



Synthesis and Charactersation of Biodegradable Polyesteramide Based on poly (ϵ -caprolactone) and Study of Degradation

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ABSTRACT

Poly (ϵ -caprolactone) macromonomers carrying amide end groups were synthesised via a polycondensation reaction of functional carboxylic diacid terminated poly(ϵ -caprolactone) prepolymers (HOOC-PCL-COOH) with 1,4 phenylene bis(2-Oxazoline). The prepolymers were produced by the ring opening polymerisation of ϵ -caprolactone by adipic acid. This polymerisation used a new one-step method that eliminated the need to isolate of the telechelic prepolymer intermediate. The expected structures of the polymers were confirmed by FTIR. Hydrolytic and enzymatic degradations of the materials were performed.

Key words: degradation , poly (ϵ -caprolactone), chain extender, coupling reaction.

INTRODUCTION

There has been recent interest in using end functionalised low molecular weight polyester prepolymers in the synthesis of biodegradable networks. This synthesis involves an initial ring-opening polymerisation of a cyclic ester, such as lactide, glycolide and/or ϵ -caprolactone, with a polyol initiator to produce hydroxyl-telechelic polyesters. The latter have been used in various post-polymerisation reactions to produce a variety of polymers containing end groups suitable for cross-linking. In particular, it has been reported that

(carboxylic acid)-telechelic polyesters can be synthesised using a two step synthesis^[1] that consist of isolation of the hydroxyl-telechelic prepolymer followed by a reaction with succinic anhydride. The functional (HOOC-PCL-COOH) can participate in an addition coupling reaction with heterocyclic compounds, such as bisoxazolines, which present good thermal stability and high reactivity toward the carboxy end groups of poly(ϵ -caprolactone). The foci of this paper are the (following: 1) synthesis of (carboxylic acid)-telechelic poly(ϵ -caprolactone) polymers using a new, one-step method that eliminates the isolation of the hydroxyl-telechelic

prepolymer intermediate and (2) the reaction between chain coupling agents (cc) and oligomers such as PCL with reactive end-groups (carboxyl groups) .. Hydrolytic and enzymatic degradation of the synthesised polymers were compared and discussed.

EXPERIMENTAL

Materials

ϵ -Caprolactone (ϵ -CL), adipic acid, heptanes and dioxane, were purchased from FLUKA chemical., 1,3 phenylene BIS (2-oxazoline)(PBO) was purchased from Mikuni Chemical, Tetrahydrofuran (THF) and methanol were purchased from Biochem (Chemopharma) and Techanal respectively.

Synthesis of poly(ϵ -Caprolactone)

PCL was prepared according to Scheme 1, via a ring opening polymerisation of ϵ -Caprolactone (0.4 mol) in the presence of adipic acid (0.1 mol) as a catalyst. The PCL was purified by THF and precipitated from methanol. The polymer was obtained after vacuum filtration. Viscosimetric mass was determined by viscosimetry.

Synthesis of polyesteramide by a coupling reaction

PCL was mixed an equimolar ratio with chain extender PBO and the reaction was performed at 210°C for 120 min under inert atmosphere (N_2).

Polyesteramide was followed by viscometry in samples taken along the reaction. The samples were dissolved in dioxane and a viscometric value was determined. The final polyesteramide (PEA); was dissolved in THF and precipitated from using heptane.

Viscometric study

The viscosimetric study was conducted with an Ubbelohde viscosimeter immersed in a water bath at 30°C using dioxane as a solvent, the Mark-Houwink parameters were: (a) 0.71, (K) 17×10^{-3} ml/g. The average molecular weights M_v of the coupling reaction polymers samples were determined by viscometry.

Study of chemical degradation of PCL and PEA

A series of acidic and basic aqueous solutions of varying pH were prepared from hydrochloric acid (HCl) and sodium hydroxide (NaOH), respectively. The prepared solutions were poured into Ubbelohde viscometers that were immersed in a water bath at 30°C, relative viscosities were measured as a function of time. As previously reported in literature².

