondenzace v tavenině katalyzované pomocí, jak organokovového, tak i nekovového katalyzátoru. Produkty reakcí byly následně charakterizovány pomocí viskozimetrických měření, gelové permeační chromatografie, diferenční skenovací kalorimetrie a infračervené spektroskopie. Dále byly metodou titrace stanoveny koncové karboxylové skupiny polykondenzátů. Bylo zjištěno, že povaha katalyzátoru silně ovlivňuje vlastnosti produktů polykondenzace připravených směsí. Výsledky ukazují významné interakce mezi kyselinou mléčnou a citronovou v podobě inhibice kondenzačních reakcí. Bylo zjištěno, že laktóza způsobuje vznik vysoce větvených či dokonce 3D polymerních struktur. Bílkovinné znečištění, ve srovnání s kyselinou citrónovou a laktózou, výrazněji neovlivňuje strukturu polykondenzátů.

Klíčová slova: polylaktid, polykondenzace, nečistoty, kyselina citrónová, laktóza, syrovátková bílkovina, porovnání katalyzátorů

ABSTRACT

Presented diploma thesis is dedicated to investigation of selected impurities presence effect, (citric acid, lactose and whey protein concentrate) on resulting properties of lactic acid polycondensates. The samples were prepared by direct melt polycondensation catalyzed by both organometallic and non-metal based compounds. The products of the reaction were characterized by viscometric measurements; gel permeation chromatography; differential scanning calorimetry and Fourier transform infrared spectroscopy. In addition, carboxyl end groups determination was done by titration method. It was observed that catalyst type strongly influences character of the polycondensation products. The results show significant interactions between lactic acid and citric acid due to inhibition of condensation reactions. Lactose was found to be involved into highly branched and possibly 3D polymer structure formation. Proteinic impurity does not affect the polycondensates structure as noticeable as citric acid and lactose.

Keywords: polylactic acid, polycondensation, impurities, citric acid, lactose, whey protein, catalysts comparison.

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"If you don't make mistakes,
you're not working on hard enough problems.
And that's a big mistake."
-- F. Wikzek --

I hereby declare that the print version of my Master's thesis and the electronic version of my thesis deposited in the IS/STAG system are identical.

CONTENTS

INTRODUCTION

Biodegradable polymers have attracted increasing interest over the past two decades both in the fundamental research and also in practical use. Polylactic acid (PLA) is synthetic biodegradable polyester and it represents its potential applicability in a growing number of technologies such as orthopaedics, drug delivery, sutures and scaffolds, due to its excellent biocompatibility and bioresorbability [1; 2]. The synthesis of PLA can be carried out either through ring opening polymerization of lactones or through direct polycondensation of lactic acid (LA) monomer. The former method can be characterized by high purity requirements set on reactants and enables production of products with high and medium molecular weight. On the other hand, the latter does not provide high molecular weight products. Both of the mentioned methods require the presence of catalyst [3]. There are many publications dealing with PLA synthesis using metal-based compounds as catalysts, frequently based on tin [4]. However, the residual metal catalyst can poses a problem as regards both thermal stability and biocompatibility of PLA [5]. These concerns have led to investigation of non-tin based catalyst for PLA preparation [6; 7].

This diploma thesis follows the project carried out at Polymer Centre Faculty of Technology, Tomas Bata University in Zlin, which has been dealing with production of PLA from dairy industry by-product, whey. In this case, the real product of biotechnological whey processing, lactic acid, (monomer for PLA preparation) can contain various impurities in form of other simple organic acids, residual lactose and protein based components [8]

The presented work deals with investigation of selected impurities effect on resulting PLA properties. The preparation of polymer was carried out by direct melt polycondensation using both conventional tin based (tin octoate) and non-metal based catalysts (methanesulphonic acid). The influence of the impurities (citric acid, lactose and whey protein concentrate) on polycondesation products were proceeded by means determination of molecular weight (viscometric measurements, gel permeation chromatography), thermal properties (differential scanning calorimetry) and structural analysis (Fourier transform infrared analysis and determination of end groups by titration method).
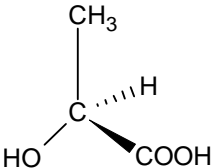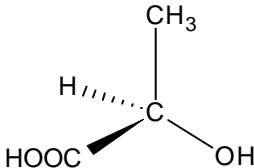
I. THEORY

1   LACTIC ACID

Lactic acid (2-hydroxypropionic acid or 2-hydroxypropanoic acid), $CH_3CHOHCOOH$, is the most widely occurring carboxylic acid, having a prime position due to its versatile applications in food, pharmaceutical, textile, leather, and other chemical industries. [9]

1.1   Structure and properties

It is one of the major carboxylic acids presents in nature. Lactic acid exists in two optically active isomeric forms. L(-) lactic acid and D(+) lactic acid, see Figure 1. Mixture of both L:D (1:1) is called racemic lactic acid. [10]

Table 1 - *Physical properties of lactic acid [10]*

L – Lactic Acid	Property	Unit	D – Lactic Acid
	Molecular weight **Melting point** **Boiling point** **Dissociation const., Ka at 25ºC** **Heat of combustion, ΔHc** **Specific Heat, Cp at 20ºC**	90.08 g.mol^{-1} 16.8 ºC 122 ºC at 2 kPa $1.37. 10^{-4}$ 1361 KJ/mole 190 J/mole/ºC	

1.2   Chemical synthesis

Traditionally lactic acid has been produced through either chemical synthesis route or microbial route (fermentation).

Chemical synthesis is performed through the hydrolysis of lactonitrile. At first step, hydrogen cyanide (HCN) is added to acetaldehyde (CH_3CHO) in liquid phase in presence of a base catalyst under high pressure when lactonitrile is produced. Crude lactonitrile is then purified by distillation and subsequently hydrolyzed to lactic acid by hydrochloric acid or sulfuric acid. The process is often dependent on other by-product industries and, considered expensive where petroleum based raw material is the major cost-contributor. Moreover, chemical synthesis route produces a mixture of L-lactic acid and D-lactic acid whereas in most of the cases, L-lactic acid is the desirable product. The problems of high cost of raw materials, impurity of the product and dependence on other industries for raw materials could be overcome in fermentation based process. [11]

1.3   Microbial production

Lactic acid can be produced by bacteria, yeasts, and molds including genetically modified forms of these organisms using carbohydrate substrates. Agricultural by-products

are suitable substrates for some species of lactic acid bacteria. Microbial process includes fermentation, product recovery and purification steps. [10; 12]

Since the conventional microbial process is quite complicated due to number of downstream treatment process like precipitation, conventional filtration, acidification, carbon adsorption, evaporation only simple scheme of traditional LA production process is presented. [12]
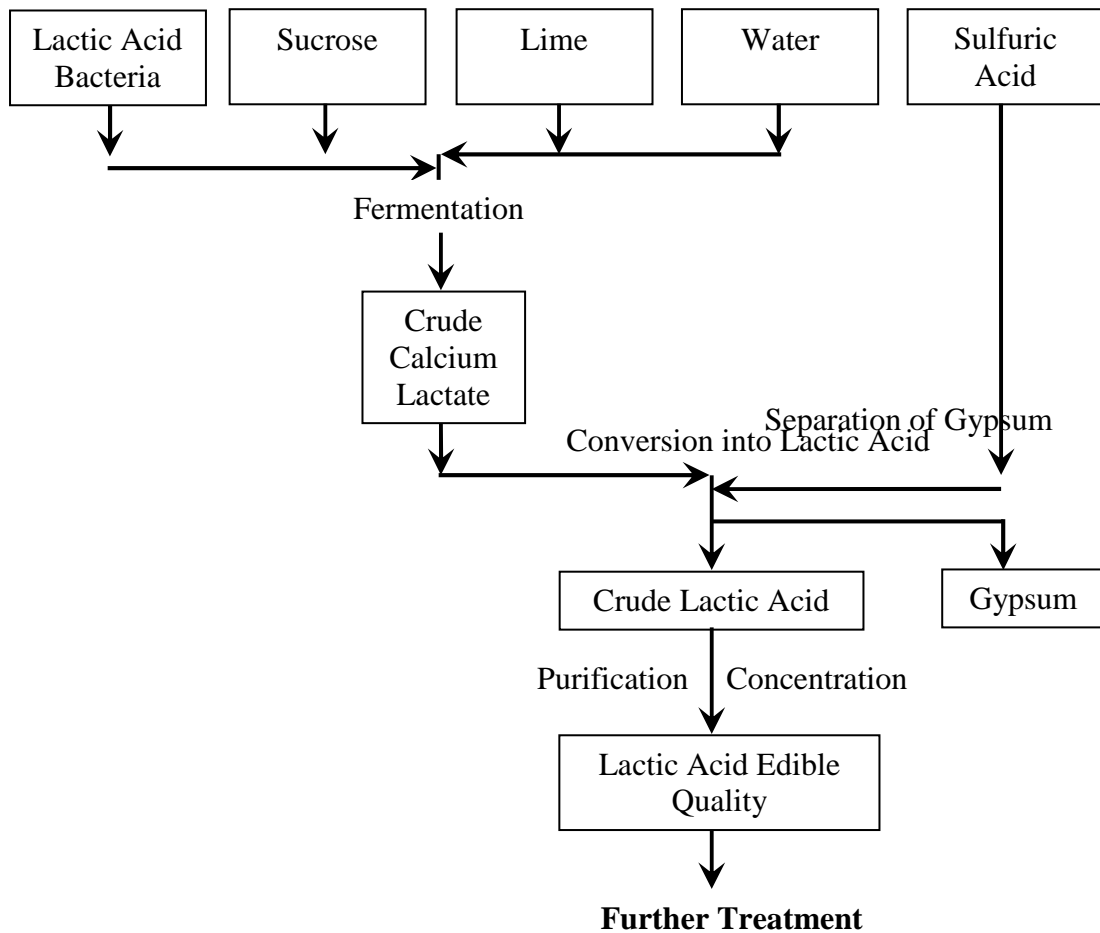
Figure 1 – *Scheme of traditional LA production process. [12]*

At first, substrate must be prepared. Hence, lactic acid bacteria, sugar and water are mixed and put into the fermentor. During fermentation lime (or other bases) must be continuously added. It leads to convert incipient LA into calcium lactate (or other corresponding salt – reaction 1).

$$2CH_3CHOHCOOH + Ca(OH)_2 \longrightarrow 2CH_3CHOHCOOCa + 2H_2O \qquad (1)$$

Calcium lactate is than converted into lactic acid with addition sulfuric acid according to reaction (2)

$$(2CH_3CHOHCOO^-) Ca^{2+} + H_2SO_4 \longrightarrow 2CH_3CHOHCOOH + CaSO_4 \qquad (2)$$
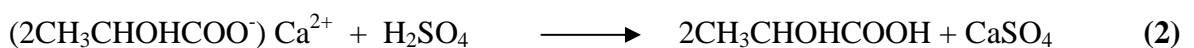
Emerging $CaSO_4$ (gypsum) is separated by filtration and crude lactic acid is further purified and concentrated into desirable quality. [12]

1.3.1 Microorganisms for Production

Bacteria are the most common organisms for lactic acid fermentation process. Especially Lactobacillus species prove good results and are commercially utilized.

Temperature, pH, lactic acid tolerances, substrate specificity, yields (grams/gram of substrate utilized), productivities (grams/liter/hour), and bacteriophage resistance as well as the desired isomer, low-cost sources of carbohydrate substrates are important criteria for selecting suitable strains for commercial production. On the same hand, heterofermentative (produce lactic acid and other metabolic products) or homofermentative (produce lactic acid only) behavior of microorganisms also influences the choice between the selections of organisms. The homofermentative bacteria are of greatest interest for commercial lactic acid production, for obvious economic reasons. From the standpoint of oxygen requirements, lactic acid bacteria are facultatively anaerobic or microaerophilic and grow at low oxygen concentrations. They require complex nitrogen sources of amino acids and vitamins. [13; 14; 15]

1.3.1.1 Molds and yeasts

Are able to produce L(+) Lactic acid from various sources. Their mainly advantages are lover claims on supplement sources during fermentation, better tolerance to acidic pH then bacteria, easily recovery process and fact that some of these species are able to convert polysaccharides like a starch to lactic acid without previous hydrolysis to glucose. Limitations can find mostly in lower yields of LA and forming co-products (ethanol, glycerol etc.) during fermentation. [10; 15]

1.3.2 Raw materials

There exist many suitable materials from purified sugars such as glucose, sucrose, and lactose and food-processing wastes containing these sugars such as waste banana pulp, pineapple wastes, sugar cane juice, and cheese whey to agricultural and industrial wastes containing complex carbohydrates like starches, hemicelluloses, or cellulose. Raw materials such as woody biomass containing hemicelluloses, cellulose, and lignin must be converted by chemical, physical, or enzymatic methods to yield fermentable sugars. [10; 15; 16]

Glucose produced by acid and enzyme conversion of corn starch has been the preferred raw material for commercial lactic acid production by microbial fermentation on the bases of cost, availability, and ease of product recovery and purification. Owing to the nutritional requirements of the lactic acid bacteria, glucose or sucrose must be supplemented with sources of amino acid peptides and vitamins such as corn steep liquor from the corn wet milling process, or protein hydrolysates or yeast extracts or both. These nutrients are cost consuming and can affect the final cost of LA to 30%. [17; 18]

1.3.3 Cheese whey and permeate

Cheese whey is the liquid remaining following the precipitation and removal of milk casein during cheese-making. The most abundant components are lactose (4.5-5% w/v), soluble proteins (0.6-0.8% w/v), lipids (0.4-0.5% w/v) and mineral salts (8-10% of dried extract). Cheese whey also contains appreciable quantities of other components, such as lactic (0.05% w/v) and citric acid, non-protein nitrogen compounds (urea and uric acid), B group vitamins, and so on. Two main whey varieties produced are acid (pH <5) and sweet (pH 6-7) whey, according to the procedure used for casein precipitation. [19]

Whey has commonly been considered as a waste product. Although several possibilities for cheese-whey exploitation have been assayed over the last 50 years, approximately half of world cheese-whey production is not treated, but is discarded as effluent. Despite the fact that a research in the field of whey utilization continues, only about 50% of total cheese-whey production is now treated, principally into various liquid and powdered food products. [19]

Cheese whey permeate (deproteined cheese whey). Is the liquid product obtained after passing through the membrane during ultrafiltration. Membrane pass only molecules with lower molecular weight (usually water, lactose, minerals), in residue (retentate) protein and possibly remain fats. Permeate composition depends on the size of the pore membrane. Pass substances with molecular weights up to 3 000 or up to 10 000. Permeate is a source of lactose. Composition of permeate (and retentate) differs according to treatment; 98–99% total solids, 79–81% lactose, 8–10% ash and 2.5–4% protein. [19; 20]

1.3.4   Fermentation

Fermentation is the process of transformation. Suitable substances – usually carbo-hydrates are exposure to microorganisms. In consequence of metabolic activities of these micro-organisms, lactic acid is produced.

In commercial scale lactic acid bacterial processes, the cells are kept in suspension in the production medium by mixing with mechanical agitators or circulating the medium from the bottom to the top of the fermentor by pumping. It was pointed out previously that *Lactobacillus* spp. require low oxygen concentrations for growth and lactic acid produc-tion. Generally, agitation during the fermentation provides sufficient oxygen.

Batch mode fermentation is operated for many years in commercial scale. Typical glucose concentrations range from 15 to 20% with final lactic acid concentrations of 14% temperature and pH values are 45 °C and 5.5–6.0, respectively. Standard yields of lactic acid from glucose in batch fermentations under continuous monitoring and control of tem-perature and pH exceed 0.90g of lactic acid per gram of glucose utilized with LA concen-tration 120-130 $g.L^{-1}$ and productivity $3 – 5$ $g.L^{-1}.h^{-1}$. Process controls in lactic acid pro-duction by fermentation include temperature; pH; and concentrations of microbial cells, substrates, and lactic acid. [10; 11; 15]

1.3.5   Cheese whey fermentation

Since the 1930s, numerous processes have been investigated for the microbial con-version of lactose in cheese whey. Cheese whey permeate from ultrafiltration membrane separation processes that also contains lactose is a potential raw material for lactic acid production.

Lactic acid bacteria do not have sufficient proteolytic enzyme activity to utilize milk proteins in cheese whey. Consequently, cheese whey and cheese whey permeate must be also supplemented with sources of amino acids and vitamins. Pretreatment of cheese whey proteins with proteolytic enzymes may aid in partial or complete replacement of pro-tein hydrolysates. [11]

Owing to the complex composition of lactose and whey proteins and the presence of bacterial contaminants in cheese whey and permeates, these substrates should be steri-lized before inoculation. A major problem in utilizing cheese whey and permeate for lactic acid production is the lack of microbiological stability of these materials. The costs asso-ciated with refrigerated transport and storage makes it uneconomical to collect these raw materials (7% solids) from far located cheese manufacturing plants. Also, owing to its low

solubility in water (saturation at 10.6% - lactose in H_2O at °C), lactose may crystallize out of whey at refrigerated transportation temperatures. For commercial production of lactic acid from cheese whey or permeate the lactic acid plant should be located adjacent to a large cheese manufacturing plant that could produce an economically viable supply of these raw materials. In this manner, production could take place throughout the year without incurring the costs and problems associated with the collection and refrigerated storage and transportation of the cheese whey. Lactic acid yields from lactose in cheese whey or permeate in batch fermentations are similar to those obtained in glucose-based fermentations ($0.90g.g^{-1}$ of lactose utilized). [11; 19]

1.3.6 Product Recovery and Purification

The major technology barrier in cost-effective production of high purity lactic acid is its down-stream separation and purification. The recovered product should meet quality standards for food, cosmetic, personal care product, pharmaceutical, medical, and biodegradable polymer applications. For these uses, lactic acid must be free of residual sugars, other organic acids, amino acids, proteins, vitamins, complex organic nitrogen compounds, heavy metals, residual organic solvents, and Maillard´s reaction products between carbohydrates and amino acids. [15]

The recovery of lactic acid from the fermentation medium is a major cost item for the entire process. It has been estimated that lactic acid purification and recovery costs may account for 50% of the final cost of manufacturing this product. [15]

Based on purpose the product is usually available in four grades (Figure 2):

*that will not discolor when heated to 200°C for a few hours.
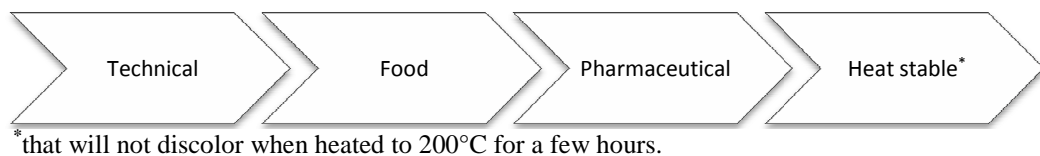
Figure 2 – *Base lactic acid grades [15]*

The greater purity, more complex and expensive recovery process. The first step in the conventional recovery processes is heating the fermentation beer to 80–100°C and increasing the pH to 10 to kill the bacteria, coagulate proteins and maintain calcium lactate's solubility in the beer. The crude calcium lactate solution is then filtered, decolorized with activated carbon, and concentrated by evaporation. Lactic acid is recovered by addition of sulfuric acid. This process leads to lactic acid in edible quality. If pharmaceutical or heat stable grade is desirable more purification steps must be done. [15; 21]

