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Polymer 46 (2005) 2133–2140
polymer
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Properties of L-tyrosine based polyphosphates pertinent to potential biomaterial applications

Anirban Sen Gupta[1], Stephanie T. Lopina*

Department of Chemical Engineering, The University of Akron, 302A Whitby Hall, Akron, OH 44325, USA

Received 18 June 2004; received in revised form 8 December 2004; accepted 12 January 2005
Available online 28 January 2005

Abstract

Since their introduction by Kohn and Langer et al. in 1984, L-tyrosine based 'pseudo' poly(amino acids) have undergone extensive research in the area of polymeric biomaterials. Starting from L-tyrosine based diphenolic monomers, polyiminocarbonates, polycarbonates and polyarylates have been developed by Kohn and co-workers and are being investigated for potential orthopedic biomaterial applications. Mao et al. have reported development of L-tyrosine based polyphosphates and polyphosphonates in a patent, however, detailed structural and physico-chemical characterization studies on such polymers have not been reported yet. For the purpose of the current paper, using a novel solid phase process for synthesis of L-tyrosine based diphenolic monomers and adapting the polymerization process described by Mao et al., L-tyrosine based polyphosphates were developed and investigated for their pertinent bioengineering properties. The properties investigated consist of chemical solubility, hydrophilicity and hydrolytic degradation. The results of this investigation are crucial to validate further investigation of biomaterial applications of these polymers.

Keywords: L-tyrosine; Polyphosphates; Degradation

1. Introduction

L-tyrosine based 'pseudo' poly(amino acids) were introduced as a novel class of polymeric biomaterials by Kohn and Langer in 1984 [1,2]. The unique chemistry of these polymers is the combination of enzymatically degradable peptide bonds with hydrolytically degradable non-peptide bonds in the polymer backbone. Normal homo(polyamino acids) developed from natural amino acids, although biocompatible, have been reported to exhibit prominent bioengineering and processing difficulties like insolubility in common organic solvents, poor hydrolytic degradability, unpredictable water permeability and swelling, high thermal transition temperature ranges, etc. [3,4]. Introduction of specific non-peptide bonds alternating with peptide bonds in a natural amino acid based polymer backbone, has been found to provide a way to customize the engineering properties of such polymers and thereby improve chemical and thermal processability, solubility, water permeation and resultant hydrolytic degradation. Promising results have been found with the incorporation of iminocarbonate, carbonate and arylate moieties in L-tyrosine based polymer backbone [5–8] and have set the path for investigation of incorporating other non-peptide moieties in such L-tyrosine based polymer systems. Among the different categories of polymers developed and investigated for biomaterial applications, biodegradable polymers remain as a category of prime importance. In that context, introduction of easily hydrolysable non-peptide moieties in an L-tyrosine based peptidic backbone would provide a way to obtain a natural amino-acid based polymer with customizable degradation properties. The choice of the non-peptide moiety would also provide a way to manipulate the related engineering properties mentioned previously. Following this idea and to exploit the already established hydrolytic nature of the 'phosphoester' moiety in such a situation, attempts were made to develop L-tyrosine based

* Corresponding author. Tel.: +1 330 972 7255; fax: +1 330 972 5856.
E-mail address: lopina@uakron.edu (S.T. Lopina).
[1] Current address: Department of Biomedical Engineering, Case Western Reserve University, Cleveland, OH 44106, USA.

0032-3861/$ - see front matter © 2005 Published by Elsevier Ltd.
doi:10.1016/j.polymer.2005.01.023