Study of enzymatic degradation

The PCL and PEA were purified by dry sterilisation in an oven at 170°C, polymers degrade beyond 300°C.

Growth media

The composition of the degradation medium was the following:

(Per litre of distilled water) 1 g/l yeast extract, 0.35 g/l KH_2PO_4 , 1.825 g/l Na_2HPO_4 , 0.244 g/l $(NH_4)_2SO_4$, 0.015 g/l NaCl, 0.0198 g/l $CaCl_2 \cdot 2H_2O$, 0.0614 g/l $MgSO_4 \cdot 7H_2O$, 0.0032 g/l $FeCl_2 \cdot 4H_2O$. The medium was supplemented with 14g/l agar to generate solid medium

The PCL was inoculated with each bacterial strains (*Bacillus subtilis* F6), *Pseudomonas Aërogenosa*: (P1, P2, P5) with different bacterial activity and the solid media was incubated at 30°C for 48H. Polymers were deposited on the surface of the solid media. The degree of degradation was characterised by viscosimetric weight loss according to (Equation 1)

$$\text{Weight loss\%} = (M_{v_0} - M_v) / M_{v_0} \times 100$$

RESULTS AND DISCUSSION

Synthesis and characterisation of PCL and PEA

PCL was synthesised via the ring opening polymerisation of ϵ -caprolactone with adipic acid. The polyester and polyesteramide were characterised by FTIR and 1H NMR.

The FTIR spectra (Fig. 1) shows the absorption bands at 1722.3 cm^{-1} and 1430 cm^{-1} that were attributed to the linear ester and carboxy terminal groups respectively. For the polyesteramide the amide bands were noted respectively at 1541

Table 1 : Viscosimeter mass of PEA samples

Time(mn)	$[\eta](\text{dl/g})$	Mv(gr /mol)
5	0.228	2404.2
12	0.239	25186.74
15	0.279	31741.78
27	0.448	61565.46
90	0.375	44794.65
120	0.35707	39593.07

cm^{-1} and 3456cm^{-1} . The signal at 1660.6cm^{-1} was attributed to the CO of the amide.

Viscosimetry study: the viscometric mass of PCL, Mv (PCL) was determined: 16829,33 gr /mol

The figure 2 shows that the viscosimetric masses increase up to 27mn and then decrease until the end of the reaction. After this time we note that the optimum polyesteramide deteriorates due to the formation of macrocyclic oligomers (polyester) or blockage of polymer chains. Many studies have noted this behaviour and the lack of completed reaction.

Chemical degradation of PCL and PEA

Chemical degradation of both polymers was carried out in different pHs for 30 days. The PCL solutions were monitored as a function of time, and the results were plotted in Fig. 3.

This graph revealed that at pHs ranging from 1,13 to 7,6 the relative viscosity of the PCL solutions decreased to a plateau. This pattern indicated that, PCL undergoes hydrolysis at a neutral pH without using a catalyst. The rates of hydrolysis are very similar for the three selected pHs.

The range (pHs: 1,13 and 7,6) is slow beyond 30 days the relative viscosity of the solutions did not change.

Fig. 4 shows the viscosity of PCL solutions at various pH conditions. In the pH range of 11.1-12 appreciable viscosity changes due to hydrolysis were observed with time. The relative viscosity increased because of the increased volume of polymers by the charge effect of salt

formation. There was a slight decrease in relative viscosity at the beginning of the hydrolysis of PCL;

The same variations were observed for solutions of polyesteramide. The relative viscosity decreased in acidic pH (Fig. 5) and near neutrality with time. A increase in relative viscosity at a basic pH was observed. After 2 days the relative viscosity decreased then stagnated (Fig. 6).

