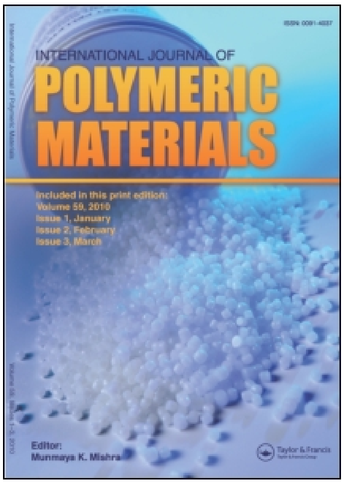

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Microwave-Assisted *In Situ* Synthesis of Poly L-Lactic Acid with Nanoparticles of Calcium Phosphate

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A microwave reactor was used to polymerize lactic acid and/or calcium lactate in the presence of phosphoric acid or ammonium hydrogen phosphate. The polar nature of lactic acid makes it a strong absorber of microwave radiation, and when present in a nonpolar solvent such as chloroform, it is possible to conduct the polymerization reaction in a microwave reactor. Using calcium lactate and ammonium hydrogen phosphate it is possible to create nanoparticles of calcium phosphate within the matrix of poly lactic acid *in situ* during the polymerization. FTIR spectroscopy and gel permeation chromatography were used to follow the polymerization process, and transmission electron microscopy was used to determine the size distribution of the calcium phosphate particles.

Keywords biodegradable polymers, calcium phosphate, microwave synthesis, nanoparticles, poly lactic acid

INTRODUCTION

Poly (L-lactic acid) (PLLA), poly(glycolic acid), poly(caprolactone), and their copolymers have attracted wide attention for their biodegradability in the human body. However, the mechanical strength, toughness, and elastic modulus of these polymers are lower than those of natural cortical bones. Thus, for preparing a desired material that presents high mechanical performance to

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match natural bones, inorganic fillers can be introduced into biodegradable polymers to fabricate ceramic/polymer composites, such as hydroxyapatite (HA), β tricalcium phosphate, or bio-ceramics [1–7].

Polymer/ceramic composite scaffolds mimic natural bone to some extent. Natural bone is composed of inorganic compounds (mainly partially carbonated HA on the nanometer scale) and organic compounds (mainly collagen). The nanometer size of the inorganic component (mainly bone-like apatite) in natural bone is considered to be important for the mechanical properties of the bone [8]. Some studies have suggested that better osteoconductivity would be achieved if synthetic hydroxyapatite could resemble bone minerals more in composition, size and morphology [9,10]. In addition, nano-sized hydroxyapatite may have other special properties due to its small size and huge specific surface area. Webster et al. [11,12] have shown significant increase in protein adsorption and osteoblast adhesion on the nano-sized ceramic materials compared to traditional micron-sized ceramic materials.

A commonly encountered problem with almost all polymer ceramic composites is poor adhesion, and in a PLLA-hydroxyapatite composite synthesized by blending, only physical adsorption is achieved between hydroxyapatite particles and a PLLA matrix. After transplant to a human body the interfacial layer between the filler and the polymer matrix is destroyed first, thus hydroxyapatite particles may easily disengage from the organic matrix, resulting in a sharp decrease of mechanical properties in a short time. Consequently, improvement of the interfacial adhesion between the hydroxyapatite particles and the PLLA matrix has become the key technique in preparing hydroxyapatite-PLLA composites.

One would certainly conclude from the literature surveyed [1–12], that a solution of the existing problem would be to synthesize a composite containing hydroxyapatite reinforcement uniformly distributed in a PLLA matrix, which is chemically attached to the polymer matrix.

Microwave-assisted synthesis of nanoparticles of hydroxyapatite from different calcium and phosphate groups containing compounds under microwave irradiation (13–22) has been reported. In an earlier study we have shown that microwaves can be used to synthesize PLLA from L-lactic acid precursors [23]. In addition microwaves can be used to create blends of PLLA and PGA [24] as well as block copolymers [25,26]. If the two processes can be carried out simultaneously in such a way that the starting material for PLLA synthesis and the source of calcium is the same or a mixture of reactants, then by adding a phosphate group-containing compound, a calcium phosphate type material (like hydroxyapatite, or tricalcium phosphate) can be synthesized in situ while polymerizing PLLA.