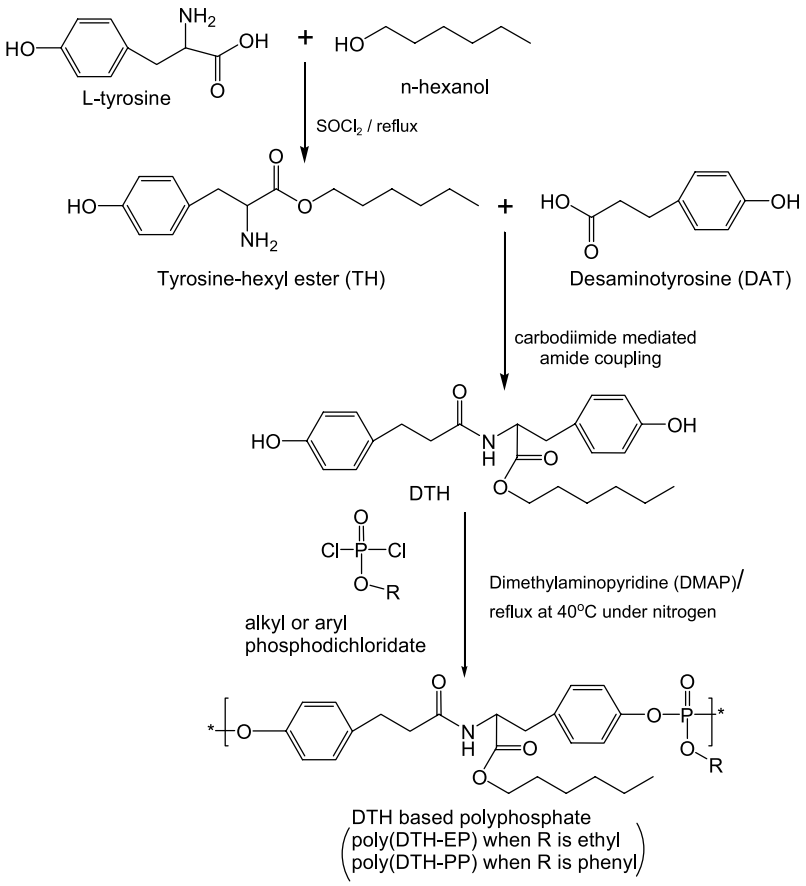


Fig. 1. Chemical structures of L-tyrosine based starting compounds, diphenolic monomer and corresponding polyphosphate polymer.

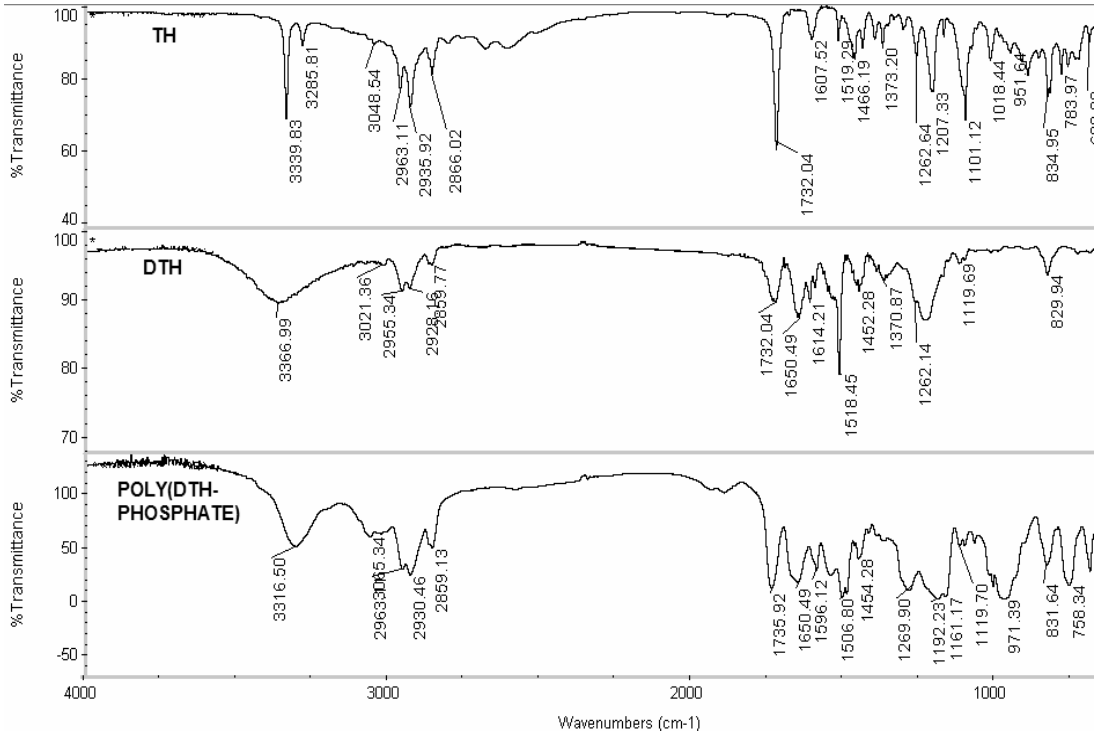


Fig. 2. Stacked FTIR spectra of tyrosine-hexyl ester (TH), the diphenolic monomer desaminotyrosyl-tyrosine-hexyl ester (DTH) and the corresponding poly(DTH–ethyl phosphate) polymer.