Enzymatic degradation of PCL and PEA

The study of the degradation of PCL was conducted on a solid medium. The same components were used for the preparation of the liquid media only without the agar. The solid and liquid media were inoculated with bacterial strain L_9 (*Bacillus megaterium*).

The study of biodegradation of PEA was conducted on solid media inoculated with bacterial strains: P_5 and L_9 PCL added to media growth inoculated with L_9 .

For PEA, we noted a clear zone of hydrolysis on solid media inoculated with L_9 (*Bacillus megaterium*) strain.

The method of clear zone was used by Jarerat *et al.*,³ to isolate *Saccharothrix waywayandensis*, this organism had the ability to degrade up to 95% of PLA after 7 days of incubation in a liquid medium. The results obtained in this study of the degradation of PCL and PEA is consistent with work of Jarerat *et al.* The most important zone of hydrolysis was noted on the solid inoculated with the strain L_9 PCL were the source of glucose for the strains used. They were supplemented into the medium of degradation and were the source of glucose for the L_9 (*Bacillus megaterium*). To estimate the rate of biodegradation for PCL we determined the viscosimetric mass loss of PCL and absorbed from the liquid medium at the end of the biodegradation process. The results obtained are shown in the graph (Fig. 10).

The graph shows that the absorbance which measures the activity of bacterial strains is inversely proportional to the viscosimetric mass loss. The L_9 strain degraded the most polymer 45% and,

it was 47% less active than the P_5 strain the P_5 strain resulted in mass loss as measured by the viscosimeter of 10.87% . The invasion of polymers by microorganisms can highlight the sensitivity of the polymers studied to microbial attack. The 3118 strain of the *genus Amycolatopsis*⁴ was able to degrade 50% of a PLA film after 8 weeks at 30°C.

The estimation of biodegradability is based on the on the material observed that is directly invaded by microorganisms.

The biodegradation rate of PCL on the surface of seeded nutrient agar medium was observed. At the end of the process biodegradation, the medium was incubated for 48 h at 30°C .