Presented is a proof of concept work that shows under microwave irradiation *in situ s*ynthesis of calcium phosphate reinforcement particles while polymerizing L-lactic acid to poly (L-lactic acid). This is an exploratory work and presented are the initial results. The process requires optimization, but the results obtained show *in situ* synthesis of nano scaled calcium phosphate particles during polymerization of poly (L-lactic acid).

EXPERIMENTAL PROCEDURE

Materials

85% L-lactic acid solution and calcium lactate pentahydrate (calcium-L-2-hydroxy-propionate) containing 6.8% calcium was obtained from Purac. The catalyst for polymerization, stannous octoate (Sn 2-ethylhexanoate), 98% phosphoric acid, ammonium hydrogen phosphate and chloroform, were purchased from Sigma Aldrich.

Measurements

Molecular weight (Mw) of the polymer was determined by gel permeation chromatography (GPC) on a Varian high-pressure liquid chromatographic system, a model 2410 refractive index detector, and a Jordi DVB mixed bed column. Chloroform was used as an elutent at a flow rate of 1.0 mL/min. GPC data was calibrated by standard polystyrene. FTIR measurements were carried out on a Bruker Vector 22 under diffuse reflectance mode. For all measurements the sample was ground with KBr and made into a pellet for measurements in diffuse reflectance mode, 256 scans and $2\,\mathrm{cm}^{-1}$ resolutions. Transmission electron microscopy studies were carried out to study the size and morphology of the calcium phosphate particles synthesized.

Apparatus

The apparatus used for polymerization was a Panasonic domestic microwave oven (2450 MHz, 1300 W) with different power levels from ~130 W to 1300 W. The oven was modified so that Teflon tubing (10 mm diameter) could be introduced into the cavity of the oven without sacrificing the safety of the oven due to radiation leakage. All reactions were carried out in a hood with strong ventilation.

Procedure

Since calcium lactate was in a solid powdered form, it was difficult to process it as-is in the microwave, so it was dissolved and diluted with L-lactic acid. 10 g of L-lactic acid and 4 g of calcium lactate were carefully measured and added to a filtering flask. In order to keep Ca/P ratio 1.66, for 4 g of

Table 1: Heating duration and power level used for polymerization of L-lactic acid, calcium lactate and H_3PO_4 or ammonium hydrogen phosphate

Stage	Approx. power level	Heating intervals (before sample was cooled)	Total time
Dehydration	P1 $\sim$ 130 W	5 × 1 minute	5 minutes
Low power polymerization	P1 $\sim$ 130 W	11 × 5 minutes	55 minutes
High power polymerization	P3 $\sim$ 400 W	5 × 2 minutes	10 minutes
Low power chain extension	P1 $\sim$ 130 W	2 × 30 minutes	60 minutes

calcium lactate, 43.64 mg of 98% H_3PO_4 or 0.69 g of ammonium hydrogen phosphate was added. The flask was placed at the center of a domestic microwave oven. The flask was sealed from the top using a stopper made of polytetrafluorethylene. Polytetrafluoroethylene tubing connected to a roughing pump was attached to the neck of the filtering flask. A slight negative pressure of about 400 Torr was generated inside the reaction flask that aided the removal of volatiles during the reaction process.

During the reaction procedure the weight of the sample was continuously monitored. Monitoring the weight can be very useful at predicting the reaction progress, as we shall see in the Results and Discussion section. Samples were heated in the microwave for a predetermined time, and after the reaction was completed, the reaction products were stored in an airtight container flushed with N_2 and then placed in a freezer at 0°C for further analysis. Samples were polymerized with the aid of a catalyst, after the initial dehydration process (30 min), the catalyst was added to the monomer keeping 1000:1 molar ratio between the monomer and catalyst.

Since the reactions were carried out in a domestic microwave, the temperature inside the polymerization flask could not be controlled. In order to avoid this overheating that may result in an explosion of the flask, the samples were intermittently removed from the microwave cavity and cooled to room temperature before the sample was placed back. Overheating of the reaction chamber can also result in burning of the reaction product or explosions inside the microwave cavity.

Presented in Table 1 is the polymerization procedure. Microwave heating can be divided into 4 stages: (i) dehydration, (ii) low power polymerization, (iii) high power polymerization, and (iv) low power chain extension.