A lot of processes for lactic acid purification have been developed. Broad utilize is distillation. Simple distillation however isn´t a good approach because lactic acid have higher boiling point than water, hence remain in distillation apparatus and undesirable condensation can occurs. Also it is energy intensive. Vacuum conditions for the distillation, to lower the temperature of the distillation are preferred because lactic acid condensation to dimers or oligomers is reduced. Much more preferred approach is converting LA to ester by reaction with methanol which is more volatile than lactic acid. Ester is then hydrolyzed back to the lactic acid (see reactions 3 and 4). [11; 15; 21]

$$2CH_3CHOHCOOH + CH_3OH \longrightarrow CH_3CHOHCOOH + H_2O \qquad (3)$$

$$CH_3CHOHCOOCH_3 + H_2O \longrightarrow CH_3CHOHCOOH + CH_3OH \qquad (4)$$
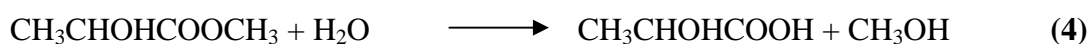
These purification processes are often combined with liquid-liquid extraction, ion exchange, and adsorption on solid adsorbents. However, for every ton of fermentation lactic acid produced by the conventional recovery process, about one ton of gypsum ($CaSO_4 \cdot {_2}H_2O$) by-product is also produced. Ecological and economical disposal thus becomes a problem for a large-scale production facility. [10; 15]

More recent methods for lactic acid recovery include centrifugation, or membrane filtration using either microfiltration (0.2 mm pore size) or ultrafiltration to separate the cells and higher-molecular weight residues such as peptides in the spent production medium. Heavy metals are removed using cation exchange resins. Lactic acid can be extracted from the fermentation medium using a strong anion exchanger. Advantage is fact that no by-product as gypsum is produced but high cost and regeneration of ion-exchanger are disadvantages. [15; 21]

Advances in membrane technology allowing low-energy desalting-electrodialysis have led to a proprietary method for production of fermentation lactic acid from carbohydrates without calcium salt production. This new technology can be operated as a continuous process and scaled up to large-volume recovery of lactic acid from the fermentation beer and manufacture of lactide polymers. Combination of membrane filtrations and electrodialysis method is sketch on following schema (Figure 3). [15]
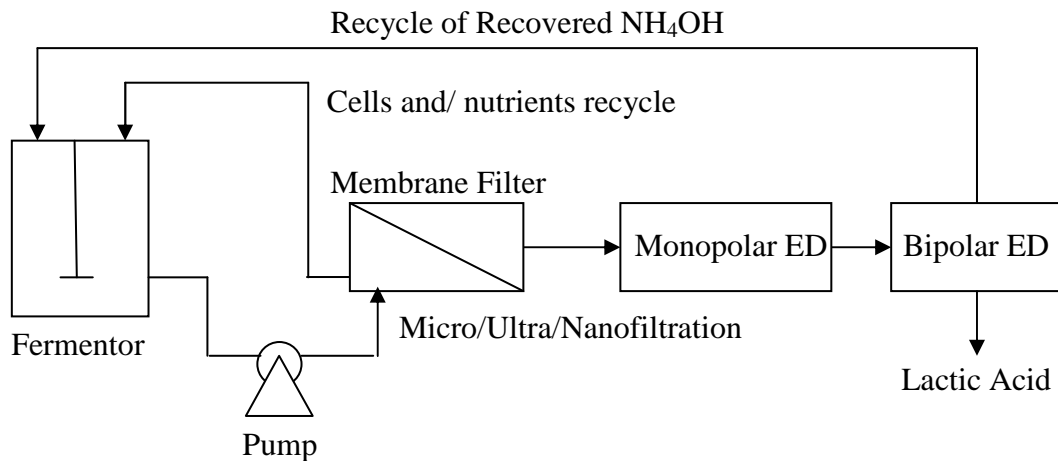
Figure 3 – *Schematic diagram of fermentative production of lactic acid integrated with Micro/Ultra/Nanofiltration membrane in the first stage and electrodialysis (ED) membranes in the second stage. [11]*

There must be mention that cost of LA purification process can reach 80% from total cost of LA production. Hence, purification and also fermentation processes are still big challenge for researchers. And development in this ways still continues. Also there is clear relation between price and purity. More pure LA – higher price. [13]

1.4 Applications

Lactic acid is used as acidulant/ flavouring/ pH buffering agent or inhibitor of bacterial spoilage in a wide variety of processed foods. It is non-volatile odorless and is classified as GRAS (generally regarded as safe) by FDA in the US. It is a very good preservative and pickling agent. Addition of lactic acid aqueous solution to the packaging of poultry and fish increases their shelf life. Technical grade lactic acid is used as an acidulant in vegetable and leather tanning industries. Various textile finishing operant and acid dying of food require low cost technical grade lactic acid to compete with cheaper inorganic acid. Lactic acid has many pharmaceutical and cosmetic applications and formulations in topical ointments, lotions, anti acne solutions, humectants, parenteral solutions and dialysis applications, for anti carries agent. [10; 15; 17]

2 POLYLACTIC ACID

Polylactic acid belongs to the family of aliphatic polyesters (Figure 4) commonly made from α-hydroxy acids. PLA is a high strength and high modulus thermoplastic. For its properties it is being used for number of applications from biomedical to conventional thermoplastics. Since, the mechanical properties of high molecular weight PLA are comparable to other commodity thermoplastics like polystyrene and PET.
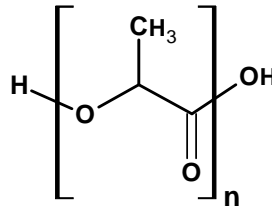

Figure 4 – *Chemical structure of PLA*

Therefore it has large opportunities to replace these polymers for numerous applications. But its high cost has prevented it from being used in other spheres. But now latest technological advances have given rise to PLA that are commercially viable and can compete with petrochemical plastics. [10; 22; 23]

2.1 Properties

2.1.1 Legislative definitions

PLA is considered as biodegradable, bioresorbable and compostable polymer that can be made from renewable sources. Before description PLA as a whole, all these terms should be explained. [24]

Degradable plastic (ISO 472) - A plastic designed to undergo a significant change in its chemical structure under specific environmental conditions, resulting in a loss of some properties that may vary as measured by standard test methods appropriate to the plastic and the application in a period of time that determines its classification. [25]

Biodegradable plastic (ISO 472) - A degradable plastic in which the degradation process results in lower molecular-weight-fragments by the action of naturally occurring microorganisms such as bacteria, fungi and algae. [25]

Bioresorbable - Are solid polymeric materials and devices which show bulk degradation and further resorb in vivo; i.e. polymers which are eliminated through natural pathways either because of simple filtration of degradation by-products or after their metabolization. [26]

Compostable plastic (ASTM D6400) - A plastic that undergoes degradation by biological processes during composting to yield carbon dioxide, water, inorganic compounds, and biomass at a rate consistent with other known compostable materials and leaves no visually distinguishable or toxic residues. [27]

2.1.2 General properties

PLA is thermoplastic polyester which can by prepared either crystalline or amorphous. At normal conditions PLA is brittle and hard polymer with glass transition temperature between 55-65 °C and melting point around 160-170 °C. Its comparison with other plastic shows Figure 5. Chemical, physical, mechanical and other properties are function of temperature, molecular weight, chemical composition (copolymer presence), additives, and processing methods and so on. [28]
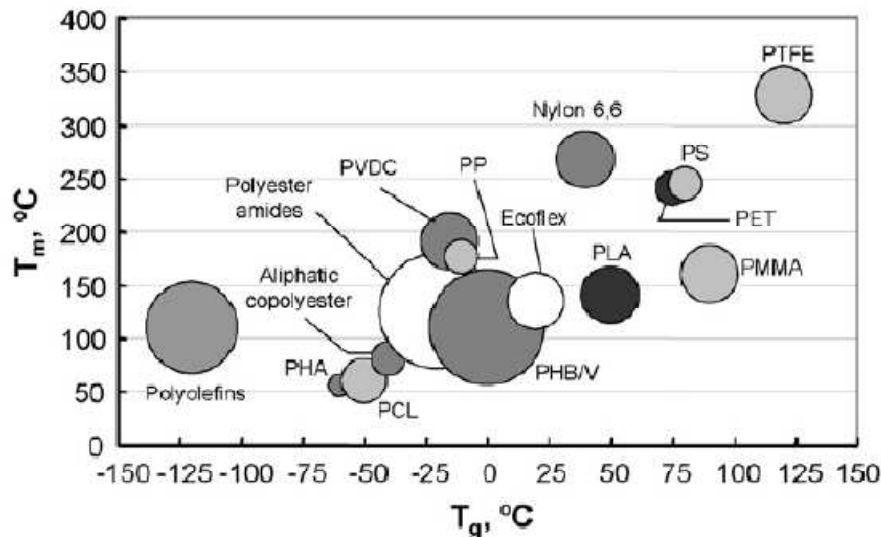
Figure 5 - *Comparison of glass transition and melting temperatures of PLA with other thermoplastics. [29]*

Interesting is a crystallinity of PLA. Because of existing stereo regularity LA, polylactide also can exist in 3 forms. L-PLA, D-PLA and LD-PLA. All these three form could have different properties depend on isomer composition. PLLA is crystalline whereas PDLLA is completely amorphous polymer. Because of the crystallinity, poly(L-lactide) of same molecular weight has better mechanical properties than poly(DL-lactide). Pure L-PLA can crystallize but if more than 15% D form is present in polymer chain, only amorphous material is possible to obtain. Crystallinity also affects biodegradation rate due to better H_2O diffusion into polymer backbone. L-PLA is the most abundant form using for practical application then other forms. [23; 24; 28] For list of more general properties see Table 2.

Table 2 – *General properties of PLA [23; 24; 28; 30]*

Properties	Description	Remarks
Electrical	Insulator	Volume resistivity = 5.10^{16} Ω.cm Dielectric constant = 3.8 Dielectric loss = 0,020
Thermal degradation	Is realized hrough: Thermal hydrolysis, depolymerization, cyclic oligomerization and intermolecular and intramolecular transesterification.	Improve by: Deactivation of catalyst, removal of the non-polymeric contents and blocking the hydroxyl end groups increasing the purity of the polymer also increase thermal stability.
Solubility	<u>Soluble</u> in chloroform, acetone, dichloromethane, benzene <u>Nonsoluble</u> in cyklohexane, methanol, ethanol. Good resistance against fats and oils.	Solubility parameter: δ_p at $25\ ^o$C = 19-20,5 $J^{0,5}$ $cm^{-1,5}$
Modification	Copolymerization, chain extending, end groups reaction	Glycolic acid and caprolactone are often co-monomers. Diisocyanates, bis-2-oxazolines could be use as the chain extenders.
Blending	PLA has been blended with different plasticizers and polymers (biodegradable and non-biodegradable) to achieve desired mechanical properties.	LDPE, PHAs, PCL, Starch and more systems has been studied.
Barrier properties	Weak	In comparison with PET and LDPE 8-10 times worse.
Processing	Can be processed using the conventional production infrastructure with minimal equipment modification.	Prior to melting processing of PLA, the polymer must be dried sufficiently to prevent excessive hydrolysis. Injection holding, stretch blow molding, thermoforming, foaming, fiber spinning are common processing methods.

2.1.3 Mechanical Properties

The mechanical properties of PLA can be varied to a large extent ranging from soft, elastic plastic to stiff and high strength plastic. With the increase of molecular weight the mechanical properties also increase. Table 3 shows values of common mechanical properties and its comparison with other commodity plastics. [31]

Table 3 – *Typical mechanical properties of PLA[22]*

Property	Units	PLA Standard grade	GPPS General Purpose PS	PET
Tensile strength	[MPa]	68	45	57
Elongation at break	[%]	4	3	300
Flexural modulus	[MPa]	3700	3000	2700
Flexural strength	[MPa]	98	76	88
Izod impact	[J.m^{-1}]	29	21	59
Density	[kg.m^{-3}]	1.26	1.05	1.4

2.1.4 Biodegradability

Take place in 2 steps. At first, ester bond into polymer chain is hydrolyzed and low molecular weight PLA arise. Secondly, these fragments could be utilized by microorganism to produce CO_2, H_2O, biomass and other compounds. Degradation rate depends on many factors such temperature, humidity, crystallinity, size and shape of a product and molecular weight. Average degradation time in terms of real-compost is about 30 days. Products of biodegradation are completely harmless. [31; 32]

2.2 PLA synthesis

There are several ways for poly(lactic) acid synthesis. Differences are mainly in polymerization conditions, methods and resulting polymer properties. Figure 6 depicts various routes for PLA synthesis.

Carbohydrates

Fermentation

Lactic Acid

Oligomerization — **Oligomer** — **Solution Polycondensati-** — Melt State — **Oligomer (SSP)** — **Direct Condensation Polymerization**

Dimerization

Lactide

Purification

Solid State

Pure

Ring Opening Polymerization

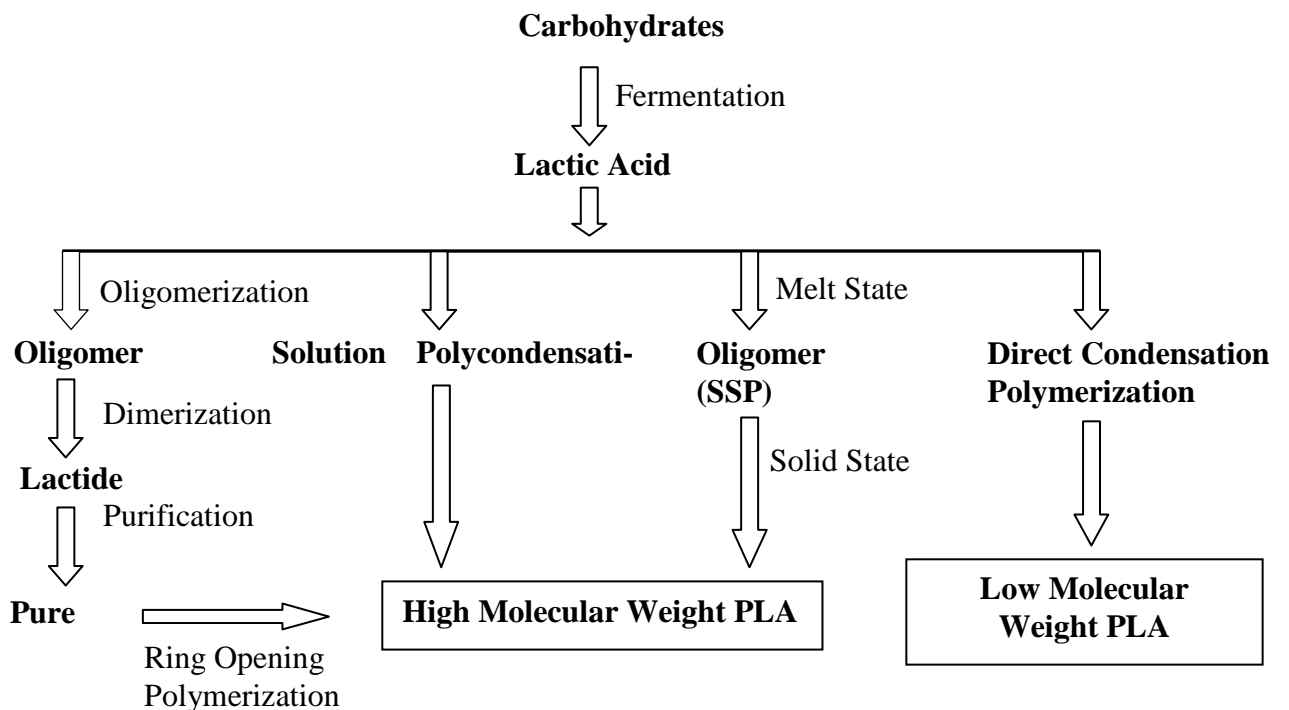**High Molecular Weight PLA**

Low Molecular Weight PLA

Figure 6 - *Various routes for PLA synthesis [24]*

2.2.1 Ring opening polymerization (ROP)

The ring-opening polymerization route includes polycondensation of lactic acid followed by a depolymerization into the dehydrated cyclic dimer, lactide (3,6-dimethy-1-4-dioxane-2,5-dione), which can be ring-opening polymerized into high molar mass polymers (Figure 7). The depolymerization (Figure 8) step is conventionally done by increasing the temperature and lowering the pressure, and distilling off the produced lactide. Due to the two stereo-forms of lactic acid the corresponding optically active lactide can be found in two different versions, i.e D,D-lactide and L,L lactide. (In addition lactide can be form one D- and L-lactic acid molecule yelding D,L-lactictide (meso-lactide). [10; 24; 33; 34]

The preparation of lactic acid based polymers by ROP has successfully been carried out by solution, bulky, melt and suspension polymerization. The polymerization mechanism can be of ionic, or coordination type depending on catalyst system used. The ROP can be catalyzed by transition metal compounds of tin, aluminum, lead, zinc, bismuth, iron, and yttrium. Tin(II) 2-ethylhexanoate is the most frequently used catalyst. ROP occurs under milder reaction conditions, and it sometimes proceeds as a "living" manner, that is, without side reactions to give polyesters of controlled molecular weight. [10; 34]

Typical reaction conditions for lactide ROP (Figure 8) are: temperature below 180 $^{\circ}$C, relatively short reaction time 2-5 hours, catalyst, and presence of initiator. [23]

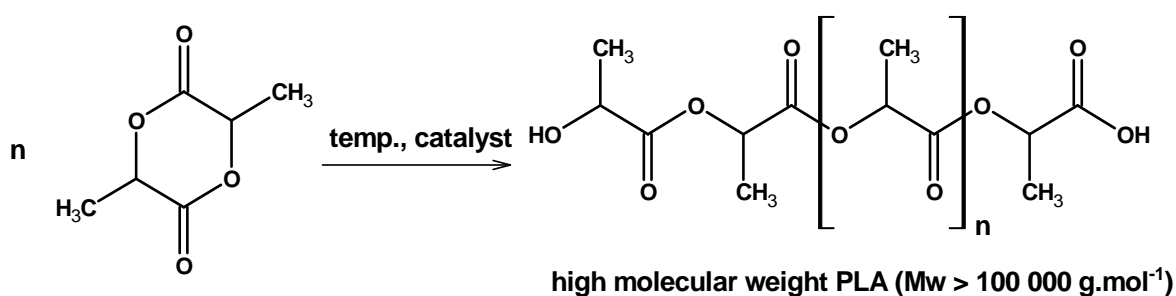high molecular weight PLA (Mw > 100 000 g.mol^{-1})