Table 1
List of solvents in which the L-tyrosine based polyphosphates were found to be soluble and partially soluble; pure poly(L-tyrosine) was insoluble

Solvents	Soluble	Partially soluble	Insoluble
Dichloromethane	✓		
Chloroform	✓		
Tetrahydrofuran	✓		
Ethanol		✓	
Water		✓	

polymers with alternating peptide and phosphoester bonds in the polymer backbone. The resultant L-tyrosine based polyphosphates were investigated for their solubility, hydrophilicity and hydrolytic degradation.

2. Materials and methods

The synthetic procedures for the development of L-tyrosine based diphenolic monomers and the subsequent polyphosphate polymers, as well as, the characterization studies for their respective chemical structures, have been described elsewhere [9,10]. Briefly, L-tyrosine is converted to L-tyrosine-hexyl ester (TH) and the latter is coupled with desaminotyrosine (DAT), a natural metabolitic L-tyrosine analog, through cabodiimide mediated solid phase amide coupling on a 1-ethyl-1′-dimethylaminopropylcarbodiimide (EDC) modified polystyrene–divinylbenzene (PS–DVB) resin. Details of the solid-phase resin based reaction for the synthesis of L-tyrosine based diphenolic monomer have been descried elsewhere [10]. The monomer thus obtained was desaminotyrosine-tyrosine-hexyl ester (DTH). DTH was further reacted with suitable alkyl and aryl phosphodichloridates, like ethyl phosphodichloridate (EP) and phenyl phosphodichloridate (PP), in 1:1 stoichiometric ratio, to induce dehydrochlorination–polycondensation reaction in presence of suitable acid acceptors like dimethylaminopyridine (DMAP), to produce the subsequent DTH-based polyphosphates, named poly(DTH–EP) and poly(DTH–PP). Such synthesis procedures were adapted from the work described by Penczek et al. [11] on general polyphosphates, Leong et al. [12,13] and Renier et al. [14] on Bisphenol A-based polyphosphates and Mao et al. [15] on similar L-tyrosine based polyphosphates. L-tyrosine, desaminotyrosine, *n*-hexanol, resin-bound carbodiimide, suitable phosphodichloridates, DMAP and all reaction solvents were obtained from Sigma-Aldrich (St Louis, MO). All solvents were freshly distilled before conducting reactions. The chemical structures of the monomer and the corresponding polymers are shown in Fig. 1. TH, DTH and corresponding poly(DTH–phosphates) were characterized by NMR and FTIR for their chemical structures, by GPC for their molecular weight distributions, by DSC for their glass transition temperatures and by TGA for their thermal degradation properties. Results of these characterization studies have been reported elsewhere [9]. For the purpose of emphasizing the successful synthetic development of the polymers in this paper, Fig. 2 shows representative stacked FTIR spectra of TH, DTH and poly(DTH–ethyl phosphate). The pertinent IR bands would be briefly mentioned in the results section.

The solubility properties of the polymers were determined by adding fixed weight of the polymer, in powder form, into fixed volumes of solvents at room temperature, with slight shaking on a gyratory shaker (60 rpm). The investigation was meant to be more qualitative in nature, than quantitative, in an attempt to identify solvent systems, which can be used to perform chemical reactions in the solution phase of the polymers. Pure homopolymeric poly(L-tyrosine) was found to be insoluble in most of these solvent systems and hence, the L-tyrosine based polyphosphates, because of their soluble nature, would have an obvious engineering advantage regarding ease of chemical processing. Table 1 shows a list of solvents in which the L-tyrosine based polyphosphates were found to dissolve readily.

A verification of the 'hydrophilic' nature of the L-tyrosine based polyphosphates was obtained by dynamic contact angle measurements by sessile-drop goniometry on a Ramé–Hart automated goniometer. For this purpose, clean silica plates were submerged in corresponding polymer solutions for 12 h to ensure uniform coating of the surface with polymer film. The surfaces were then dried under vacuum for ~8 h and subsequently dried under a flow of nitrogen for 1 h prior to dynamic contact angle analysis. Average of five readings was noted both for advancing and receding contact angles.