RESULTS AND DISCUSSION

Two sets of experiments were carried out, one using ammonium hydrogen phosphate and one using phosphoric acid as the source of phosphorous. Gravimetric analysis showed that both the samples followed a similar weight loss

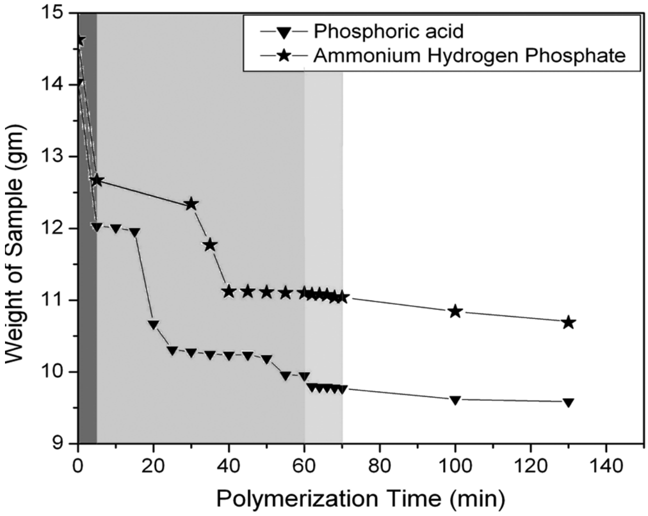

Figure 1: Change in weight of sample with time. Each shaded region correspond to different exposure energy state detailed in Table 1.

profile during the entire heating period. Figure 1 shows the weight loss for the two samples studied. The top curve in Figure 1 is when ammonium hydrogen phosphate was used and the bottom one is when phosphoric acid was used. It is clear from the figure that although the weight loss profiles for the two cases are similar, higher total weight loss was observed when phosphoric acid was used. During the polymerization process it was observed that when phosphoric acid was used, the reaction vessel was getting heated more than when ammonium hydrogen phosphate was used, resulting in higher loss due to evaporation.

FTIR Studies

A small amount of sample was taken out at different stages of polymerization to observe changes in the chemical structure of the sample as the polymerization process continues. Figures 2 and 3 show the FTIR spectrum of a sample taken out at different stages during polymerization when the source of phosphorous is H_3PO_4. Based on Table 2, we see that that the peaks corresponding to polymer formation can be observed in the C-O-C region in the form of doublet $1115\,cm^{-1}$ and $1152\,cm^{-1}$ as well as the shift in the C=O peak from $1769\,cm^{-1}$ to $1789\,cm^{-1}$ as the microwave heating time is increased. Another important observation is the appearance of doublet at $2996\,cm^{-1}$ and $2946\,cm^{-1}$ due to CH twisting vibrations. This peak is not very clear and well-resolved compared to the baseline. The reason could be the presence of strong acid (phosphoric acid), which may be limiting the chain growth.

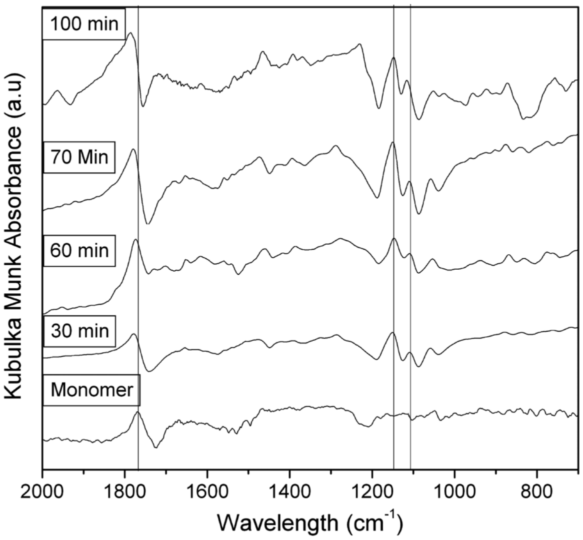

Figure 2: FTIR spectrum of L-lactic acid + calcium lactate + H_3PO_4 polymerization under microwave heating. Spectra from 700 cm^{-1} to 2000 cm^{-1}.

Figures 4 and 5 show the FTIR spectrum of lactic acid polymerization when ammonium hydrogen phosphate is used as the source of phosphorous. A similar observation can be made for this polymerization process as well. The only difference is in the peaks corresponding to the CH twisting vibration around 2994 cm^{-1} and 2942 cm^{-1}. These peaks as mentioned earlier arise due to long chain hydrocarbon, and are much clearer than when phosphoric acid is used.