Figure 7 - *Ring opening polymerization. [23]*

This technique provides high molecular weight polylactide with good properties and is widely used. But complicated and expensive lactide manufacturing and purification steps are big weaknesses of this process. Company NatureWorks LLC (owned by Cargill) use ROP for production its PLA. [23; 35]
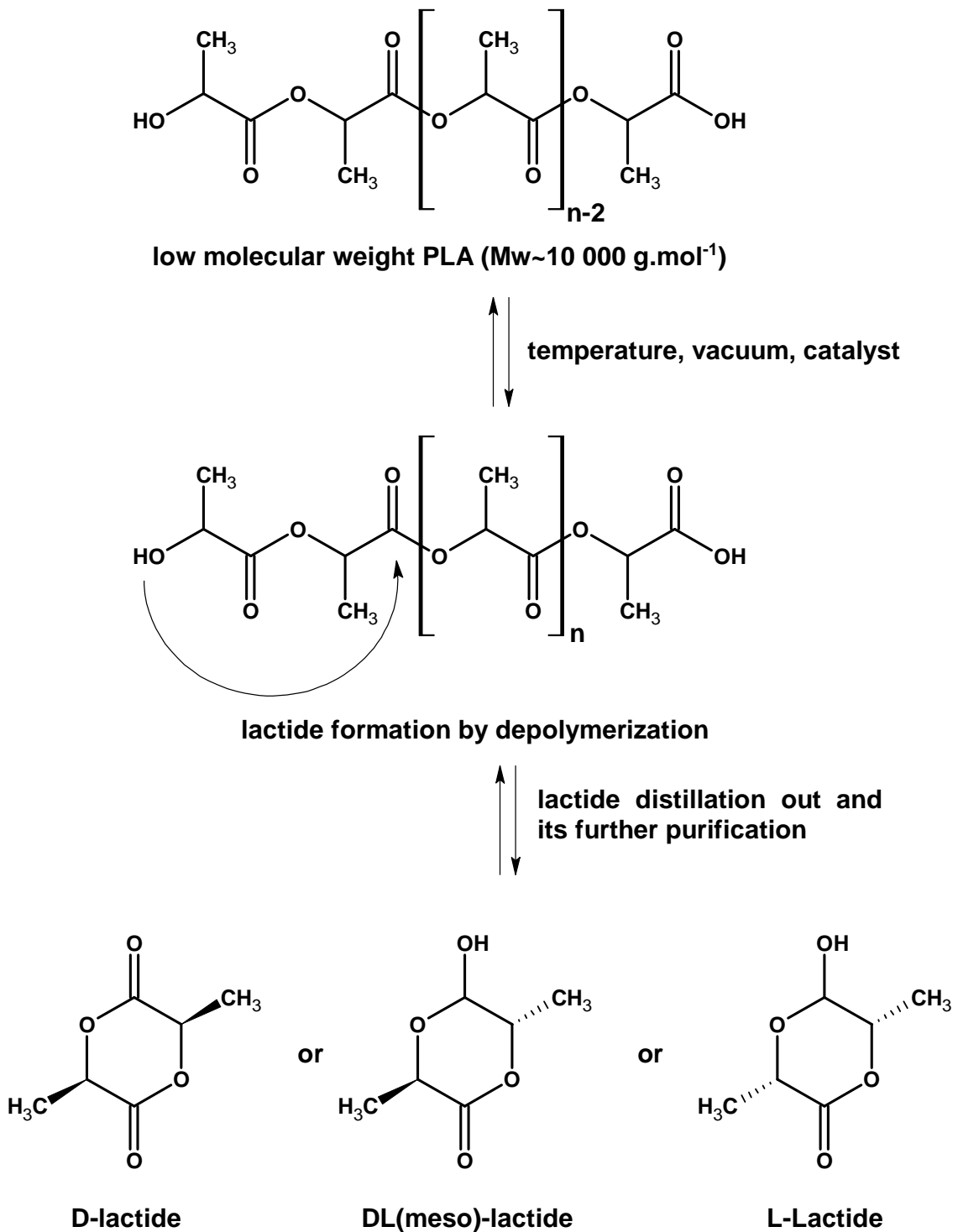
low molecular weight PLA (Mw~10 000 g.mol^{-1})

temperature, vacuum, catalyst

lactide formation by depolymerization

lactide distillation out and its further purification

D-lactide   or   **DL(meso)-lactide**   or   **L-Lactide**

Figure 8 - *Lactide formation. [23]*

2.2.2 Direct melt polycondensation

Is the simplest way of polylactic acid production. Polycondensation of LA in the presence of catalysts yields PLA and water as a byproduct. Because problem with removing water it is usually hard to yield high molecular weight polymer. However simplicity,

low cost process and possibility to driving end groups character are the biggest benefits of this route. [23] Direct melt process will be further discussed in chapter 3.

Further possibilities for PLA production are mainly based on modifications in direct polycondensation or its further developing.

2.2.3   Solution polycondensation (SP)

SP is a method which can afford PLLA with a high molecular weight ($M_w \sim 1.10^5$ g.mol^{-1}). But relatively long reaction time and high temperature is required and simultaneously use of solvents results in complexity of the process control, leaving the price of PLLA expensive. Also, it is hard to remove solvent completely from the end product. [23]
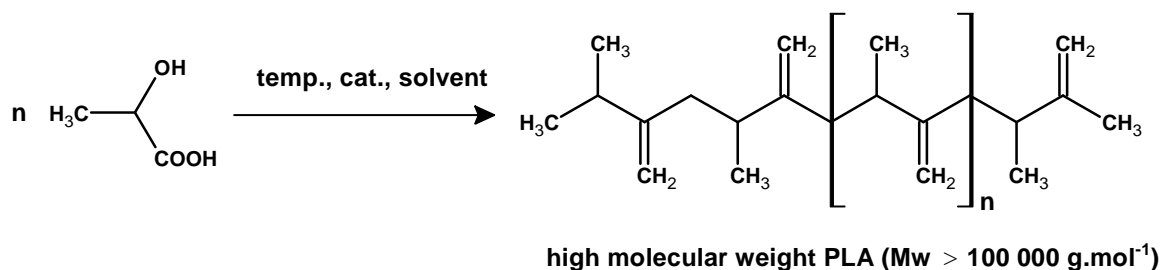

Figure 7 – *Scheme of solution polycondensation of PLA. [23]*

For example Dutkiewicz *et al.* in his paper "Synthesis of poly(L(+) lactic acid) by polycondensation method in solution" [36] works with   o-dichlorobenzene, p-xylene, o-chlorotoluene, and diphenyl ether as solvents and he reached M_n varied from 2.6×10^4 to 4.0×10^4 kg.mol^{-1}.

Solution polycondensation method uses the Japanese company Mitsui Toatsu Chemical and product called LACEA. [37]

2.2.4   Solid state polymerization (SSP)

SSP (or ester interchange reaction) appears to be an effective route for PLA synthesis when compared with ROP and simple polycondensation. It is based on catalyzed reactions between hydroxyl and carboxyl end groups inside amorphous region of low molecular weight prepolymer which leads to increasing in molecular weight. Process looks as shows Figure 10. [24; 38]

A semi crystalline solid prepolymer, of relative low molecular weight, in powder, pellet, chip or fiber form is prepared. Then heated to a temperature below T_m (5-15 °C) but

above T_g (to improve mobility and subsequent reaction of the end groups) in the presence of a suitable catalyst. [39; 40]
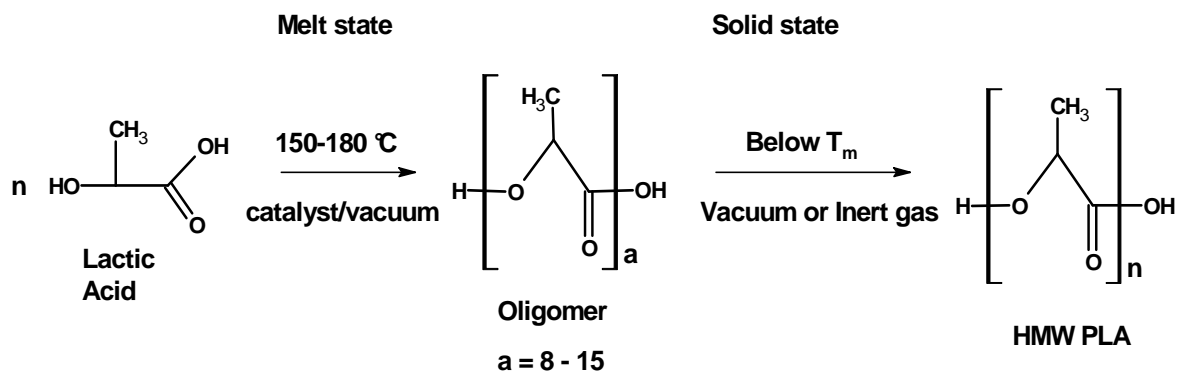

Figure 8 – *SSP process [40]*

Under these conditions end groups are able to react which leads to increasing in molecular weight. Simultaneous removal of the byproduct of condensation from the surface of the material is necessary, after it diffuses out from the bulk, either by evaporation under reduced pressure or by driving it away by a carrier gas. Plausible mechanism of reaction inside amorphous region is shown on Figure 11.
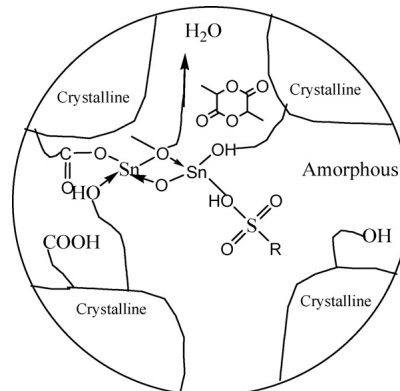

Figure 9 – *Mechanism SSP inside amorphous region [41]*

Molecular weight can reach 5.10^5 g.mol^{-1} but reaction time is relatively long (~30 h). The advantages of SSP include low operating temperatures, which control over the side reactions as well as thermal, hydrolytic, oxidative degradations along with reduced discoloration and degradation of the product. SSP polymers often have improved properties, because monomer cyclisation and other side reactions are limited. There is practically no environmental pollution, because no solvent is required. [40; 41]

2.2.5 Chain extending

Chain extending is a method based on extending oligomeric prepolymers with chain extenders. Chain extenders are usually difunctional low-molecular-weight chemicals that can increase the molecular weight of polymers in a fast reaction without producing any byproducts requiring a separate purification step. The chain extending process is typically performed in the molten stage, e.g. in an extruder in the case where the reaction rate is high enough. As effective chain extenders can be used isocyanates or bisoxazolines. [41]

Whole process depicts Figure 10. At first, lactic acid is heated with difunctional alcohol e.g. 1,4-butanediol to produce low molecular weight (oligomeric) PLA. 1,4 –butanediol creates OH terminated chain which is desirable if we want extending with diisocyanates because reaction between OH and NCO is very fast. If we use more than two functional alcohols, branched structure can be obtained. If we want to use oxazolines as the chain extenders is good to have carboxyl terminated chains. [42]
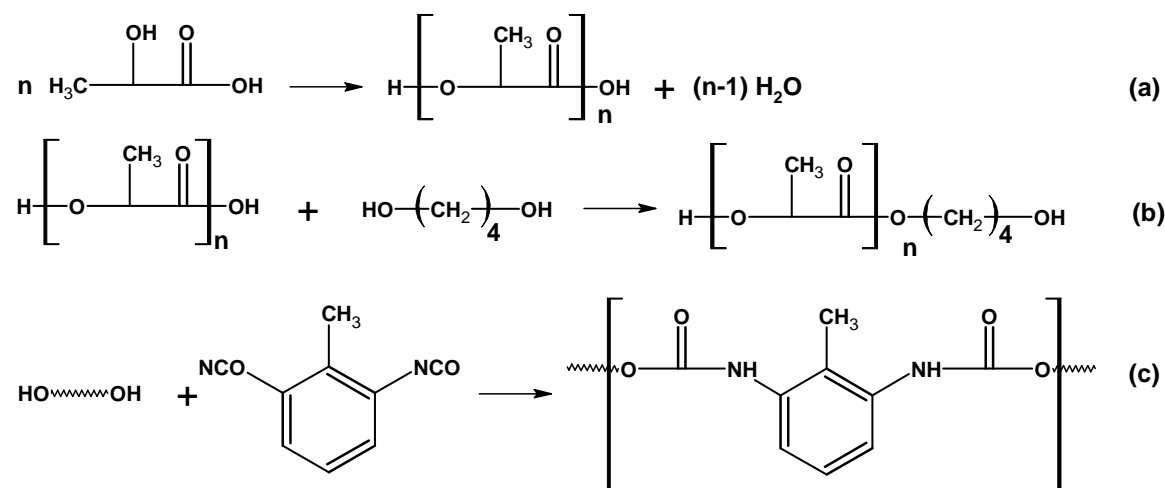

Figure 10 – *Reactions of lactic acid polycondensation (**a**), hydroxyl termination (**b**), and chain extending (**c**). [42]*

Chain linking is very promising technique for high molecular weight PLA producing. As was mentioned reaction is very fast and is possible to do it in extruder. Also molecular weights can be very high ($M_w \sim 300 kg.mol^{-1}$ was achieved). But some disadvantages also exist. For instance low thermal stability, undesirable reactions during extending (branching) or possible toxicity during biodegradation (isocyanates are poisons). [42; 43]

It was brief review of the most studied methods and routes for PLA production. But not all of them are industrially use. Only ROP and solution polymerization are widespread.

2.3 Applications

PLA has many applications and many possible applications emerging. Figure 11 depicts all possible sectors for PLA utilization.
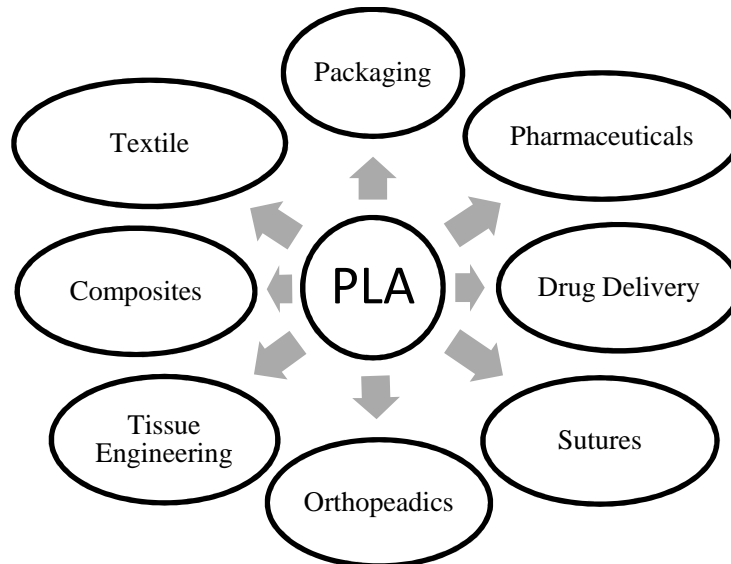
Figure 11 – *Application of PLA. [24]*

PLA and its copolymers have been used for applications like drug delivery system, protein encapsulation and delivery, development of microspheres, hydrogels, etc. In fracture fixation, metal devices are generally used to align bone fragments into close proximity, and control the relative motion of fragments so that union can take place. However, complete healing of the bone depends on its bearing normal loads, which is prevented as long as the device bears part of the load. Furthermore, sudden removal of the device can leave the bone temporarily week and subject to refracture. However, in the case of PLA based devices, degradation reduces in cross-section area as well as the elastic modulus and the load is transferred gradually to the healing bone and after the complete degradation the device will be completely absorbed, and a second surgical procedure is not necessary. PLA based fracture device has considerably lower tensile modulus than metal fixature device, but it can be improved by careful attention by fabrication using high modulus fibre reinforcement. PLA has been used for application like biodegradable/bioabsorbable fibrous articles for medical applications, orthopedic screw. The commercially available devices are BioScerw[®], PHUSILINE[®] and SYSORB[®] interference screw, BIOFIX[®] and PL-FIX[®] pins. The other biomedical applications of PLA include the development of scaffolds, biocomposite material, sutures, prosthesis, etc. Moreover, low molecular weight PLA is used for tissue engineering. [23; 24; 43]

Since, the mechanical properties of high molecular weight PLA are comparable to other commodity thermoplastics like polystyrene and PET, and therefore it has large opportunities to replace these polymers for numerous applications. Fibers and nonwovens, films, thermoformed and injection molded articles are examples. Articles have transparency equivalent to or higher than PS and PET. Fibers of PLA made by thermal spinning possess physical properties similar to PET and nylon. Moreover, PLA is aliphatic polyester and does not containany aromatic ring structures. Hence, moisture regains and wicking properties are superior to those of PET, and garments made from PLA or with wool or cotton are more comfortable with silky touch. Being a nonflammable polymer, the fiber shows improved self extinguishing characteristics. Articles have transparency equivalent to or higher than PS and PET. Fibers of PLA made by thermal spinning possess physical properties similar to PET and nylon. Moreover, PLA is aliphatic polyester and does not contain any aromatic ring structures. Hence, moisture regains and wicking properties are superior to those of PET, and garments made from PLA or with wool or cotton are more comfortable with silky touch. Being a nonflammable polymer, the fiber shows improved self extinguishing characteristics. [23; 24; 43]

PLA also finds applications in agricultural films, degradable rubbish bags, thermoformed trays for fruits and vegetables, disposable plates and cups, toys, celery. In agriculture for applications like mulch films, temporary replanting pots, delivery system for fertilizers and pesticides. [24; 43]

3   DIRECT MELT POLYCONDENSATION OF LACTIC ACID

In direct melt polycondensation, lactic acid is transferred into PLA by condensation reactions between hydroxyl (OH) and carboxyl (COOH) groups without using any solvents or external coupling agents (chain extenders).

Due to its structure one hydroxyl and one carboxyl group in chain, LA can reacts with itself and 1:1 equilibrium of reaction groups always will keep. However, it is hard to synthesize high molecular weight polymer in a satisfactory time due to:

1. Low equilibrium constant

High equilibrium constant (K_c) is one of the basic requirements which must be fulfilled in order to prepare a high molar mass polymer. If the equilibrium constant is not sufficiently high (typically $K_c < 10$) the condensation side products (usually water) must be removed from the reaction mixture in order to obtain a reasonably high degree of polymerization. This is generally problem for many polycondensation systems. According to the theory: Equilibrium constant is defined as:

$$K_C = \frac{[...-C(O)O-\cdots][H_2O]}{[...-C(O)O-\cdots][HO-\cdots]} \tag{5}$$

The number average degree of polymerization (DP_n) is related to K_C by a simple equation (5), which can be derived starting from the expression defining K_c:

$$DP_n = K_C^{0,5} + 1 \tag{6}$$

Since $K_C \sim 10$ the number average degree of polymerization $DP_n \sim 4$ would result in the equilibrium polymerization. On the other hand:

$$DP_n = 1/(1-p) \tag{7}$$

where p is a degree of conversion of the reactive groups defined by Carothers. This means that for $K_C = 10$, only 76% of hydroxyl and carboxylic group would react until equilibrium is reached. For majority of polyesters, $DP_n > 100$ is needed in order to obtain the required physical properties. This corresponds to degree of conversion p not less than 0.99. [44]

2. High viscosity of the melt

At higher conversion high viscosity of melt hampers removal of the low molar mass byproduct (water). Low pressure and higher temperatures are favourable. Low pressure facilitates removal of water and higher temperature causes lower viscosity. However at high temperature side reaction like lactide formation (Fig. 8) or decomposition can occur. [23]

For these reasons simple LA polycondensation leads only to low molecular weight products (M_w~5000 kg.mol^{-1}). If the high vacuum and catalyst are conducted higher molecular weights and better product properties could be achieved. Especially catalysts have been intensively studied in the past and a lot of various compounds were tried. [15; 16; 31]

It is well known, that catalysts and impurities can significantly affect polycondensation reaction. During polycondensation growth of the chain is a series of separate reactions. Concentration of growing chains is high but the average degree of polymerization is low until the last stage, when it is rapidly increase. Even very small concentrations of foreign substances (impurities) may cause that at the end of the growing macromolecules is linked to the reactive substance and the reaction stops. Three-dimensional structures can be developed if the impurities are 3 or more functional. Otherwise, impurities can run or support other adverse reactions (cyclization, depolymerization). For instance, if 100 000 monomers must be incorporated into polymer chain, concentration of monofunction impurities must be lower than 10 p.p.m. [45]

3.1   Mechanisms and catalysts

For the catalyzed esterification there are two general mechanisms involving either acyl-oxygen or alkyl-oxygen bond cleavage. Both types can be either acid or base-catalyzed. Acid-catalyzed mechanisms involving acyl-oxygen bond cleavage is frequently accepted and reads schematically as follows on Figure. 12. [44] This scheme is generally suitable for MSA catalyzed polyesterification.