Investigation of in vitro hydrolytic degradation of the polymers was conducted by exposing the polymer to phosphate buffered saline (pH 7.4) at physiological temperature (37 °C) adapting a method reported previously by Richards et al. [12]. For this purpose, disks were compressed out of powdered polymer, using a pelletizer mold. For each disk, about 100–150 mg of finely powdered polymer was put into the mold (Harrick Scientific, NY) and subjected to 15,000 psi pressure on a hydraulic press (Carver Inc., IN) for 20 min at room temperature. Disks, each about 13 mm in diameter and 2 mm in thickness were obtained in this way. Picture of some typical disks made in this process and of the pelletizer mold is shown in Fig. 4. For each set of experiment, six such disks were weighed and put into six 20 ml scintillation vials. The weight of each vial along with the dry pellet was also measured. For each batch of experiment, the six vials were filled up with 15 ml of PBS solution each, sealed and kept at 37 °C with slight shaking (60 rpm) on a gyratory shaker. The physical mass loss of the pellets relative to the initial dry mass, was followed daily over a period of 6 days, for each batch. The corresponding

molecular weight changes of the pellets were investigated by gel permeation chromatography (GPC) techniques.

Drastic changes of local pH as a result of acidic degradation products, has been found to have a detrimental effect both on the polymer properties and on the surrounding tissue, regarding several intended biomaterial applications of clinically established biodegradable polymers like polylactate (PLA), polyglycolate (PGA), polycaprolactone (PCL), etc. [16,17]. In this regard, the effect of the L-tyrosine based polyphosphate degradation on local pH was considered to be an important factor to be studied for judging potential biomaterial applications. The study was performed by immersing L-tyrosine-polyphosphate disks in PBS (pH 7.4) at 37 °C and measuring the change in pH with time, with the help of a temperature-corrected pH probe. Pressure-molded disks of PLGA (50:50), with a reported M_w of 5000–15,000, obtained commercially from Sigma-Aldrich, were put to the same test for comparison purposes.

3. Results and discussion

3.1. Pertinent IR bands confirming the chemical structures of monomer and polymer

Fig. 2 shows stacked representative FTIR spectra for TH, DTH and poly(DTH–ethyl phosphate). Detailed chemical characterization studies of the starting compounds, the monomeric DTH and the subsequent polyphosphate compounds have been reported in a previous publication [9]. Briefly, the polyphosphate repeating unit structure was confirmed by hydrogen bonded (P=O) stretching at around 1190 cm^{-1}, non-hydrogen bonded (P=O) stretching at around 1160 cm^{-1}, asymmetric (P–O–C) stretching at around 970 cm^{-1} and symmetric (P–O–C) stretching at around 830 cm^{-1}, along with the usual (N–H), ester (C=O) and amide (C=O) peaks from DTH. Theoretical values for phosphorus–halogen stretching bands lie around 850 cm^{-1}. Since, no recognizable peaks were obtained in that region, it was concluded that there were no remaining phosphorus–chlorine (P–Cl) moieties in the polymer and that the polymer was in all probabilities, hydroxyl terminated.

3.2. Feasible solvent systems for chemical processing of L-tyrosine polyphosphates

The L-tyrosine based polyphosphates were found to be readily soluble in a variety of common organic solvents (Table 1). On an average, over 8 g of polymer could be readily dissolved in 100 ml of any of the listed organic solvents (except ethanol) at room temperature, to give a transparent yellowish solution. With more polymer added to the system, the solution was found to become increasingly viscous and translucent. It was possible to solvent-cast films of the polymer from the corresponding solution, indicating the potential advantage of the polymers to undergo chemical processing in the event of further biomaterial device fabrication. Processes like solvent-casting, solution spinning, etc. can be envisaged for device manufacture from these polymers. The partial solubility of the polymer in water suggested amphiphilic behavior. This knowledge might be useful in investigating these polymers for aqueous-based drug delivery devices. Also, the chemical solubility of the polymers and the structural versatility provides a way for future studies regarding custom chemical derivatization of the polymer systems for specific biomaterial applications.