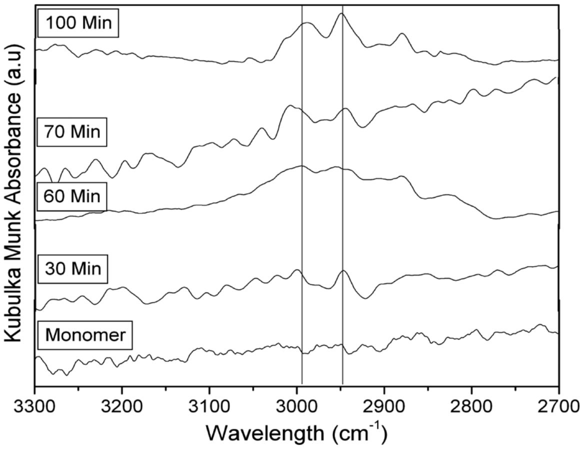

Figure 3: FTIR spectrum of L-lactic acid + calcium lactate + H_3PO_4 polymerization under microwave heating. Spectra from 2700 cm^{-1} to 3300 cm^{-1}.

Table 2: Infrared frequencies characterizing the polyesterification of L-lactic acid

Monomer	Oligomer	Polymer	Assignment
–	2996 cm^{-1} s	2996 cm^{-1} s	γ-C–H
–	2946 cm^{-1} m	2946 cm^{-1} s	γ-C–H
1465 cm^{-1} vw	1465 cm^{-1} s	1467 cm^{-1} s	δ-C–H
–	1778 cm^{-1} s	1791 cm^{-1} s	ν E(C=O)
1767 s	–	–	ν Ac(C=O)
–	1115 cm^{-1} m	1115 cm^{-1} s	ν E(O–C–O)
–	1152 cm^{-1} m	1152 cm^{-1} s	ν E(O–C–O)
1064 cm^{-1} m	1064 cm^{-1} m	–	ν Ac(C–O)
–	1057 cm^{-1} m-s	1057 cm^{-1} s	ν E(C–O)
–	1024 cm^{-1} m-s	1024 cm^{-1} s	ν E(C–O)

*AC = acid group; AL = alcohol group; E = ester group; s = Strong; m = Medium; vw = Very weak.

Formation of calcium phosphate cannot be observed from the FTIR spectrum, since the amount of calcium present in calcium lactate was very low (6.8%), in addition, the calcium lactate was further diluted by 10 ml of lactic acid, bringing down the concentration of calcium, and hence its phosphate, even lower. Phosphate group peaks can be observed in the 1100–1200 cm^{-1} region and in our sample this peak overlaps with the O-C-O bond, and hence it is difficult to detect the formation of any calcium phosphates using FTIR.

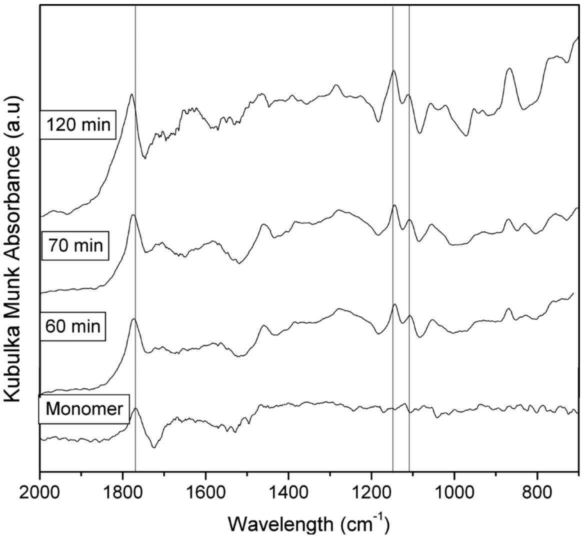

Figure 4: FTIR spectrum of L-lactic acid + calcium lactate + ammonium hydrogen phosphate polymerization under microwave heating. Spectra from 700 cm^{-1} to 2000 cm^{-1}.