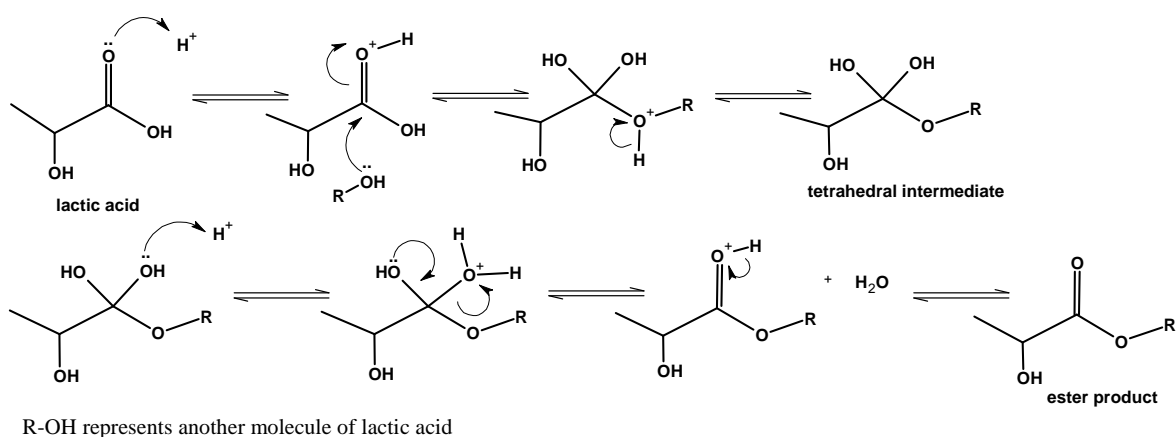R-OH represents another molecule of lactic acid

Figure 12 - *General mechanism of acyl-oxygen bond cleavage [44]*

As was mentioned, polyesterification is acid-catalyzed, either self-catalyzed or by the addition of a protonic acid. Covalent metal alkoxides are also used but this mechanism

depicts at Figure 13. However metal alkoxides have advantages in facts that are good stable and less inclined to support the side reactions connected with decreasing in molecular weight, undesirable by-products formation and discoloration. [44]
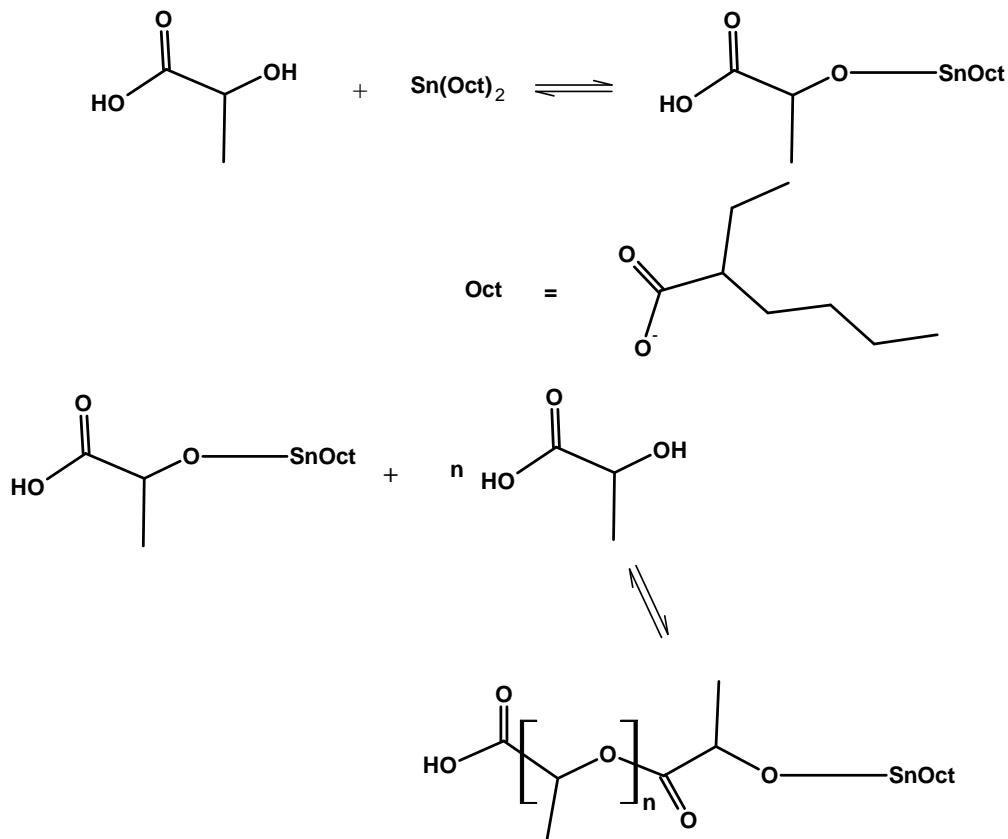

Figure 13 – *Mechanism of esterification with Sn(Oct)$_2$*

3.2 Description of impurities

The impurities are obviously considered these substances that are undesirable and adversely affect the reaction.

3.2.1 Citric acid (CA)

Citric acid (2-hydroxypropane-1,2,3-tricarboxylic acid) is produced commercially via fermentation, with an approximate annual production of 7×10^5 tons CA (Figure 13) has two primary and one tertiary carboxylic acid groups, the latter obviously being less reactive than the primary acid groups.
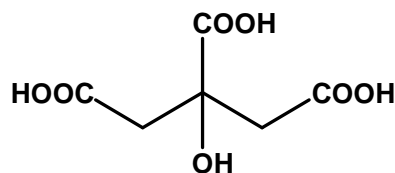
Figure 14 – *Formula of citric acid.*

In addition, it contains a sterically hindered tertiary hydroxyl group. This low cost compound is a solid at room temperature and has a melting temperature of 153 °C. [15]

Thermal Stability and Reactivity.

CA has limited thermal stability due to prone to dehydration and decarboxylation at elevated temperatures. These side reactions start to take place at an appreciable rate above 180 °C. Due to high volume resistance is almost impossible for citric acid to react with itself to form polymer structure. [46; 47]

3.2.2 Lactose

Lactose is the principal carbohydrate in the milks of all mammals. Lactose is a disaccharide consisting of galactose and glucose, linked by l-4 glycosidic bond (Figure 15).
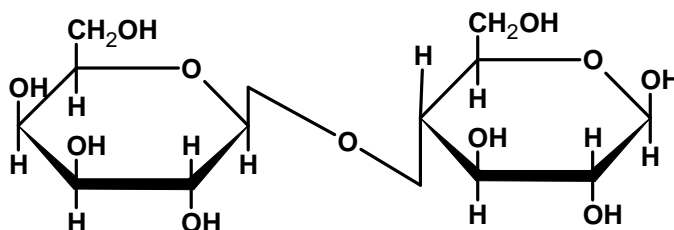

Figure 15 – *Formula of lactose sugar.*

The hemiacetal group of the glucose moiety is potentially free (i.e. lactose is a reducing sugar). Lactose has several applications in food products, the most important of which is probably in the manufacture of humanized infant formulae. It is used also as a diluent for the tableting of drugs in the pharmaceutical industry. Production of lactose essentially involves concentrating whey by ultrafiltration or vacuum concentration, crystallization of lactose from the concentrate, recovery of the crystals by centrifugation and drying of the crystals. [48]

Reactivity

The lactose molecule contains a number of reactive sites (e.g., glycosidic linkage, reducing group of glucose, free hydroxyl groups). On heating at temperatures above

l00 °C, lactose is degraded to acids with a concomitant increase in titratable acidity. Formic acid is the principal acid formed. [48; 49]

By the action of heat under acid conditions hydrolysis of the lactose 1-4 linkage can take place resulting in formation of glucose and galactose. It requires extremely acidic conditions (pH <1.5) and very high temperatures (up to 150 °C). [50; 51]

As a reducing sugar, lactose can participate in the Maillard reaction, leading to non-enzymatic browning. The Maillard reaction involves interaction between a carbonyl (in this case, lactose) and an amino group (etc. from proteins) to form a glycosamine (lactosamine). [48]

3.2.3   Whey Protein

Whey protein is heterogeneous protein (containing several different proteins). All whey proteins are relatively rich in sulphur (1.7%). Lactoglobulin is major protein and representing about 50% of total whey proteins. Lactalbumin small protein with a molecular mass of 14kDa represents about 20% of the proteins Blood serum albumin, Immunoglobulins are minority proteins. [20]

On a commercial scale, whey protein-rich products are prepared by: Ultrafiltration/diafiltration of acid or rennet whey to produce whey protein concentrates (30-80% protein). Ion-exchange chromatogramy to yield whey protein isolate, containing about 95% protein. [20]

Reactivity

Due to complicated structure, reactivity of whey proteins is depends on many factors. But in conditions of high temperature, long time and low pH degradation processes take place.

The whey proteins are relatively heat labile. Moderate heat treatment (60-70°C) results in structural unfolding of whey proteins. At higher temperatures, depending on compositional factors, protein aggregation occurs. By heating at 90 °C for 10 min. proteins are completely denatured. Prolonged treatment caused partial hydrolysis of peptide bonds and decrease in high partial hydrolysis of peptide bonds and decrease in high molecular weight components molecular weight components. In presence of reducing sugar, Maillard's reactions can take place during heating. [48, 52]
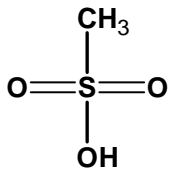
3.3   Catalysts

In this thesis, 2 different catalysts were used and their effect was observed.

3.3.1 Methanesulfonic acid (MSA)

MSA is a strong organic acid, non-oxidizing, stable at high temperatures (up to 180 °C). Moreover, the MSA is nontoxic and is readily degradable with formation of sulfates and CO_2. These properties, especially high thermal stability and low tendency to oxidize organic compounds could be useful in catalysis lactic acid polycondensation. General properties are summarized at Table 4. [53]
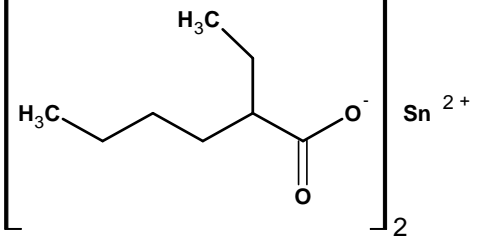
Table 4 – *Methane sulfonic acid properties.* [53]

Formula	Property	Unit
CH_3	Molecular Weight	96.10 g.mol^{-1}
O=S=O	Density	1.5 g.cm^{-3}
OH	Boiling point	167 °C
	Stability	Stable under ordinary conditions.

3.3.2 Stannous octoate - Sn(Oct)$_2$

Stannous octoate (Tin 2-ethylhexanoate; Stannous-2-ethyl hexanoate) belongs to the group of metallic carboxylates (metal salts of carboxylic acids). Sn(Oct)$_2$ is used as a catalyst during chemical reactions (polymerization, esterification, oxidation, condensation, hydrogenation and other reactions). Its advantages are related to the commercial availability, low sensibility to impurities, and relatively high selectivity even in bulk, high-temperature polymerizations. General properties are summarized at tale 5. [54]

Table 5 – *Stanous octoate properties.* [54]

Formula	Property	Unit
H_3C, H_3C, O$^-$ Sn^{2+}, O, 2	Molecular Weight	405.10 g.mol^{-1}
	Density	1.251 g.cm^{-3}
	Decompose at	202 °C
	Stability	Stable under ordinary conditions.

4   AIMS OF THE WORK

On the basis of assigned tasks and literature survey, the goals of this diploma thesis can be specified in the following items:

1) Preparation of lactic acid polycondensates catalyzed conventional organometallic and non-metallic based compounds
   - Stannous 2-ethylhexanoate (tin octoate)
   - Methanesulphonic acid

2) Polycondensation of lactic acid contaminated with below mentioned impurities, which can occur in lactic acid prepared through fermentation of dairy industry by-product-whey. Selected impurities are:
   - Citric acid
   - Lactose
   - Whey protein concentrate

3) Characterization of polycondensation product and evaluation of catalyst role in this process. The analysis of the samples will be focused on determination of:
   - Changes in molecular weight (viscometric measurements, gel permeation chromatography)
   - Changes in thermal properties (differential scanning calorimetry)
   - Structural changes (Fourier transform infrared spectroscopy, polymer end group determination-titration method)

4) Discussion of the results and their presentation in experimental part of this thesis

II.   ANALYSIS

5   MATERIALS AND METHODS

5.1   Materials

L-lactic acid $C_3H_6O_3$ (LA) 80 % water solution, optical rotation $[\alpha]=10.6°$ (measured by the polarimeter Optech P1000 at 22 °C, concentration of 10%) was purchased from Lachner Neratovice, Czech Republic. Stannous 2-ethylhexanoate $(Sn(Oct)_2)$ $(C_{16}H_{20}O_4Sn; 95\%)$ and methanesulphonic acid $(CH_4O_3S; 99.5 \%)$ was supplied by Sigma Aldrich, Steinheim Germany. The solvents acetone (C_3H_6O), dichloromethane (CH_2Cl_2), methanol (CH_4O), ethanol (C_2H_6O), indicator bromothymol blue $(C_{27}H_{28}Br_2O_5S)$, potassium hydroxide (KOH) and anhydrous citric acid $(C_6H_8O_7)$ (CA) (all analytical grade) were bought from IPL Lukes, Uhersky Brod, Czech Republic. Chloroform $(CHCl_3)$ (HPLC grade) was purchased Chromspec, Brno, Czech Republic. Anhydrous Lactose (L) $(C_{12}H_{22}O_{11})$ (food grade) and protein concentrate (PROT) (certified composition: proteins-75 wt. %, lactose-17.5 wt. %, minerals 7.5 wt. %) were kindly provided by Milcom a.s. Prague, Czech Republic. All chemicals were used as obtained without further purification.

5.2   Sample Preparation

A typical procedure was as follows: relevant portions of LA and individual components representing impurities (CA, L and PROT) were added into a double necked flask (250 ml) equipped with a Teflon stirrer. The compositions of the reaction mixtures are shown below in Tables 6, 9 and 12.Total mass of the mixture at the beginning of reaction was 50 g (water is not included). The flask was then placed in an oil bath heated by magnetic stirrer with heating and connected to a laboratory apparatus for distillation under reduced pressure. The dehydration step followed at 160 °C, reduced pressure 15 kPa for 4 hours. After that, the reactor was disconnected from the vacuum pump and the relevant amount (0.5 wt. %, related to initial mass of the reactants) of the catalyst $(Sn(Oct)_2)$ or MSAwas added dropwise under continuous stirring. The flask with dehydrated LA/impurity/catalyst mixture was connected back to the source of vacuum (100 Pa) and the reaction continued for 24 hours at the temperature 160 °C. The resulting product was allowed to cool down at room temperature and dissolved in acetone then. The polymer solution was precipitated in a mixture of chilled methanol/distilled water 1:1 (vol./vol.). The obtained product was filtrated, washed with methanol and dried at 45 °C for 48 hours. The pH value of the filtrate after polymer separation was checked to be sure that unreacted impurities are not present in the polymer. The scheme of the apparatus is shown in Figure 15.
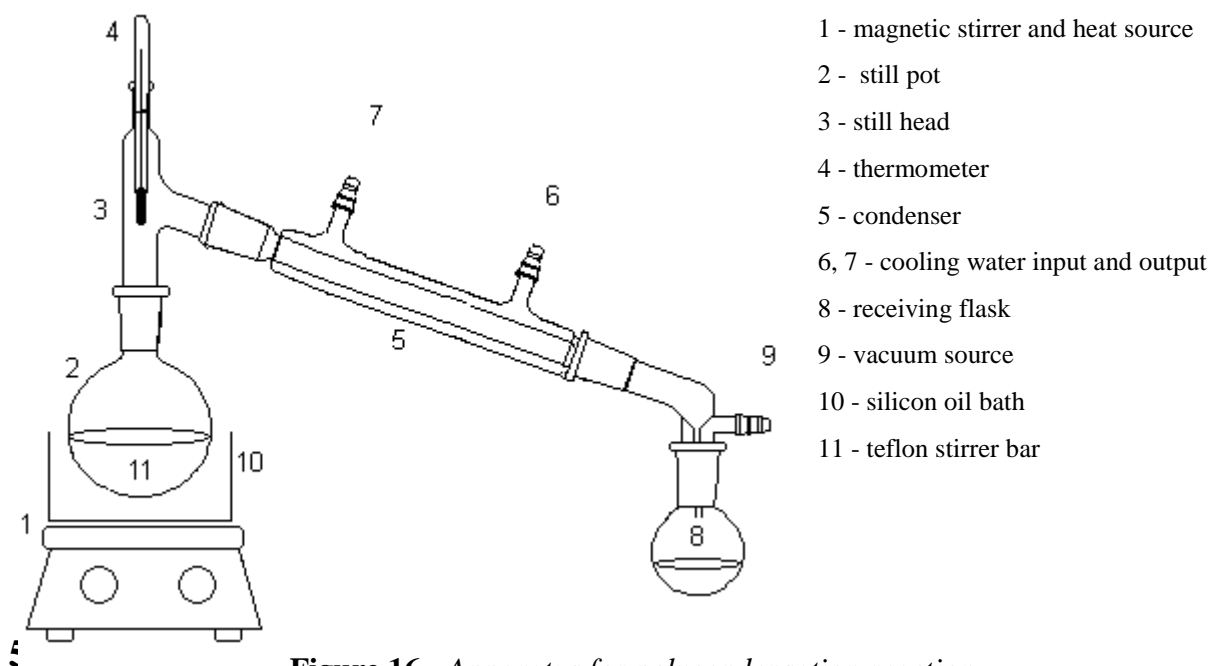
Figure 16 - *Apparatus for polycondensation reaction.*

1 - magnetic stirrer and heat source
2 - still pot
3 - still head
4 - thermometer
5 - condenser
6, 7 - cooling water input and output
8 - receiving flask
9 - vacuum source
10 - silicon oil bath
11 - teflon stirrer bar

Following subchapter introduces the methods, which were used for in this work. A short theoretical background of each method is given before detailed experimental settings description.