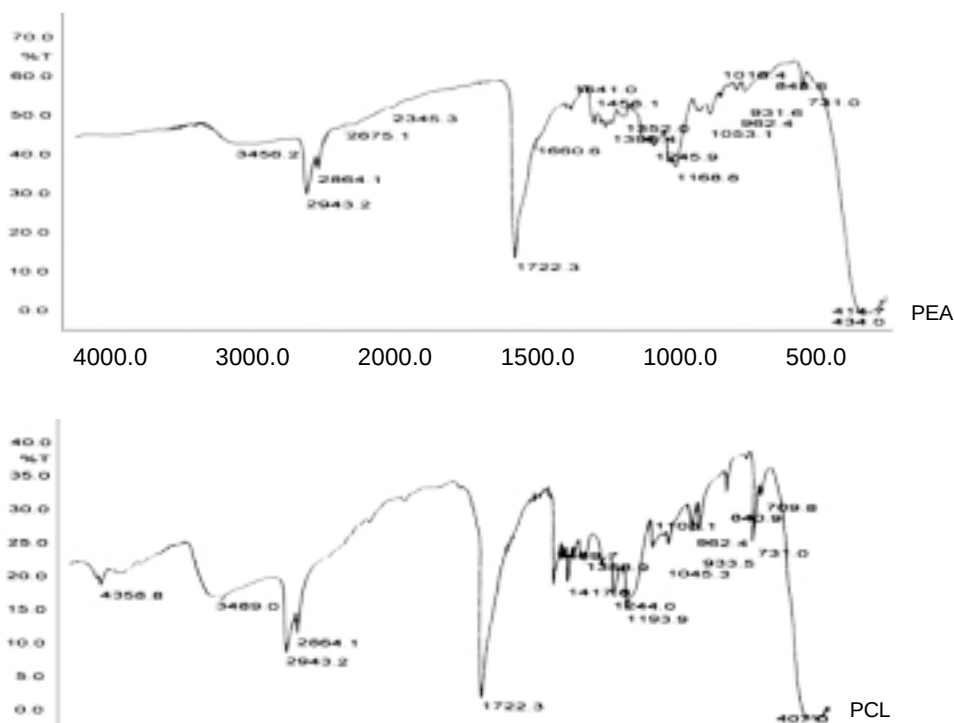


Fig. 1: FTIR spectra of polyesteramide PEA and poly(ϵ -caprolactone) PCL

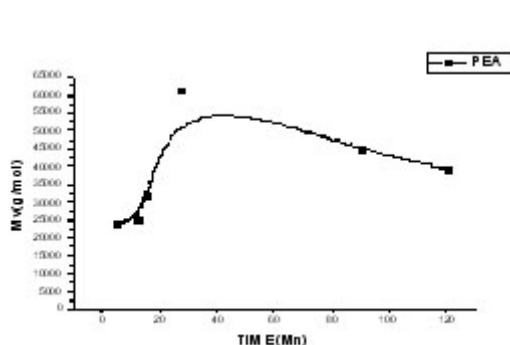


Fig. 2: Kinetic monitoring of the formation of polyesteramide

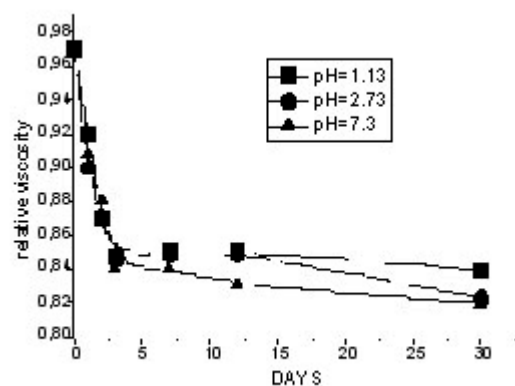


Fig. 3: variation of relative viscosities of PCL in acidic pH

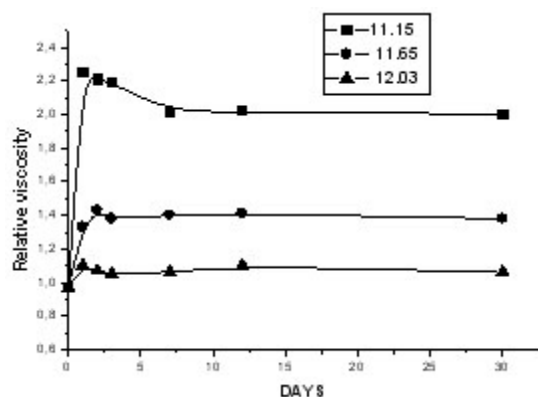


Fig. 4: variation of relative viscosities of PCL in basic pH

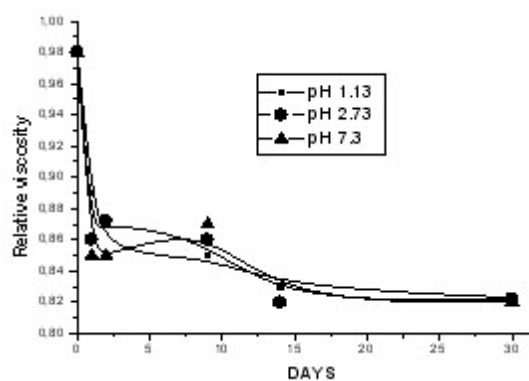


Fig. 5: variation of relative viscosities of PEA in acidic pH