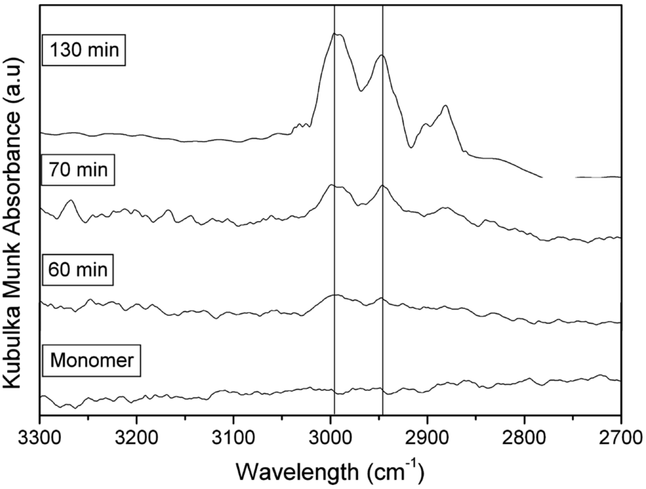

Figure 5: FTIR spectrum of L-lactic acid + calcium lactate + ammonium hydrogen phosphate polymerization under microwave heating. Spectra from 2700 cm^{-1} to 3300 cm^{-1}.

Gel Permeation Chromatography

A 5 mg/ml solution of final polymer was prepared in chloroform and was filtered using a 0.02 μ polypropylene filter and was injected into the chromatography column. Flow rate was maintained at 1 ml/min. Figures 6 and 7 are the GPC trace of the final sample, the molecular weight calculated using a universal calibration curve was 11.825 kD when H_3PO_4 was used, and 19.04 kD when ammonium hydrogen phosphate was used. As seen in the FTIR

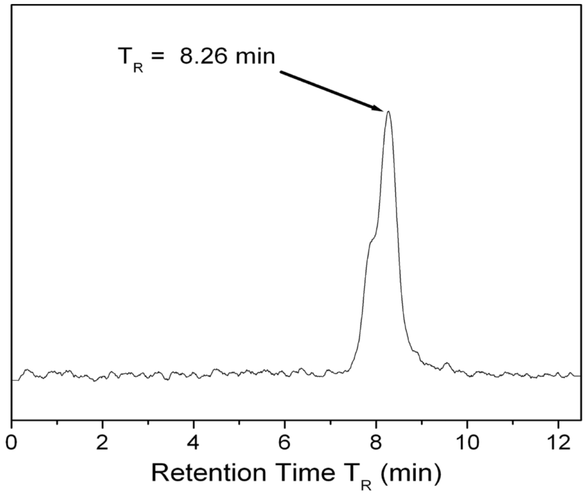

Figure 6: GPC trace of L-lactic acid + calcium lactate polymerization using H_3PO_4 using conditions detailed in Table 1.

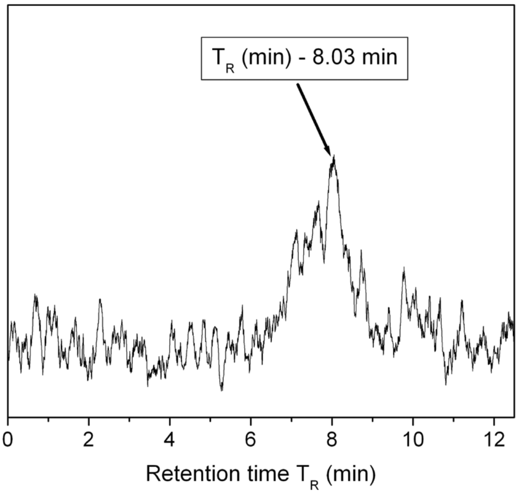

Figure 7: GPC trace of L-lactic acid + calcium lactate + ammonium hydrogen phosphate polymerization using conditions detailed in Table 1.

spectrum, the molecular weight is lower when H_3PO_4 is used mainly due to the use of concentrated acid solution that if left un-reacted even in minute quantity can cause acidic hydrolysis of the polymer resulting in a low molecular weight polymer. On the other hand with ammonium hydrogen phosphate there is no degradation of the polymer.

Transmission Electron Microscopy of Particles when Ammonium Hydrogen Phosphate is Used

When the final polymer was dissolved in chloroform, after dissolution the solution was not clear, and had some particles suspended in the solution. A pipette collected these particles, and a few drops of the solution were placed on a polymer film and placed on a transmission electron microscope grid and the particles were observed. Figure 8 is the dark field TEM image of the particles showing an even distribution of nanosized calcium phosphate particles that were originally embedded *in situ* within the PLLA matrix.