5.3.1 Viscometric measurement

Viscosity of a polymer solution depends on concentration and size (i.e., molecular weight) of the dissolved polymer. By measuring the solution viscosity is possible to get an idea about molecular weight. The most common solution viscosity term is: The intrinsic viscosity [η] which is a unique function of molecular weight (for a given polymer-solvent pair). Measurements of [η] can be used to measure molecular weight. [55]

Viscosity measurements were performed in chloroform at 30 °C in an Ubbelohde viscometer with capillary 0a. The intrinsic viscosity, [η], was calculated using the equation (8):

$$[\eta] = \lim_{c \to 0} \frac{\eta_{rel} - 1}{c} \qquad \textbf{(8)}$$

where η_{rel} is the relative viscosity, which is equal to the ratio of polymer solution and pure solvent viscosities and c is the concentration of the polymer solution (0.4, 0.8 and 1.2 g/100 ml).

5.3.2 Gel permeation chromatography (GPC)

Gel Permeation chromatography belongs in the group of separation methods, which is based on the ability of molecules to move through a column of gel that has pores of clearly-defined sizes. The larger molecules can not enter the pores, thus they pass quickly through the column and elute first. Slightly smaller molecules can enter some pores, and so take longer to elute, and small molecules can be delayed further. The technique can be used to determine the molecular weight of polymers and its distribution. [56]

GPC analyses were performed using a chromatographic system Breeze (Waters) equipped with a PLgel Mixed-D column (300x 7.8 mm, 5µm) (Polymer Laboratories Ltd). For detection, a Waters 2487 Dual absorbance detector at 239 nm was employed. Analyses were carried out at room temperature with a flow rate of 1.0 mL.min^{-1} in chloroform. The column was calibrated using narrow molecular weight polystyrene standards with molar mass ranging from 580 to 480 000 g.mol^{-1} (Polymer Laboratories Ltd). A 100 µL injection loop was used for all measurements. The sample concentration ranged from 1.6 to 2.2 mg.ml^{-1}. Data processing was carried out using the Waters Breeze GPC Software (Waters). The weight average molar mass M_w, number average molar mass M_n and polydispersity (M_w/M_n) of the tested samples were determined.

5.3.3 Differential scanning calorimetry (DSC)

The Differential Scanning Calorimeter is a thermal analysis method that determines the temperature and heat flow associated with material transitions as a function of time and temperature. Sample and comparative standard material are maintained at the same temperature and a heat flow is measured. The result of DSC measurement is a graphic dependent differential rate of heating (in J.s^{-1}) on temperature. The DSC can be used to determine: glass transition, melting temperature, heat of fusion, percent crystallinity, crystallization kinetics and phase transitions, [57]

For the determination of glass transition temperature (T_g), melting point (T_m) of the polycondensates the differential scanning calorimetry (DSC) was used. Approximately 8 mg of the sample were placed in an aluminium pan, sealed and analyzed on NETZSCH DSC 200 F3, calibrated in terms of temperature and heat flow, using indium. The experiments were performed under nitrogen atmosphere (60 ml.min^{-1}) in two scans in the temperature range of 0 – 180 °C and at the heating rate of 10 °C.min^{-1}.

5.3.4 Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy belongs into the group of absorption spectroscopy techniques. It is based on the interaction of the molecules with electromagnetic radiation in infrared region (λ=0.3-300µm). In principle a radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some of it is passed through (transmitted). The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. Like a fingerprint no two unique molecular structures produce the same infrared spectrum. An infrared spectrum represents a fingerprint of a sample with absorption peaks which correspond to the frequencies of vibrations between the bonds of the atoms making up the material. Because each different material is a singular combination of atoms, no two compounds produce the exact same infrared spectrum. Therefore, infrared spectroscopy can result in a positive identification (qualitative analysis) of every different kind of material. In addition, the size of the peaks in the spectrum is a direct indication of the amount of material present. With modern software algorithms, infrared is an excellent tool for quantitative analysis. [58]

To identify the physico-chemical structure of LA polycondensation products, Fourier transform infrared analysis (FTIR) was carried out. The investigation was conducted on by NICOLET 320 FTIR, equipped with attenuated total reflectance accessory (ATR) utilizing Zn-Se crystal and the software package OMNIC over the range of 4000-650 cm^{-1} at room temperature. The uniform resolution of 2 cm^{-1} was maintained in all cases.

5.3.5 Acidity number (AN)

The concentration of terminal carboxyl groups was expressed as acidity number (AN), which represents the amount of KOH (in mg) for neutralization of 1 gram of a substance. [59] AN can be calculated according following equation:

$$AN = \frac{M_{KOH} \times c_{KOH} \times V_{KOH}}{w \times 1000} \tag{9}$$

where M_{KOH} = 56.11 g.mol^{-1}, c_{KOH} represents concentration of KOH solution (mol.L^{-1}), V_{KOH} is volume of titration solution at equivalence point (L) and w stands for weight of the sample (g).

In this study, AN was determined by titration of the sample in methanol/dichroloromethane solution (1:1 vol.) with 0.01 M KOH ethanol solution. Bromothymol blue was used as an indicator.

5.4   Results and discussion

The following chapter is divided into three parts – subchapters. Each subchapter is dedicated to description of the individual impurity effect on LA polycondensation products from the changes of molecular weight, thermal properties and structural characteristics point of view. In addition, significant attention is paid to comparison of the catalytic abilities of two selected compounds – $Sn(Oct)_2$ and MSA.

5.4.1   Effect of citric acid (CA) on lactic acid (LA) polycondensation products

This study covers the investigation of LA/CA polycondensation products (PLACA). The experiments were carried out for eight LA/CA ratios (molar ration 1 – 211; i.e. 0-20 wt. % CA). The detailed compositions of the investigated systems can be seen in Table 6. Water presence is not considered in calculation of the LA/CA systems compositions.

Table 6 - *Compositions of the reaction mixture (at the beginning of reaction) and designation of the samples for LA/CA systems.*

Sample designation	LA concentration (wt. %)	CA concentration (wt. %)	LA:CA molar ratio
PLA	100	0	-
PLACA 1%	99	1	211.2
PLACA 3%	97	3	69.0
PLACA 5%	95	5	40.5
PLACA 7%	93	7	28.3
PLACA 10%	90	10	19.2
PLACA 15%	85	15	12.1
PLACA 20%	80	20	8.5

The basic characteristics of the samples prepared by melt polycondensation of LA and CA are shown in Table 7. In case of $Sn(Oct)_2$ catalyst, it can be noticed that increasing content of CA in the reaction mixture leads, beside the appearance change of the product, to significant reduction of [η]. While the pure PLA has [η] = 0.47 dL.g^{-1}, PLACA 5% in the mixture causes noticeable reduction of [η], which poses more than 55 % of the value obtained for pure PLA. However, noticeable reduction of [η] was observed already for

PLACA 1% (more than 45 %). The samples which were prepared in above 10 wt. % CA presence are characteristic with low [η], which corresponds to their waxy like appearance unlike powder form of the polycondensation products prepared at lower concentration of tricarboxylic acid.

Table 7 - *Characteristics of PLACA polycondensates.*

Sample designation	M_n* (kg.mol^{-1})	M_w* (kg.mol^{-1})	M_w/M_n*	[η]** (dL.g^{-1})	Yield (wt. %)	AN/SD*** (mg$_{KOH}$.g^{-1})
Catalyst – Sn(Oct)$_2$						
PLA	22.6	46.9	2.1	0.47	62	23.6/1.9
PLACA 1%	10.8	27.0	2.5	0.25	46	30.0/1.5
PLACA 3%	1.9	7.7	4.1	0.20	25	35.7/1.7
PLACA 5%	1.1	4.7	4.3	0.14	22	50.5/1.2
PLACA 7%	0.9	4.0	4.4	0.15	53	54.0/1.3
PLACA 10%	0.3	1.4	4.7	0.09	33	78.6/1.2
PLACA 15%	0.2	1.2	6.0	0.08	43	98.4/1.7
PLACA 20%	0.2	1.2	6.0	0.08	48	97.9/1.1
Catalyst - MSA						
PLA	7.1	12.0	1.69	0.18	62	35.0/1.1
PLACA 1%	7.9	12.6	1.59	0.19	46	71.3/2.4
PLACA 3%	1.0	4.4	4.40	0.08	25	176.6/0.5
PLACA 5%	0.8	3.0	3.75	0.10	22	209.7/3.2
PLACA 7%	0.5	2.1	4.20	0.10	53	101.0/3.4
PLACA 10%	0.3	1.3	4.33	0.10	33	117.4/1.7
PLACA 15%	0.2	0.7	3.50	0.06	43	98.4/3.0
PLACA 20%	0.1	0.6	6.00	0.05	48	173.7/1.5

*   GPC measurement;   ** Viscometry (30°C in CHCl$_3$);   *** SD-standard deviation

The reduction of [η] due to CA presence occurred in case of MSA as well. However, the maximal obtained [η] is 0.18 dL.g^{-1} (Table 7). It can be noticed that the course of [η] reduction does not seem to as steep as in previous case. It reveals that the system is as sensitive on CA presence under MSA catalysis. However, reached [η] is significantly lower, which is connected with the principles of the catalytic function of the both compound as mentioned above.

The values of molecular weight determined by GPC (Table 7) correspond to the intrinsic viscosity data. The highest M_w was achieved in case of PLA (46,900 g.mol^{-1}). The increasing content of CA in the reaction mixture decreases M_w steeply. Finally, the sample designated as PLACA 20% proves M_w only 1,200 g.mol^{-1}. In addition, polydispersity (M_w/M_n) of the polycondensates increases from 2.1 (for PLA) to 6.0 (for PLACA 20%).

Similar observations can be noticed for the PLACA series catalyzed by MSA. The lower [η] corresponds to lower M_n and M_w determined by GPC. The already mentioned differences in catalytic principles between the used catalysts (Figures 12 and 13) are also responsible for narrower distribution of PLACA molecular weights, which is proved by lower polydispersity degrees up to 5 wt. % of CA (Table 7). These variances are not relevant at higher contents of CA in the systems.

Due to fact that, CA contains three carboxylic and one hydroxyl group while LA poses one from each the ratio COOH/OH is shifted towards higher values than 1 in presence of CA in the reaction mixture. The reduction of molecular weight and increasing of polydispersity index is the logical consequence of that.

The incorporation of citric acid into the structure of product through condensation reaction can be proved by determination of carboxyl groups, which should be in excess in case the reaction took place during product formation. The titration method can provide such information in form of acidity number as presented above. The values of AN, shown in Table 7, are in accordance of the assumption of occurrence of the condensation reaction between LA and CA molecules. In case of Sn(Oct)$_2$ catalyzed samples, the rising CA content causes increase in AN up to 15 wt. % CA in reaction mixture. The AN values for PLACA 15% and PLACA 20% are more or less identical, which reveals possible limits of CA incorporation into the structure of the product. In case of MSA, noticeable rise of AN occurs for samples PLACA 1, 3 and 5%. The results for the samples with higher CA content are unclear due to high AN variation. On the other hand, M_w of these samples are low and reactions of short oligomeric fractions may play certain role here together with already mentioned limitations of CA incorporation into the structure of the molecules. This limit is connected with the inhibition of condensation reaction that leads to reduction of molecular weight of the samples (Table 7). It can be supposed that this method considers mostly the carboxyl groups appearing at the end of the polymeric chains. The amount of such terminations increases with increasing amount of short chains of the polymer or its branches.

Table 8 - *Effect of CA on thermal properties of PLA based polycondensates. Results from DSC – T_m was taken from the first heating scan, T_g - was taken from second heating scan*

Sample designation	Catalyst – Sn(Oct)$_2$			Catalyst - MSA		
	T_{m1} (°C)	T_{m2} (°C)	T_g (°C)	T_{m1} (°C)	T_{m2} (°C)	T_g (°C)
PLA	151.2	-	53.8	142.6	-	34.1
PLACA 1%	110.0	124.8	46.1	140,1	-	38.2
PLACA 3%	134.5	147.9	54.7	95.0	125.1	23.6
PLACA 5%	118.1	130.9	53.6	108.9	119.0	17.2
PLACA 7%	123.4	137.6	51.0	109.8	119.3	18.8
PLACA 10%	104.4	122.3	49.0	97.3	117.6	17.4
PLACA 15%	90.1	102.0	33.0	88.0	-	-
PLACA 20%	59.1	88.5	32.4	90.8	-	-

The summary of DSC results obtained from the first and second heating scan is shown in Table 8. In case of Sn(Oct)$_2$, all samples containing CA proved double melting exothermic peaks while pure PLA is characteristic by significant single melting peak situated at 151.2 °C. The phenomenon of cold crystallization was not observed. Similar results have been reported by Yao et al. [47]. Generally, CA presence decreases T_m of the samples and this reduction corresponds to CA concentration in the system. The only exception is created by PLACA 1%, which shows noticeably lower T_{m1} and T_{m2}. The experiment was proceeded more times with the similar result. The reason of such behavior could be found in possible obstruction of the volume CA molecules during development of organized polymer units. This may be possible under the condition that some of the CA molecules (present in low amount in case of PLACA 1%) is incorporated inside of the polymer chain; i.e. it does not terminate the chain. On the other hand, polycondensation inhibition effect becomes predominant at higher concentrations of CA. It is connected with degreasing of molecular mass (Table 7). The results (both T_m and T_g) for MSA catalyzed samples are, generally, lower in comparison to previous case. It obviously corresponds to lower M_w (Table 7). Interestingly, double melting endotherms were not observed (beside pure PLA) for PLACA 1% as well as for PLACA 15 and 20%. The former case (PLACA 1%) can be explained by co-catalytic action of CA, which is supported by increased M_w (see Table 2). The later is probably connected with formation of low molecular component with possible structural uniformity i.e. CA strongly inhibits polycondensation. These products cannot be

considered as polycondensates. Glass transition temperature T_g, of these waxy products is below the investigated temperature range (0-180 °C).
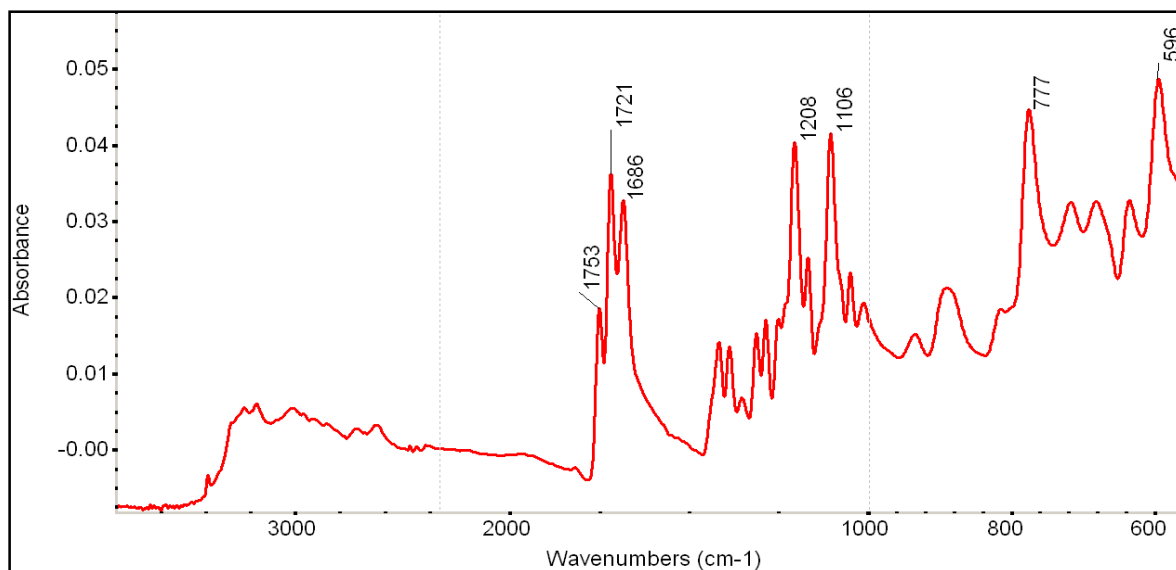

Figure 17 - *FTIR-ATR spectrum of neat citric acid (CA)*

The FTIR-ATR spectrum of neat CA is shown in Figure 17. CA as tricarboxylic acid has typical absorption peaks positioned 1753, 1721 and 1686 cm-1 (-C=O stretching). It should be mentioned that databases report only two absorption peaks in the region 1800-1650 cm^{-1}). As can be seen in the Figure 17, the intensity of the signal is low, thus the peak at 1753 cm^{-1} could be considered as random noise signal. Other significant peaks of CA were found at 1208, 1106 cm-1 (C-O stretching) and 777 cm^{-1} (C-C stretching). Typical FTIR-ATR spectra of the selected samples (PLA, PLACA 20%) are shown in Figure 18. All spectra prove typical absorptions at 2950 cm^{-1}, which corresponds to –CH- stretching. Furthermore, typical PLA absorbances were detected: 1750 cm^{-1} (C=O stretching), 1452 cm^{-1} (CH$_3$- bending), 1381 and 1361 cm^{-1} (-CH- deformations and asymmetric bending), 1265 cm^{-1} (C-O stretching – typical for PLA prepared by direct polycondensation), 1183, 1128 and 1084 cm^{-1} (C-O-C stretching), 1043 cm^{-1} (-OH bend-ing) [3].