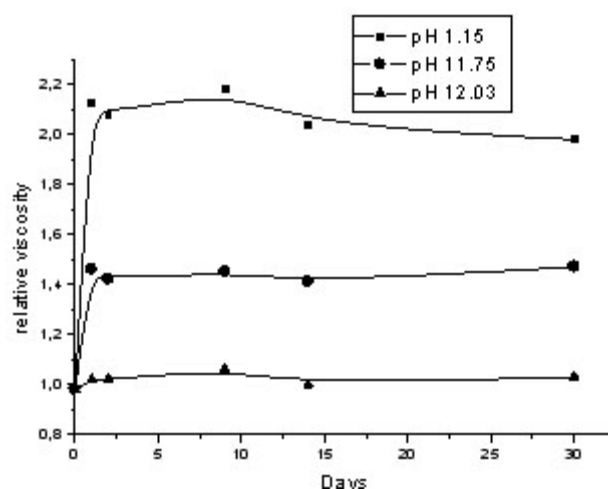


Fig. 6: variation of relative viscosities of PEA in basic pH

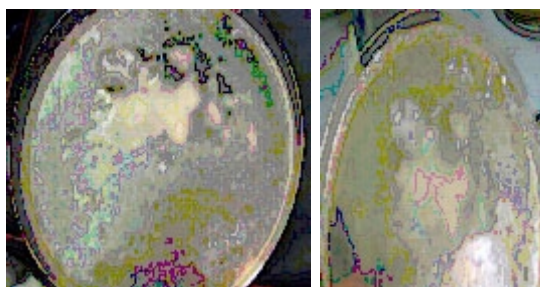


Fig. 7: PLC in a media solid inoculated with L_9

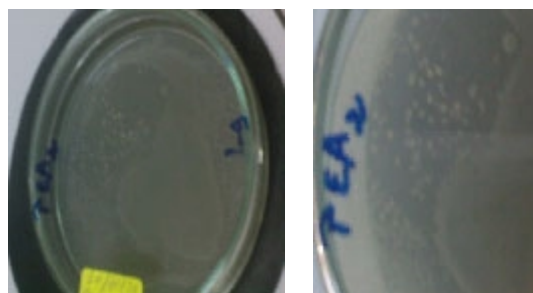


Fig. 8: Clear zones of hydrolysis of PEA inoculated with bacterial strain L_9

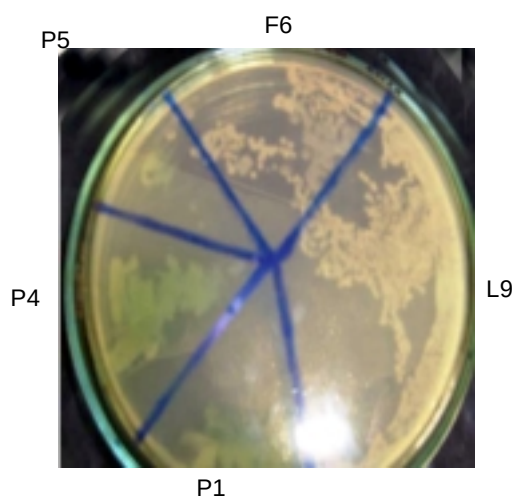


Fig. 9: Invasion of the PCL by Bacterial strains

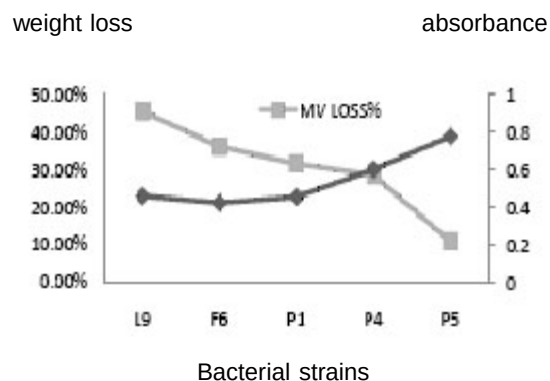


Fig. 10: Change in absorbance and the mass loss rate viscometer according to the strains