3.3. Study of hydrophilic nature of the polymers by dynamic contact angle studies

Dynamic contact angle measurements in sessile drop goniometry method verified the hydrophilic nature of the L-tyrosine based polyphosphates. The advancing contact angles and the receding contact angles were measured for water on corresponding polyphosphate surfaces and both measurements suggested considerable wetting of the surface. For poly(DTH–ethyl phosphate) the average advancing contact angle was about 70° and average receding contact angle was about 15°. For poly(DTH–phenyl phosphate) the average advancing contact angle was about 78° and the average receding contact angle was about 19°. These values suggest that both the polymer systems are prone to considerable wetting by water and are, therefore, predominantly hydrophilic. The hydrophilicity and potential hydrolytic degradability was further established by buffer incubation experiments as would be described in later sections. For pure homopolymeric poly(L-tyrosine), similar contact angle measurements could not be performed since it was difficult solvent-cast pure poly(L-tyrosine) surfaces because of the insolubility of the polymer in a variety of organic solvents.

3.4. Hydrolytic degradation studies of L-tyrosine based polyphosphates

As was mentioned previously, for in vitro hydrolytic degradation studies of the L-tyrosine based polymers, the physical mass loss of the six polymer pellets in PBS (pH 7.4) at 37 °C was followed daily over a period of 6 days. For each vial containing a polymer pellet, the buffer solution was aspirated out and then the vial containing the degraded products was subjected to freeze drying for 12 h. This ensured the complete removal of residual water from the vial and hence from the residual degraded polymer. Trace amounts of salt components from the PBS solution, however, might remain with the residual polymer. Hence, to each vial containing residual polymer, about 10 ml of chloroform was added to dissolve the residual polymer part. The polymer solution was filtered off through 0.45 μ syringe filters to ensure effective removal of the residual trace of buffer salts. The filtrate was put back into its corresponding vial and subsequently the solvent was evaporated from the

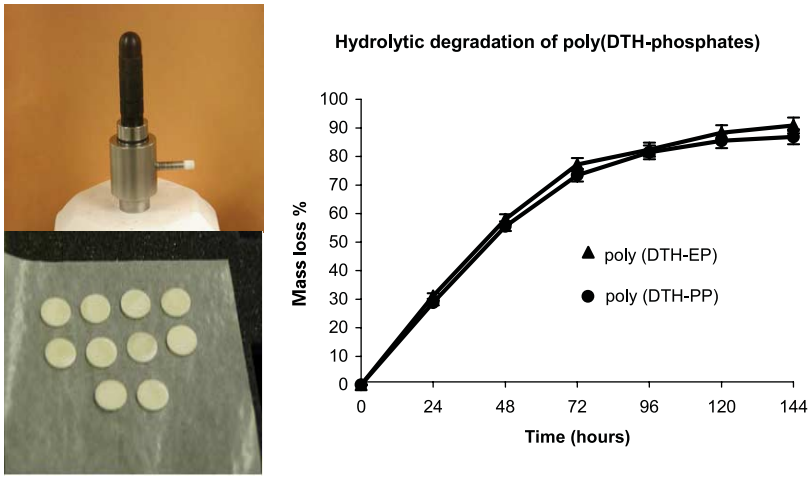


Fig. 3. Pelletizer mold (a) and polymer pellets (b) used for hydrolytic degradation study of L-tyrosine based polyphosphates; (c) shows typical mass loss data for L-tyrosine based polyphosphates due to hydrolytic degradation in PBS.

filtrate under vacuum to obtain dry residual polymer as powder. The weight of this dry polymer residue along with the vial was determined. This was compared to the initial weight of the vial plus the initial dry pellet. Hence, from these values, the mass loss of the pellet due to hydrolytic degradation could be calculated. The percentage mass losses due to hydrolytic degradation, thus calculated for both types of L-tyrosine based polyphosphates, were plotted against time. Representative mass loss data for polymer degradation are shown in Fig. 3.

As evident from the experimental results, both the polyphosphates loose over 80% of their initial mass over a period of 4 days. Poly(DTH–PP) apparently seemed to have a slightly lower degradation rate than poly(DTH–EP), although, the difference was not statistically significant. This may be attributed to the slightly more hydrophobic structure of poly(DTH–PP) compared to poly(DTH–EP), due to the presence of more aromatic groups in the former.