Figure 19 shows FTIR-ATR spectra of the selected samples catalyzed by MSA. The peak positions are more or less the same as reported for the samples in Figure 18.
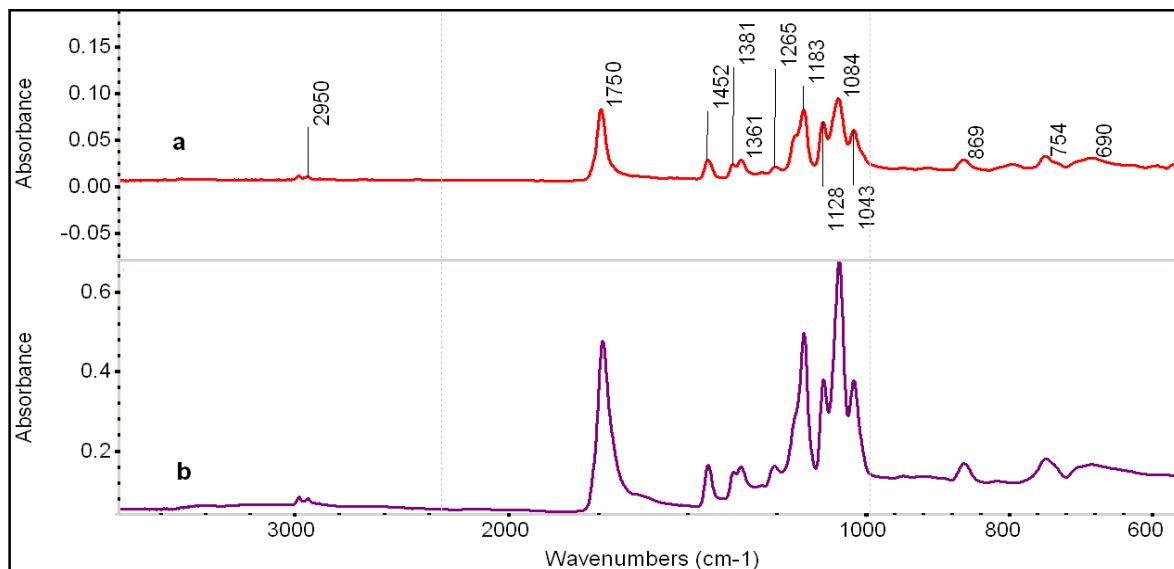
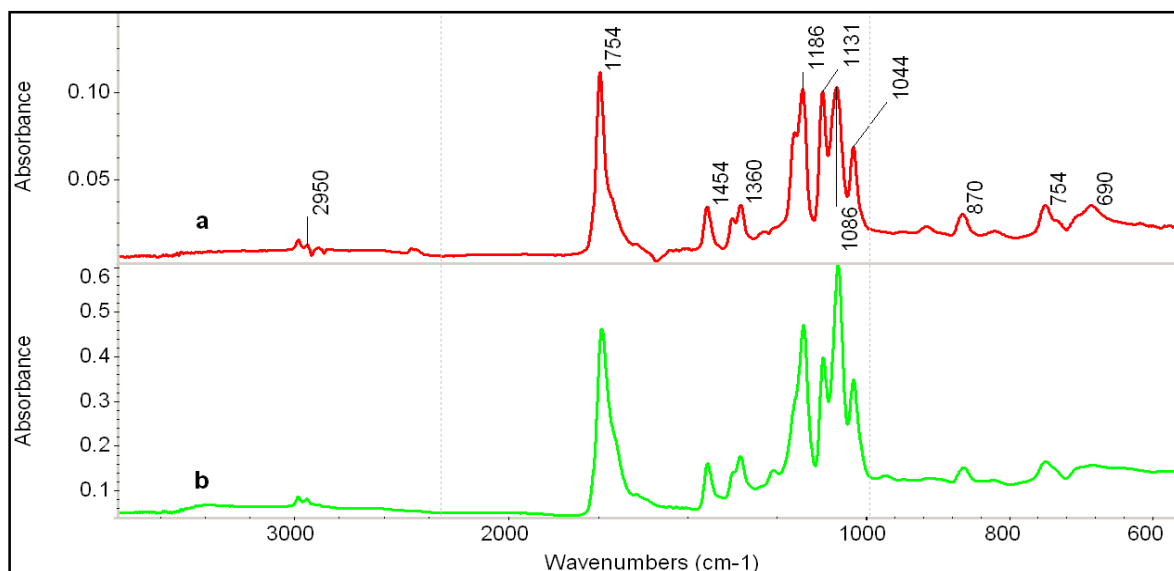**Figure 18** - *FTIR-ATR spectra of PLA (a) and PLACA 20% (b) samples, catalyst Sn(Oct)$_2$.*

Figure 19 - *FTIR-ATR spectra of PLA (a) and PLACA 20% (b) samples, catalyst MSA.*

The prove of spectra similarities is clearly visible in Figure 20 where FTRI-ATR spectra of PLACA 10% prepared under catalytic action of both MSA (a) and Sn(Oct)$_2$ (b) are depicted.

The chemical similarities of LA and CA cause difficulties in qualitative analysis of the spectra. The only evidence of a difference can be noticed in the wavenumber region 1700-1600 cm^{-1} where increase in absorbance can be found. This is evident especially in case PLACA 20% (Figures 18 and 19).
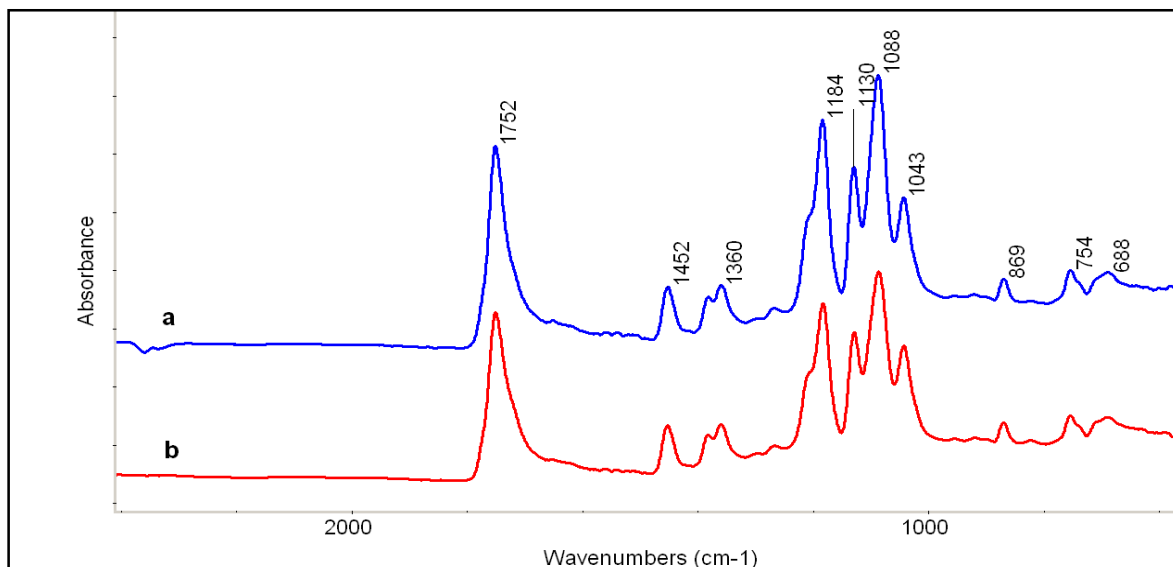
Figure 20 - *FTIR-ATR spectra of PLACA 20% samples, catalyst MSA (**a**) and Sn(Oct)$_2$ (**b**)*

It could reveal the presence of carboxyl groups in the structure of the polymer. On the other hand, the evidence of that is expected to be more distinguishable from quantitative analysis of carboxyl group. It was proceeded by comparison of peak areas corresponding to carboxyl groups presence in the region 1800-1650 cm^{-1} (A_C), normalized by area of reference peak (A_R). The peak at 1452 cm^{-1} was chosen as internal standard for spectra normalization since it has been reported as suitable for this purpose [7]. The dependence of A_C/A_R on CA content is shown in Figure 21.
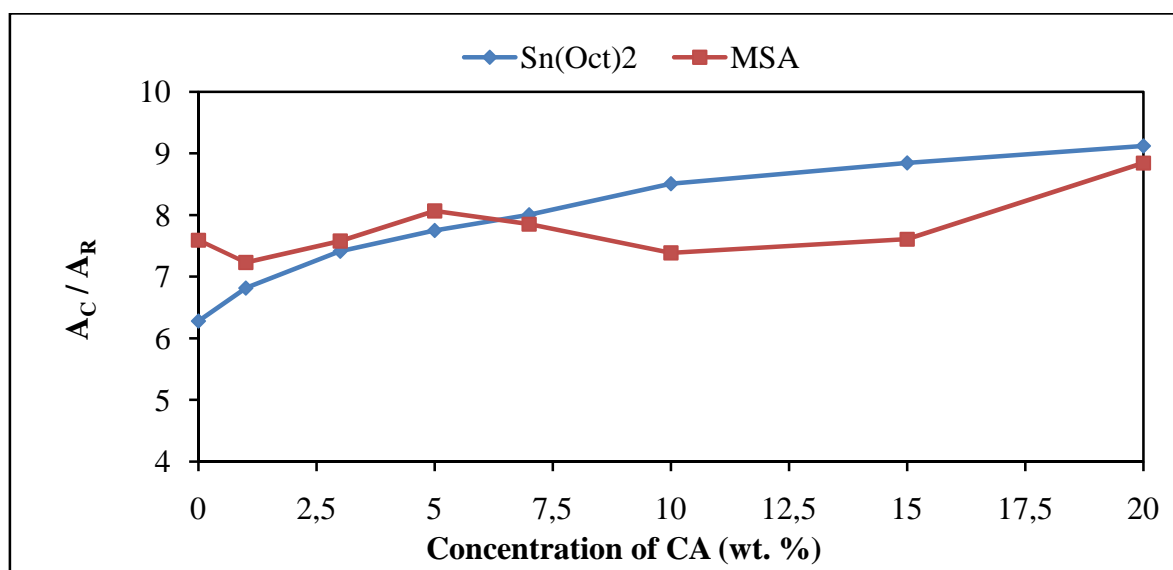
Figure 21 - *Carboxyl group presence analysis based on of FTIR-ATR spectra of PLACA samples.*

It can be noticed that in case of $Sn(Oct)_2$ catalyzed samples, the amount of carboxyl groups rises with increasing concentration of CA in the reaction mixture, which gives evidence of CA units in the polymer structure. On the other hand, MSA series are characteristic by irregular course with initial drop of carboxyl groups followed by increasing A_C/A_R ratio up to 5 wt. % of CA. The reason of the results for the samples with higher concentrations of CA is not known. However, it is in good agreement with AN data presented in Table 7.

5.4.2 Effect of lactose (L) on lactic acid (LA) polycondensation products

This part of experimental section deals with the investigation of LA/L polycondensation products (PLAL). The experiments were carried out for eight LA/L ratios (0-2 wt. % L). The detailed compositions of the investigated systems can be seen in Table 9. Water presence is not considered in calculation of the LA/CA systems compositions. The concentration range of L was chosen on the basis of the results reported in reference 8.

Table 9 - *Compositions of the reaction mixture (at the beginning of reaction) and designation of the samples for LA/L systems*

Sample designation	CA concentration (wt. %)	LA:L molar ratio
PLA	0.00	-
PLAL 0.25%	0.25	1516
PLAL 0.5%	0.50	756
PLAL 0.75%	0.75	503
PLAL 1%	1.00	376
PLAL 1.5%	1.50	250
PLAL 2%	2.00	186

The basic characteristics of the samples prepared by melt polycondensation of LA and L are shown in Table 10. In case of $Sn(Oct)_2$ catalyst, significantly more effective catalytic action was observed in comparison with MSA. It is already proved by generally higher values of [η] (Table 10). The course of [η] vs. L content dependence shows initial decrease in [η] (from 0.47 for pure PLA to 0.24 dL.g^{-1} for PLAL 0.5%). However, significant increase in [η] (with the maximum at 1 wt. % L) follows (PLAL 1%, [η]=0.72). On the other hand, MSA catalyzed series prove continuous reduction of [η] with exception at

the lowest investigated L content. These results show that $Sn(Oct)_2$ is able to catalyze the linking reaction between carboxyl of LA and hydroxyls of L. L can provide six reactive OH groups. Thus there is a possibility for creation of a polycondensate with incorporated heterocyclic units originating from L. Formation of 3D structure may be also possible.

Table 10 - *Characteristics of PLAL polycondensates.*

Sample designation	M_n* (kg.mol^{-1})	M_w* (kg.mol^{-1})	M_w/M_n*	$[\eta]$** (dL.g^{-1})	Yield (wt. %)	AN/SD*** (mg$_{KOH}$.g^{-1})
Catalyst – Sn(Oct)$_2$						
PLA	22.6	47.0	2.08	0.47	61.68	23.6/1.9
PLAL 0.25%	11.1	24.5	2.21	0.25	70.46	20.6/0.3
PLAL 0.5%	10.0	23.5	2.35	0.24	41.36	17.1/0.7
PLAL 0.75%	17.5	53.3	3.05	0.27	59.4	23.2/1.3
PLAL 1%	31.4	132.0	4.20	0.43	74.8	25.0/0.2
PLAL 1.5%	39.3	217.7	5.54	0.72	59.4	6.3/1.5
PLAL 2%	32.8	162.4	4.95	0.52	64.2	6.6/0.6
Catalyst - MSA						
PLA	7.1	12.0	1.69	0.18	64.54	35.0/1.1
PLAL 0.25%	3.9	11.1	2.85	0.24	65.2	62.3/1.3
PLAL 0.5%	7.2	20.2	2.80	0.18	67.6	93.1/0.9
PLAL 0.75%	4.5	15.6	3.47	0.16	71.4	99.3/0.3
PLAL 1%	7.6	22.8	3.00	0.17	68.4	107.7/0.1
PLAL 1.5%	9.5	31.0	3.26	0.14	63.8	68.5/0.7
PLAL 2%	4.4	11.0	2.50	0.14	69.8	59.5/2.7

*GPC measurement ** viscometry (30°C in CHCl$_3$) *** SD-standard deviation*

These results are in agreement with GPC analysis. While molecular weight of PLAL catalyzed by MSA reaches maximally M_w=31 kg.mol^{-1} (PLAL1.5%) the sample with the same composition, but catalyzed by $Sn(Oct)_2$, proves M_w almost 218 kg.mol^{-1}. The course of rising M_w can be seen in chromatograms depicted in Figure 22. The significant shift to lower elution times can be observed there. The dashed line represents peak of pure PLA. Such shift in retention time (peak position) shows on presence of structure with high hydrodynamic volume, network-like (possible formation of micro-gels). Also should be take it accounts that values of M_w and M_n calculated from such

shifted curves can hardly fit to reality because the GPC system was calibrated on linear standards (polystyrene).

The chramotgams for MSA catalyzed samples are presented in Figure 23. The data shown in Table 10 reveals that the changes in elution times were not as significant as in the case discussed above and the chomatographs in Figure 23 confims it.
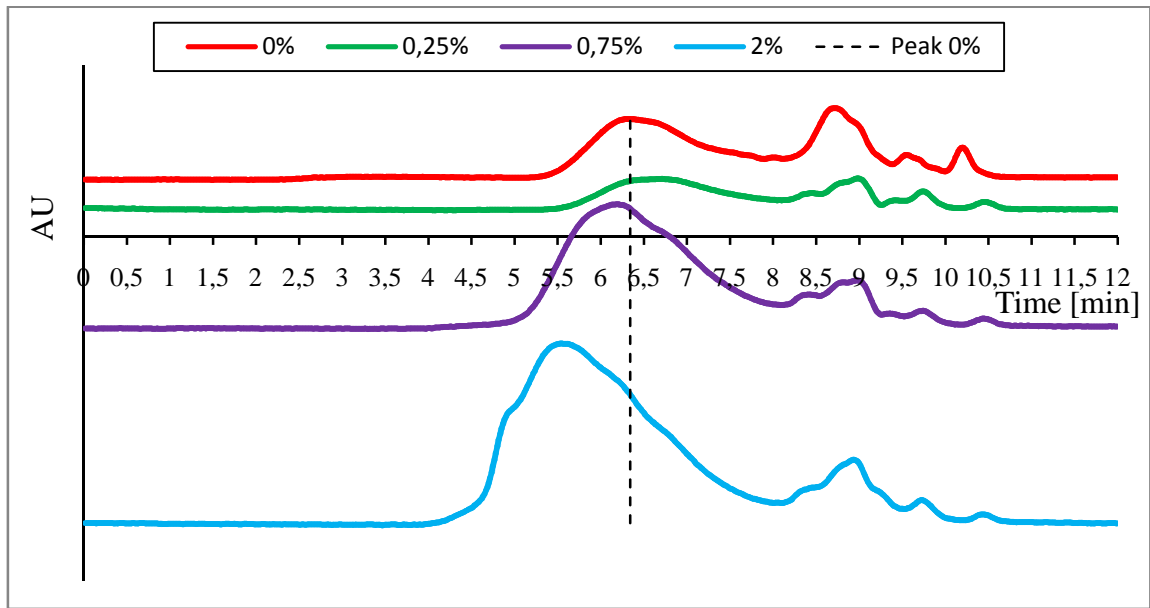

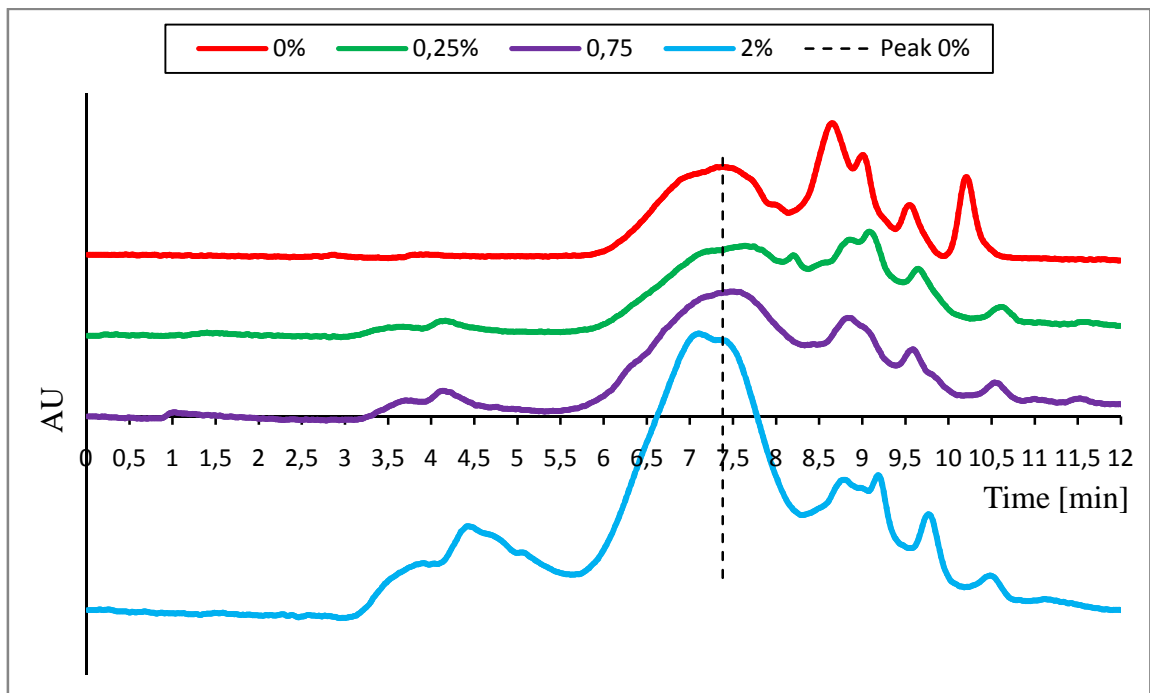**Figure 22** - *Chromatograms of selected PLAL (0-2 wt. % of L) samples catalyzed by Sn(Oct)₂.*

Figure 23 - *Chromatograms of selected PLAL (0-2 wt. % of L) samples catalyzed by MSA*

AN number of the samples shown in Table 5 reveals the decresing amount of carboxyl groups with increasing L content in case of $Sn(Oct)_2$ catalyst. Thi is in agreement with the assumtion of LA and L interaction. On the contrary, MSA calyzed samples have AN increased with maximum at 1 % of L. The reason may be in specific different activity of MSA catalyst. The possible MSA resudals may be present in the system.

The summary of DSC results is shown in Table 11. It can be noticed that L presence causes an occurrence of double melting peak almost for all samples catalyzed by $Sn(Oct)_2$. The exceptions for PLAL 0.75 and 2% were observed (one melting peak). Also it is evident that melting temperature decreases with rising content of L. While pure PLA (catalyzed by Sn(Oct)2) shows T_m=151.2 °C,   PLAL 2% has T_m only 125.1 °C. The absence of T_{m1} may be caused by structural irregularity or thermal history of the individual sample. Glass transition temperature decreases up to 0.75 wt. % of L. Further additions of L causes another increase of T_g. Finally, PLAL 2% has slightly higher T_g that pure PLA. Interestingly, PLAL 2% proves low T_m (125.1 °C). The explanation can be found in formation complicated branched structure at higher L content, which is in agreement with GPC observations. The threshold concentration seems to be 1 wt. % of L. MSA catalyzed samples (right side of Table 11) show decreasing T_m with increasing L content up to 2 wt. % where significant enhancement of T_m were observed. These samples follow the patter – the lower T_m the lower T_g. Providing we consider the assumption of complicated amorphous-like structure formation, threshold concentration is shifted to higher L concentration in case of MSA catalyzed reactions. The preliminary experiments (not presented here) show around 4 wt. %. Completely crosslinked product is formed at such a high L concentration.