Proposed Scheme of Chemical Reaction in Microwave

Figure 9 provides diagrammatically the proposed reaction that occurs with and without the presence of a phosphate source. In the absence of a phosphate source the first step of the process involves the dehydration of the lactic acid, yielding oligomeric lactide chains which on thermal cracking yields lactide rings, which on ring opening polymerization yields poly lactic acid. On the other hand when calcium lactate is used as the starting

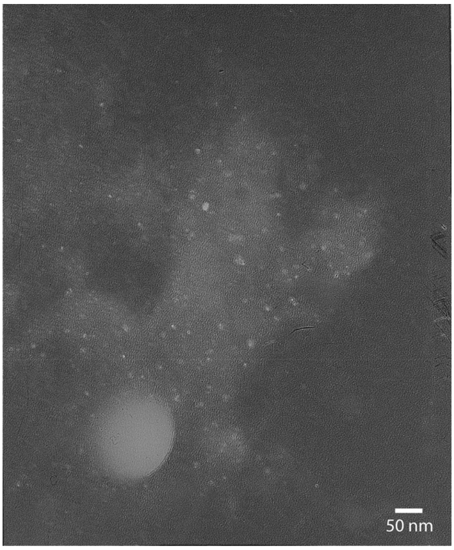

Figure 8: Dark field transmission electron image of undissolved calcium phosphate particles.

material, the dehydration of the lactate coupled with reaction with ammonium hydrogen phosphate results in the latter being hydrolyzed to yield oligomeric chains of lactide, as well as calcium phosphate and residual ammonium, which volatilizes.

$$2(CH_3CHOHCOO^-)\ Ca^{2+} + (NH_4)_2HPO_4 \rightarrow 2\ CH_3CHOHCOOH + 2Ca\text{-}H\text{-}PO_4 + 2NH_3$$

Calcium lactate +Ammonium Hydrogen Phosphate   Lactic acid+Calcium Phosphate+Ammonia

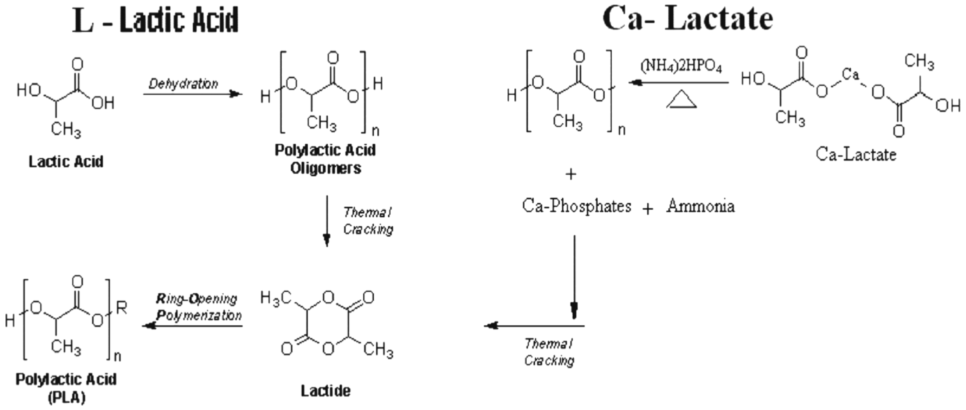

Figure 9: Proposed mechanism of *in situ* synthesis of calcium phosphates and polymerization of L-lactic acid using microwaves.

The calcium phosphate is precipitated out, and in the final stage of the process ring opening, polymerization of the lactide results in formation of PLLA with embedded calcium phosphate particles. Figure 9 details all the proposed steps in the reaction that occurs in the microwave reaction.

CONCLUSION

FTIR, NMR spectra and GPC elution curves clearly showed that calcium lactate can be polymerized to form a relatively high molecular weight polymer with Mw as high as 19 kD within 2 h of microwave heating. TEM results show the formation of solid particles rich in Ca and P that are insoluble is chloroform. These particles are approx 10–20 nm in size. A process can thus be developed where lactic acid can be polymerized along with the formation of calcium phosphate type particles, and since these particles are synthesized *in situ*, the adhesion of the particles to the polymer matrix will be much better compared to physical blending. A reaction mechanism is proposed for the synthesis of both poly lactic acid and calcium phosphate simultaneously using microwave radiation.

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