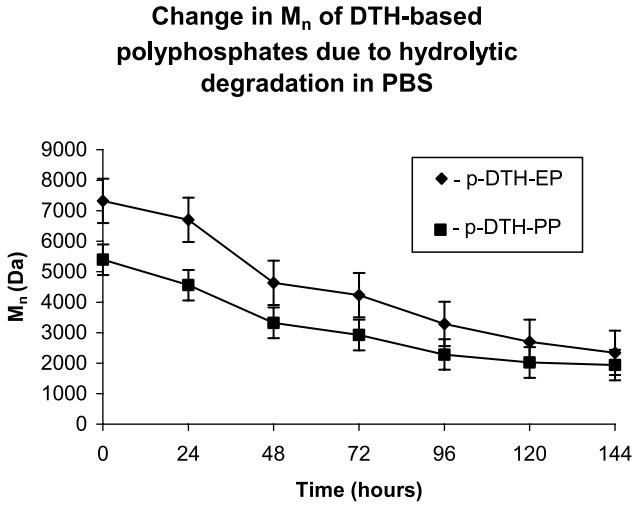
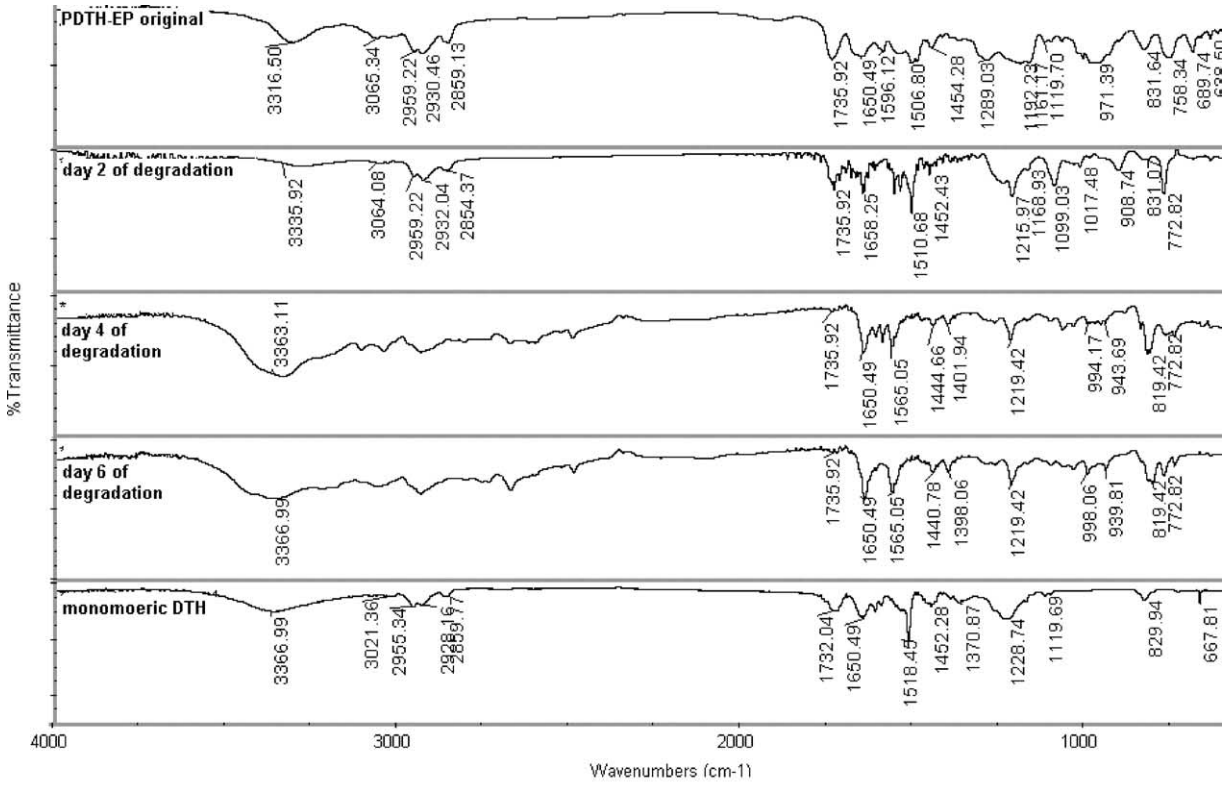