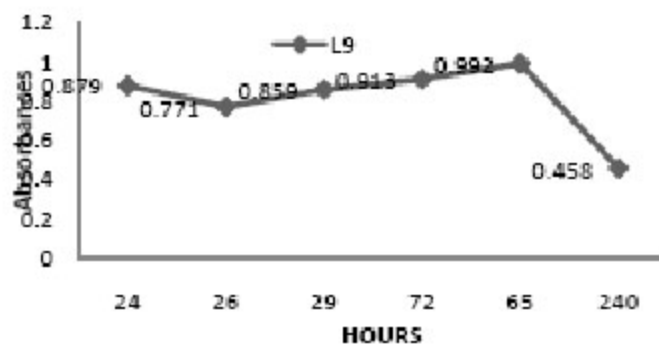


Fig. 11: Kinetic monitoring of the bacterial L9 strain

Fig. 9 shows that the rate of invasion of the polymer deposited in inoculated solid medium is greater with the L_9 strain than with the P5 strain. This result agrees with the numerical estimates of biodegradation rate by viscometric mass loss. Biological degradation of polymers is influenced by a number of factors including the nature of the polymeric substance, the kind of organism involved in the biodegradation, and the environmental conditions (eg. nutrient, supply, temperature and pH).

In this work *Bacillus megaterium* L_9 was the bacterial strain. It was able to degrade

the polyester PCL at a rate of 45.47%, PEA hydrolysis could be clearly noted. We decided to monitor the kinetics of this strain by monitoring the absorbance for 240h in a liquid medium. This strain enters the stationary phase, after 24h. After this period the nutrient medium was poor in nutrients and effected the microbe's survival. The mortality of this strain was observed by a decrease in absorbance. PCL was then supplemented to the liquid medium under vigorous agitation and was incubated at 30°C. The absorbance was measured with time.

The figure shows that the absorbance after 24h increases therefore, the strain was able to find the necessary nutrients for survival. The only source of glucose that was present was PCL. Beyond 65h, the absorbance decreases due to medium's dearth nutrients. The wavelength that was used to monitoring the kinetics of the bacterial strains was 650nm

CONCLUSION

Cyclic bis (imino ether) PBO was very reactive against the carboxyl end groups of poly (ϵ -caprolactone). The polyesteramide linkage formed by the biodegradable polymer is itself degradable to PCL and PEA. The coupling reaction was increased by improving the viscosimeter masses reaction.

The hydrolysis of the synthesised PCL and PEA were examined. Samples containing 10ml of

mother solutions of polymers in dioxane and 90ml of acidic or basic aqueous solutions of varying pH were subjected to viscosimetry analysis. The hydrolytic degradability of polyester and polyesteramide were determined under the same conditions, all of the polymers studied exhibited hydrolytic degradability, that depended on the acidic or basic conditions of solutions. The polymers showed slow degradation in a range of selected pHs, the polymers were hydrolysed even at a pH near neutral^{7,4}. The rates of acid and base hydrolysis were very for both polymers PCL and PEA polymers, due to similarity between backbones of polyester and polyesteramide. PCL and PEA were susceptible to the selected strains, the *bacillus megaterium* (L₉) strain was the most efficient for monitoring kinetics showed most, After 24h this strain starts degrading the polymers. This result leads us to consider a study of the effect of composting our synthetic polymers because this strain was isolated from the soil.

REFERENCES

1. Riccardo .P, Luigi Abis, Synthesis and characterization of poly(ester-amide)s from bis(2-oxazoline)s, Anhydrides, and diols, *Macromolecules*, **28**: 5699-5705 (1995).
2. Jae Hwan Jung, Moonhor Ree, Acid-and base catalyzed hydrolyses of aliphatic polycarbonates and polyesters. *Catalysis Today*, **115**: 283-287 (2006).
3. Jaret A, Tokiwa Y, Poly(l-lactide) degradation by *Saccharothrix, waywayandensis* *Biotechnology letters*, **25**: 401-404 (2003)
4. Ikura Y; Kudo .T, Isolation of a microorganism capable of degrading poly(L-lactide). *J Gen Appl Microbial*, **45**: 247-251 (1999).