Table 11 - *Effect of L on thermal properties of PLA based polycondensates. Results from DSC – T_m were taken from the first heating scan, T_g - were taken from second heating scan.*

Sample designation	Catalyst – $Sn(Oct)_2$			Catalyst - MSA		
	T_{m1} (°C)	T_{m2} (°C)	T_g (°C)	T_{m1} (°C)	T_{m2} (°C)	T_g (°C)
PLA	151.2	-	53.8	142.6	-	34.1
PLAL 0.25%	143.8	132.6	48.8	135.0	-	29.1
PLAL 0.5%	140.2	130.3	45.7	135.7	-	32.9
PLAL 0.75%	-	122.9	44.5	133.5	-	24.8
PLAL 1%	136.8	120.9	51.0	130.3	-	23.8
PLAL 1.5%	144.0	123.9	52.4	133.7	-	30.1
PLAL 2%	-	125.1	53.9	143.7	-	36.3

The FTIR-ATR spectrum of neat L is shown in Figure 24. L itself has relatively complicated FTIR spectrum with many absorption peaks in the wavenumber region $1300 - 800$ cm^{-1}. The most significant peak can be found at 1030 cm^{-1}, which corresponds to hydroxyl groups bending. Typical FTIR-ATR spectra of the selected samples (PLA, PLAL 2%) catalyzed by Sn(Oct)$_2$ and MSA are shown in Figure 25 and 26, respectively.
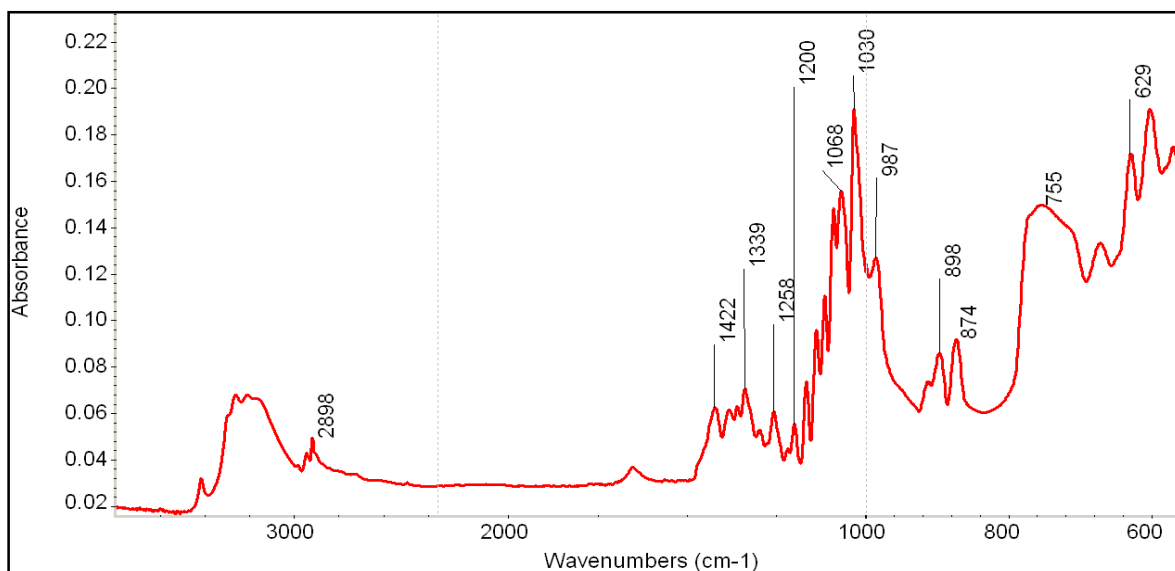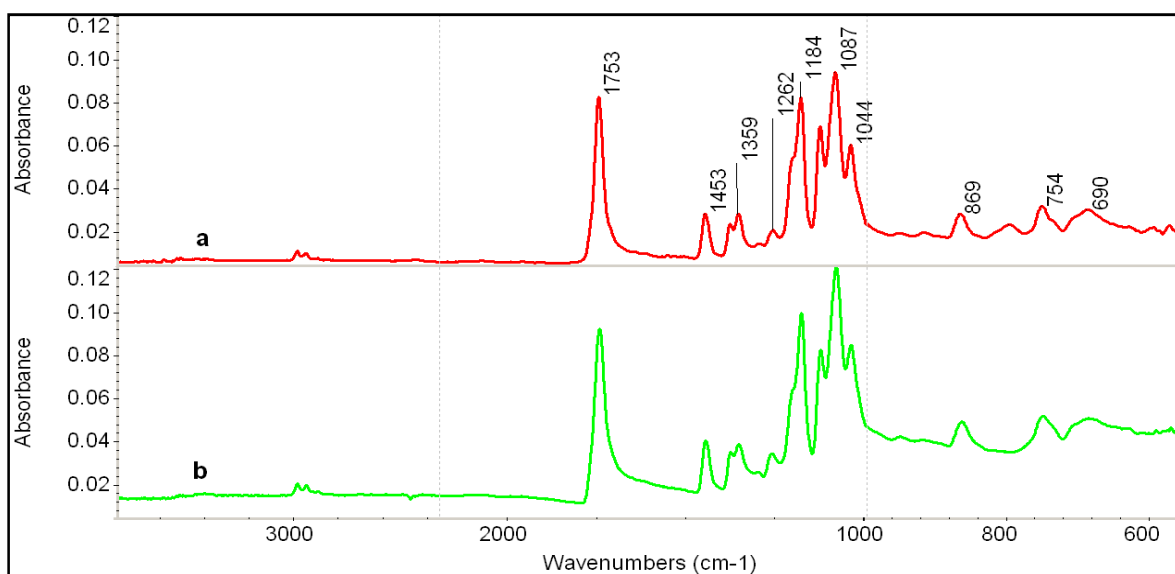

Figure 24 - *FTIR-ATR spectrum of neat lactose (L).*

Figure 25 - *FTIR-ATR spectra of PLA (**a**) and PLAL 2% (**b**) samples, catalyst Sn(Oct)$_2$.*

It can be noticed that position of the absorption peaks do not vary with presence of L and that the changes observed in case of GPS and DSC analysis are not significant here. The comparison of PLAL 2% prepared with various catalyst does not give a clear evidence of a new absorption peak as well (Figure 27). The reason of such finding comes from the chemical similarity of pure polymer (PLA) and PLA/lactose copolymer. PLA is polyester

and it is clear that absorption response of C=O, C-O-C and C-O groups will be the most significant in infrared region. L (as well as CA) absorbs strongly in at similar wavenumber. The only expected reaction, which can occur during polycondensation is interaction carbonyl vs. hydroxyl. It means that we cannot expect an appearance of new absorption peaks. On the other hand, semi-quantitative analysis of FTIR spectra is possible in this case.
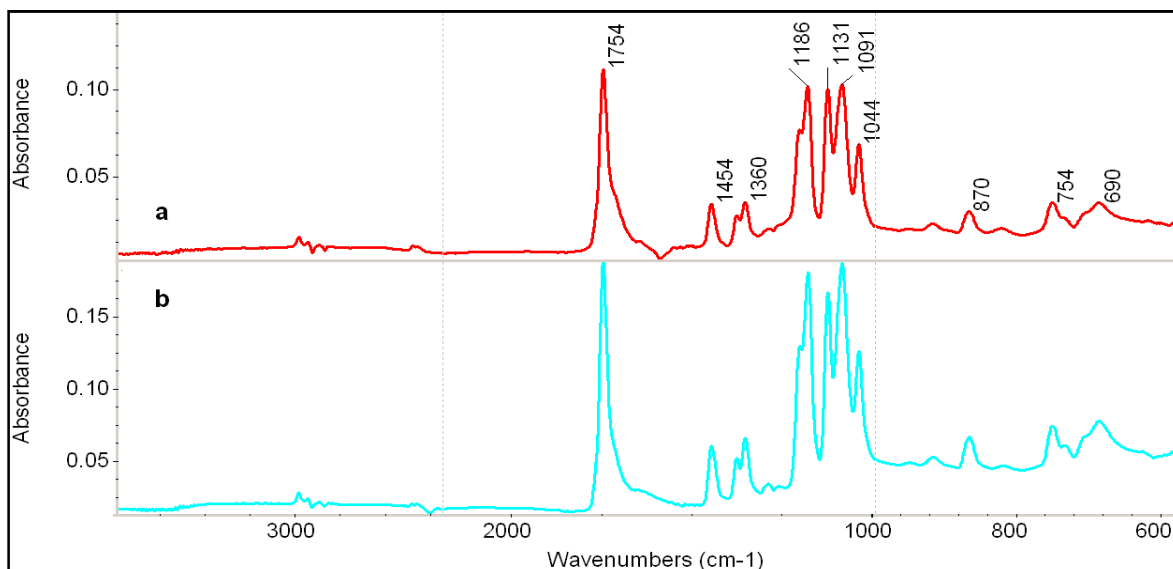
Figure 26 - *FTIR-ATR spectra of PLA (**a**) and PLAL 2% (**b**) samples, catalyst MSA.*
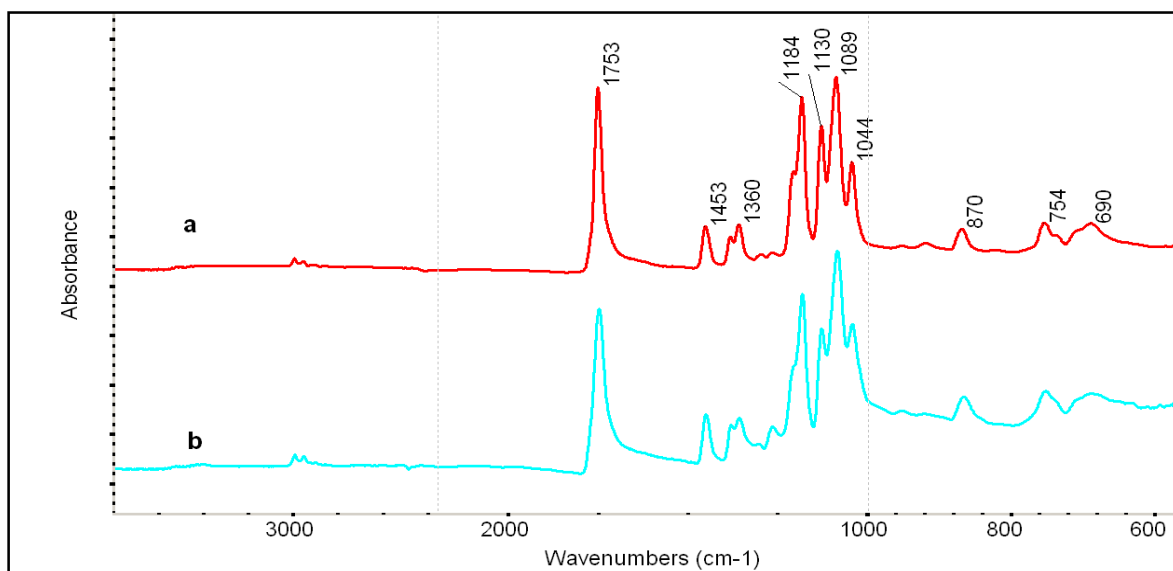
Figure 27 - *FTIR-ATR spectra of PLAL 2% samples, catalyst MSA (**a**) and Sn(Oct)$_2$ (**b**).*

The results of such analysis are presented in Figure 28. Its principles have been introduced in the previous subchapter. The dependences of normalized carbonyl absorption peak area on L concentration show slight reduction of C=O (carbonyl group connected to

carboxyl) groups in the structure of the polymer catalyzed by both $Sn(Oct)_2$ and MSA. The results in the later case are in disagreement with AN values (Table 10). It may mean that increased acidity detected by titration method do is not connected with the increase presence of carboxyl groups in the polymer structure.
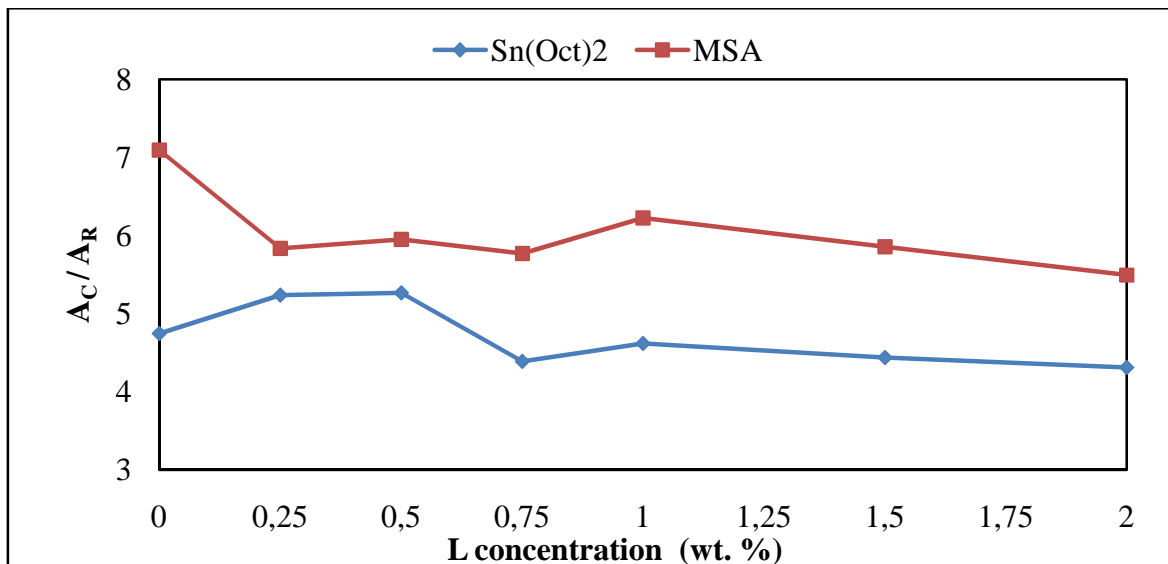
Figure 28 - *Carboxyl group presence analysis based on of FTIR-ATR spectra of PLAL samples*

5.4.3 Effect of protein concentrate (PROT) on lactic acid (LA) polycondensation

The third subchapter of results and discussion part is dedicated to investigation of the effect of protein concentrate (PROT on resulting properties of LA polycondensates. It should be mentioned that PROT originates from real biotechnological condition and it has been provided by dairy research institute within the co-operation on common project between Tomas Bata University in Zlin and Milcom a.s. Prague.

Table 12 - *Designation of the samples and characterization of the reaction mixture at the beginning of the polycondensation with protein concentrate.*

Sample designation	Protein concentration (wt. %)	Lactose concentration (wt. %)
PLA	0.00	0.00
PLAPROT 0.25%	0.25	0,06
PLAPROT 0.5%	0.50	0,12
PLAPROT 0.75%	0.75	0,18
PLAPROT 1%	1.00	0,23
PLAPROT 1.5%	1.50	0,35
PLAPROT 2.%	2.00	0,47

The basic characteristics of the samples prepared by melt polycondensation of LA and CA are shown in Table 13. In case of $Sn(Oct)_2$ and MSA catalysts, [η] decreases with increasing protein content. It corresponds to results from GPC analysis. The sample designated PLAPROT 2% can be still considered as polycondensate. In addition, presence of PROT seems to have almost negligible effect on molecular weight of the material due to insignificant M_w reduction from 12 to 10 kg.mol^{-1}.

Table 13: *Characteristics of PLAPROT polycondensates*

Catalyst – Sn(Oct)$_2$						
Sample designation	M_n* (kg.mol^{-1})	M_w* (kg.mol^{-1})	M_w/M_n*	[η]** (dL.g^{-1})	Yield (wt. %)	AN/SD*** (mg$_{KOH}$.g^{-1})
PLA	22.6	47.0	2.08	0.47	61.7	24.8/1.9
PLAPROT 0.25%	14.9	35.8	2.40	0.26	60,2	16.6/0.3
PLAPROT 0.5%	14.0	35.8	2.56	0.27	61.5	15.1/1.4
PLAPROT 0.75%	13.6	37.4	2.75	0.26	50.1	21.7/0.8
PLAPROT 1%	9.9	24.3	2.45	0.21	55.3	19.5/1.1
PLAPROT 1.5%	13.2	39.3	2.98	0.22	50.9	12.9/0.8
PLAPROT 2.%	7.8	23.4	3.00	0.18	61.9	18.6/1.0
Catalyst - MSA						
PLA	7.1	12.0	1.69	0.18	64.5	35.0/1.1
PLAPROT 0.25%	4.1	9.5	2.32	0.22	65.2	63.7/2.2
PLAPROT 0.5%	2.4	6.2	2.58	0.13	62.4	77.6/1.3
PLAPROT 0.75%	4.5	17.6	3.91	0.16	76.2	192.9/0.6
PLAPROT 1%	4.7	13.8	2.93	0.16	50.8	88.2/1.5
PLAPROT 1.5%	4.4	12.3	2.80	0.16	53.0	42.3/0.3
PLAPROT 2 %	4.5	10.0	2.22	0.11	53.6	58.5/0.2
* GPC measurement ** viscometry (30°C in CHCl$_3$) *** SD-standard deviation						

On the other hand, polydispersity of PLAPROT 2% increased form 1.69 to 2.22 in comparison to neat PLA. The molecular weight reduction about more than 50 % and increase of polydispersity degree from 2.08 (pure PLA) to 3.00 (PLAPROT 2%) was observed in case of $Sn(Oct)_2$ catalyzed polycondensates (Table 13). These facts reveal that the presence of proteinic components do not disturb the polycondensation reaction as significantly as CA. The reason could be grounded in firstly lactose presence and secondly proteins denature at temperatures higher that 40 °C. The polycondensation was carried out at 160 °C. It means that all proteinic ingredients had denatured even before polycondensa-

tion reaction started. The question is how the low molecular products of protein denaturation influences (if even) the polycondensation reaction.

AN versus PROT concentration has similar trend like in case of PLAL samples. The polycondensates catalyzed by $Sn(Oct)_2$ prove, more or less decreasing AN value with increasing content of PROT. On the other hand, irregular course can be noticed for MSA series (Table 13). The explanation may be similar like for PLAL and will be discussed further.

Table 14: *Effect of PROT on thermal properties of PLA based polycondensates. Results from DSC – T_m was taken from the first heating scan, T_g - was taken from second heating scan*

Sample designation	Catalyst – $Sn(Oct)_2$			Catalyst - MSA		
	T_{m1} (°C)	T_{m2} (°C)	T_g (°C)	T_{m1} (°C)	T_{m2} (°C)	T_g (°C)
PLA	151.2	-	53.8	145.9	-	44.0
PLAPROT 0.25%	150.5	-	49.4	137.1	-	29.9
PLAPROT 0.5%	129.1	-	49.2	135.2	-	26.7
PLAPROT 0.75%	118.3	-	46.7	133.6	-	25.4
PLAPROT 1%	135.5	-	49.6	144.5	-	42.5
PLAPROT 1.5%	110.1	-	50.1	139.5	-	41.0
PLAPROT 2 %	128.4	-	49.2	140.2	-	42.8

The summary of DSC results obtained from the first and second heating scan is shown in Table 14. The results show that $Sn(Oct)_2$ catalyzed samples are characteristic by significant reduction of T_m unlike MSA series where the changes in T_m are not as high as in the first case. On the contrary, T_g values changes are less significant for $Sn(Oct)_2$ samples. It could be assumed that presence of denatured proteinic component and lactose effect the resulting properties of the polycondensates in certain synergy. In addition, principles of catalytic action increase the complexity of the whole system. These parameters influence the resulting properties. The experimental DSC data show that crystalline phase is affected in case of $Sn(Oct)_2$ catalyzed polycondensation while the changes in amorphous phase take place during MSA catalyzed reactions between LA and present impurities.
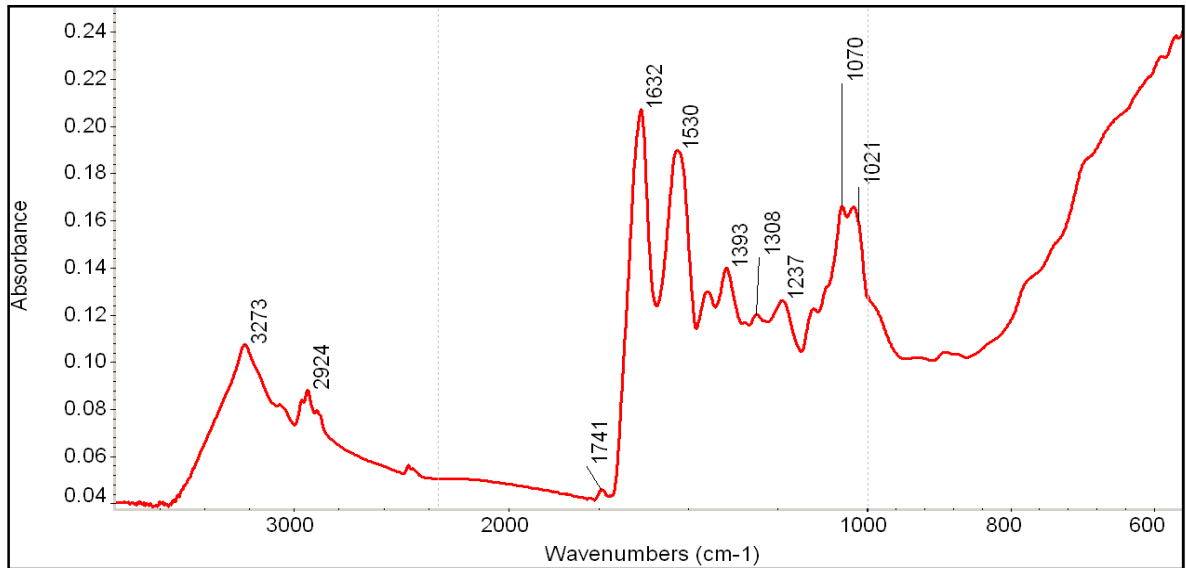
Figure 29 - *FTIR-ATR spectrum of neat protein concentrate (PROT)*

The FTIR-ATR spectrum of neat L is shown in Figure 29. PROT is characterized by several absorption peaks at 3273 cm-1 (NH stretching), 3073 cm^{-1} (OH groups – moisture presence), 2924 cm^{-1} (CH stretching), 1741 cm^{-1} (C=O stretching), 1632 and 1530 cm^{-1} (NH deformation), 1445 cm^{-1} (-CH$_2$- scissoring), 1393 cm^{-1} (CH rocking, possibly OH deformation), 1308 and 1237 cm^{-1} (C-O stretching, NH deformation), 1070 and 1021 cm^{-1} (C-O stretching). Typical FTIR-ATR spectra of the selected samples (PLA, PLAL 20%) catalyzed by Sn(Oct)$_2$ and MSA are shown in Figure 30 and 31, respectively.
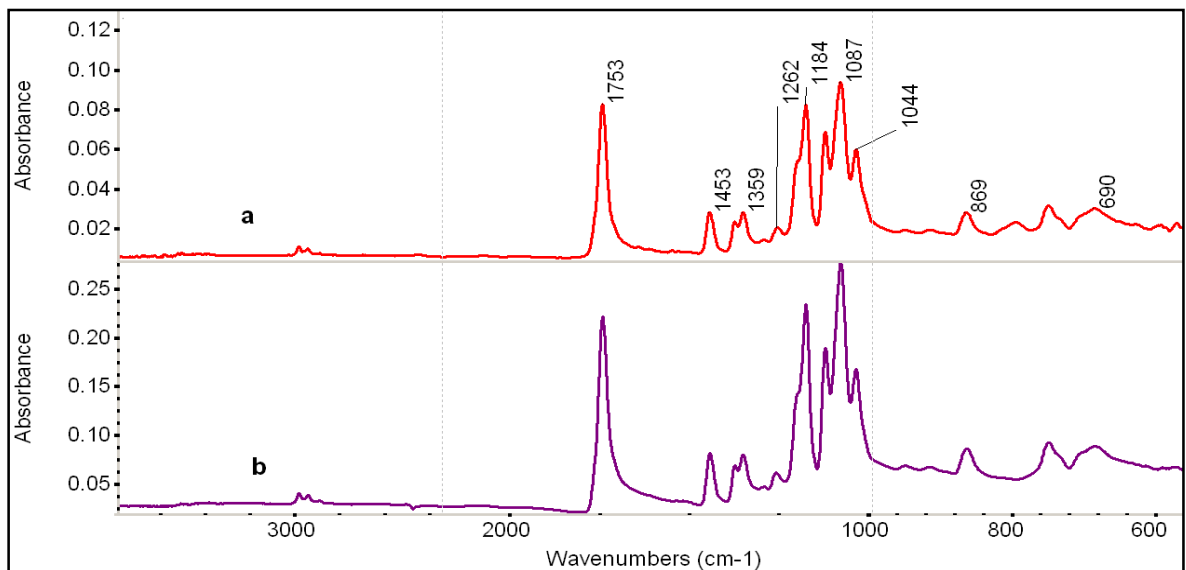
Figure 30 - *FTIR-ATR spectra of PLA (**a**) and PLAPROT 2% (**b**) samples, catalyst Sn(Oct)$_2$.*

The comparison of PLAPROT 2% sample catalyzed by both catalysts is shown in Figure 32. The spectra confirm the assumption that proteins are not involved into polycondensation reactions. The analysis of carbonyl groups presence shows, more or less, constant trend with increasing PROT content (Figure 33). It could mean that PROT impurity is not involved into polycondensation reaction by means of direct chemical reaction with LA within the investigated range of PROT concentrations.
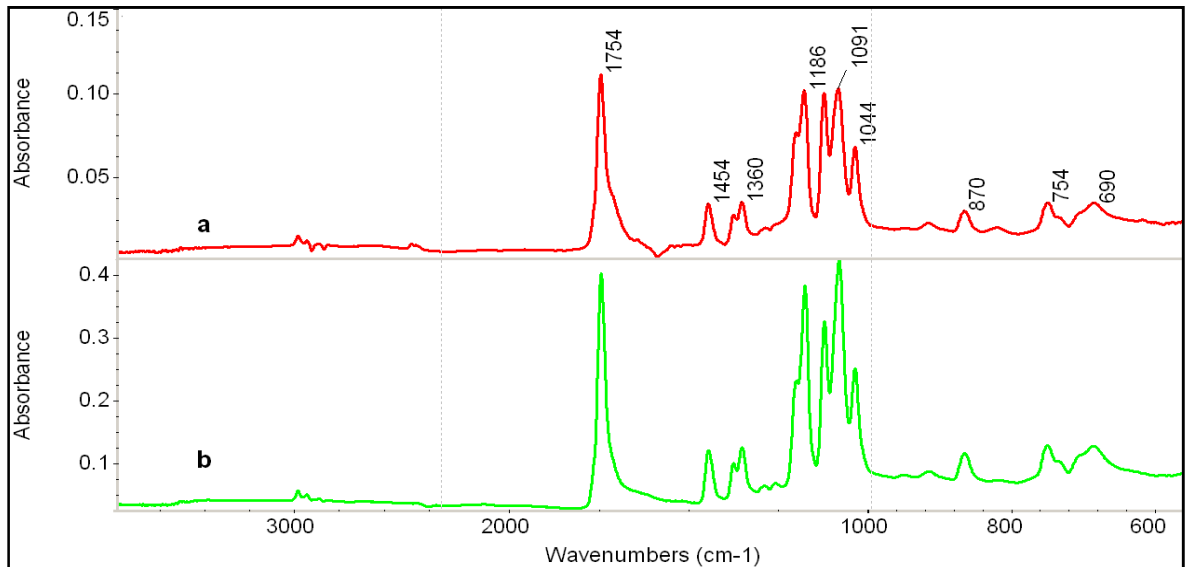
Figure 31 - *FTIR-ATR spectra of PLA (a) and PLAPROT 2% (b) samples, catalyst* MSA.
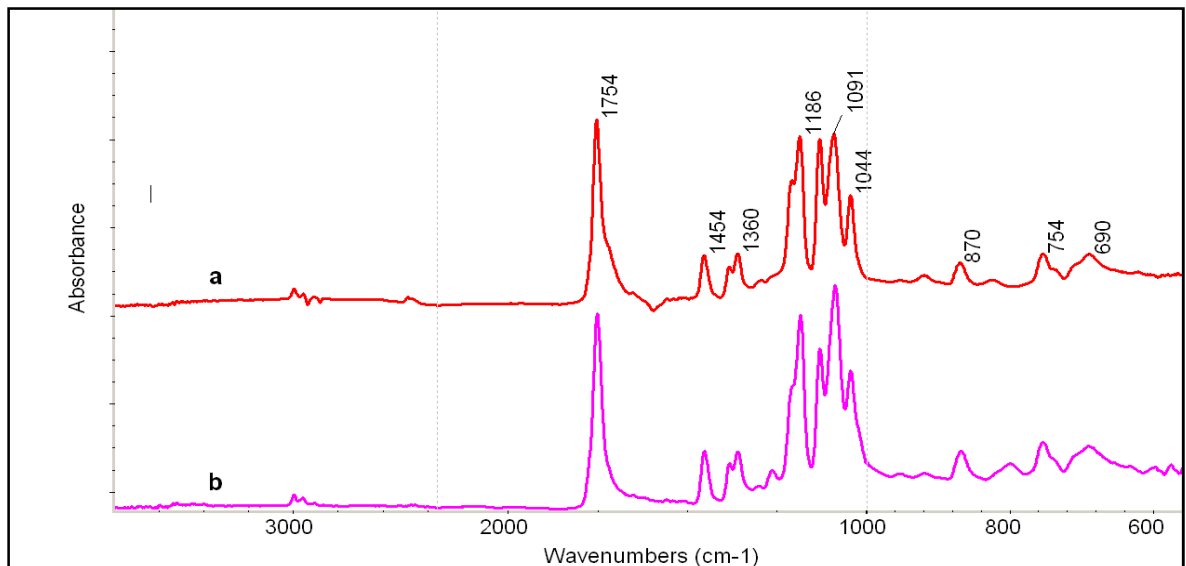
Figure 32 - *FTIR-ATR spectra of PLAPROT 2% samples, catalyst MSA (a); Sn(Oct)$_2$ (b).*
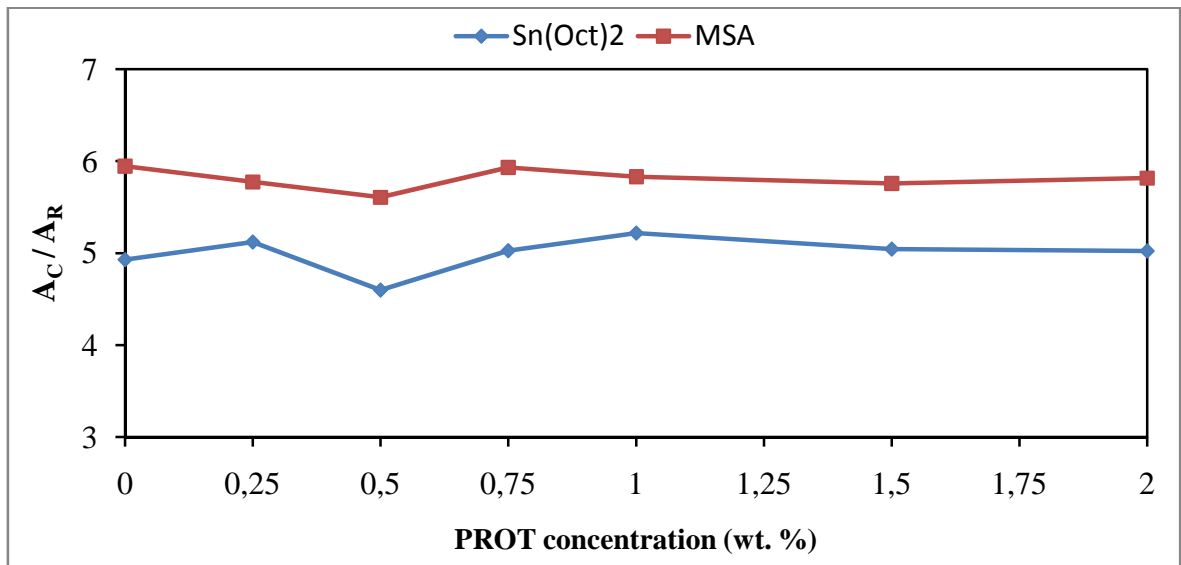
Figure 33 - *Carboxyl group presence analysis based on of FTIR-ATR spectra of PLAPROT samples.*

CONCLUSION

In recent years, biodegradable polymers have been investigated extensively for their potential applications in medical and pharmaceutical fields or packaging. Polylactid acid (PLA) is one of the most promising materials due to its availability from renewable resources. A simplified process of saccharides conversion through fermentation into lactic acid and its further polymerization is often presented. However, both literature survey and practical experience show that biotechnological preparation of lactic acid with sufficient purity to be polymerized is extremely expensive due to presence of various impurities originating from fermentation step. These impurities inhibit polymerization ability of lactic acid. In practice it means that most known method of PLA preparation (ring opening polymerization) hardly applicable as well as other methods, highly sensitive to impurities (e.g. based on azeotropic distillation).

As already mention in the introduction part, this diploma thesis follows the project carried out at Polymer Centre Faculty of Technology, Tomas Bata University in Zlin, which has been dealing with production of PLA from dairy industry by-product, whey. It has been found the major impurities, which can be present in lactic acid derived from whey fermentation (lactose present in whey) can be divided into three groups. Firstly, by-products of saccharide fermentation; i.e. other simple organic acids. The experimental study presented in reference [8] determines citric acid as the one of the most important by-product. The second group is represented by residual saccharide, lactose. Finally, proteinic residua can occur in the lactic acid as well.

This diploma work is dedicated to investigation of the presented paper deals with investigation of selected impurities (citric acid, lactose and whey protein concentrate) effect on resulting PLA properties. The range of impurities concentration (ratio lactic acid/impurity) was determined on the basis of experimental results [8]. The method of direct melt LA polycondensation was chosen for PLA samples preparation due to its lower sensitivity to impurities presence. The reaction was catalyzed by either organometallic tin octoate ($Sn(Oct)_2$) or methanesulphonic acid (MSA) and the factor of catalyst selection was studied beside molecular weight (viscometric measurements, gel permeation chromatography), thermal properties (differential scanning calorimetry) and structural analysis (Fourier transform infrared analysis and determination of end groups by titration method).

Polycondensation of lactic acid in presence of tricarboxylic citric acid (0-20 wt. % related to lactic acid) leads to significant reduction of molecular weight of the reaction products catalyzed by $Sn(Oct)_2$. On the other hand, co-catalytic action of citric acid was observed at low concentrations (1 wt. %) in case of MSA catalyst.

Presence of lactose (disaccharide that consists of galactose and glucose) in concentration range 0-2 wt. % have, generally, opposite effect on the properties of polycondensates. The results show significant enhancement of molecular weight probably due to formation of highly branched or even 3D structure due to condensation reactions between lactic acid and lactose. A threshold concentration of lactose is necessary to be present in the system to observe this phenomenon. The experimental data reveal that the threshold lactose concentration is lower in presence of $Sn(Oct)_2$ catalyst (1 wt.%).

Finally, the influence of proteinic impurities was tested in concentration range 0-2 wt. % (considering protein, related to lactic acid). Generally, slight reduction of molecular mass was observed in case of both catalysts. An effect on polycondensates structure (such as branching or crosslinking) was not found to be involved here. Thus, it can be supposed that inhibition of condensation reactions take place in this case.

EXISTING AND EXPECTED OUTPUTS

Presented work represents part of the research project assignment dealing with utilization of dairy by-product – whey for production of biodegradable polymers. The existing outcomes directly connected to this work are following:

a) Paper in impacted journal

- Sedlarik V., **Kucharczyk P**, Kasparkova V., Drbohlav J., Salakova A., Saha P. Optimization of the Reaction Conditions and Characterization of L-Lactic Acid Direct Polycondensation Products Catalyzed by a Non-Metal-Based Compound. *Journal of Applied Polymer Science,* 2010, 116, 3, p. 1597-1602.

b) Conference presentations

- Sedlarik V., **Kucharczyk P**., Kasparkova V., Drabohlav J. Preparation and characterization of polylactic acid based polyesters by method of direct polycondensation 22nd International symposium on polymer analysis and characterization, ISPAC 2009, June 22-24, 2009, Zlin, Czech Republic

- Sedlarik V., **Kucharczyk P**., Kasparkova V., Drabohlav J., Saha, P. Direct polylactic acid synthesis by using non-metal catalyst. 28th Polymer Degradation Discussion Group Meeting, September 6-10, 2009, Sestri Levante, Italy

- **Kucharczyk P.**, Sedlarik V., Kasparkova V., Poljansek I., Salakova A., Drbohlav J., Junkar I., Saha P. Lactic acid polycondensation in presence of citric acid: Effect of concentration and catalysts on resulting products. Plastko 2010, April 13-14, 2010, Zlín, Czech Republic ISBN 978-80-7318-909-9

On the basis of obtained experimental data, 2-3 manuscripts are expected to be submitted for publication in impacted journals in 2010-2011.

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LIST OF ABREVIATIONS

PLA   Polylactic acid

LA   Lactic acid

ED   Electrodialysis

GRAS   Generally regarded as safe

FDA   Food and drug administration

PET   Polyetylen terephtalate

T_m Melting temperature

T_g Glass transitiv temperature

LDPE   Low - density polyetylen

PHAs   Polyhydroxy alkanoates

 PCL   Polycaprolactone

GPPS   General purpose polystyrene

SSP   Solid state polycondensation

ROP   Ring mening polymerization

SP   Solution polycondensation

Kc   Equilibrium constant

DPn   The number average degree of polymerization

p   Degree of conversion

M_w Weight average molecular weight

M_n Number average molecular weight

M_w/M_n polydisperzity index

$Sn(Oct)_2$ Stannous octoate

CA   Citric acid

L   Lactose

$[\eta]$ Intrinsic viscosity

PROT   Protein concentrate

MSA   Methanesulfonic acid

DSC   Diferential scanning calorimetry

FTIR-ATR   Fourier transform infrared spectroscopy - attenuated total reflectance

AN   Acidity number

PLACA   Sample with defined composition of lactic and citric acid

PLAL   Sample with defined composition of lactic and lactose

PLAPROT   Sample with defined composition of lactic and protein concentrate

Ac   FTIR-ATR peak area corresponding to carboxyl groups

Ar   FTIR-ATR reference peak

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