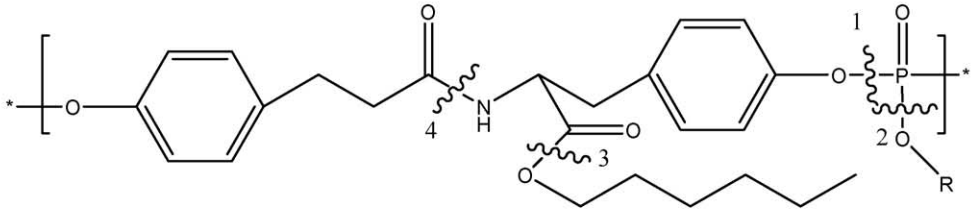
Fig. 4. Molecular weight loss data of hydrolytic degradation of L-tyrosine based polyphosphates due to hydrolytic degradation in PBS.

The fast hydrolytic degradation nature of the L-tyrosine based polyphosphates can be attributed to the presence of hydrolytically unstable phosphoester groups in the polymer backbone and also as pendant chains. Pure homopolymeric poly(L-tyrosine) showed no such hydrolytic degradation characteristics and hence the introduction of hydrolytically unstable phosphoester moieties in an L-tyrosine backbone provided a way to improve degradation characteristics of such polymers.

In conjunction with the mass loss analyses, the molecular weight loss of the DTH based polyphosphates were followed using GPC techniques. For this purpose, after measuring the mass of each (vial+ degraded polymer) system, specific amount of the residual polymeric material in the vial was dissolved in required amount of tetrahydrofuran (THF) and was passed through styrogel chromatography columns. Initial molecular weights of the respective polymers before degradation experiments were measured previously [9]. The hydrolytic degradation was found to cause a significant drop in the polymer molecular weight with time, for both L-tyrosine based polyphosphates. Fig. 4 shows representative data for polymer molecular weight loss vs. time. As evident from the figure, almost 70% loss in molecular weight was recorded in the experiments, that can be attributed to fast breakdown of the polymer backbone at the hydrolytically labile phosphoester linkages.

In order to investigate the chemical nature of the polymer breakdown, the residual degraded polymer from several batches of experiments were also analyzed by FTIR techniques. Fig. 5 shows representative FTIR analysis results of residual pellet materials from the, fourth and sixth day of a typical six-day PBS incubation experiment of DTH-based polyphosphates, stacked with the FTIR spectrum of the original polyphosphate polymer and the original DTH monomer. As evident from the stacked spectra, the most significant spectral changes for the polymer with progressive degradation, are, the weakening and





1: Backbone phosphoester bond - hydrolysis
2: Pendant phosphoester bond - hydrolysis
3: Pendant alkyl (hexyl) ester bond - hydrolysis
4: Backbone amide (peptide) bond - enzymolysis

Fig. 5. FTIR-spectra of hydrolytically degrading L-tyrosine-based polyphosphate, stacked with that of the original monomer (DTH) and original polymer (PDTH–EP) for comparison, revealing possible chemical nature of polymer degradation.

disappearance of the P–O–C symmetric and asymmetric stretching peaks in the 830 and 970 cm^{-1} regions, respectively, weakening of the organic phosphate (P=O) stretching in the 1160–1190 cm^{-1} region and the gradual weakening of the ester carbonyl (C=O) peak at around 1735 cm^{-1} region. Also the symmetric and distinct inverted bell-shaped broad stretching peak for secondary amine in the 3350–3310 cm^{-1} region seems to get overlapped by a broader irregular peak in the degraded products. This broader irregular peak even overlaps with adjacent the C–H stretching regions. There is a simultaneous development of a narrow peak in the 1215–1220 cm^{-1} region. The presence of the broader irregular peak in the 3300 cm^{-1} region is characteristic of hydrogen bonded carboxylic O–H stretching. This is supported by the development of the narrow peak in the 1215–1220 cm^{-1} region that is characteristic of carboxylic C–O stretch. This is evidence in favor of development of carboxylic acid moieties in the degraded polymer residues, which is possible if some of the hexyl esters of the DTH monomeric units get hydrolyzed. The weakening of the ester carbonyl (C=O) band at around 1730 cm^{-1} can be attributed to such hydrolysis and subsequent removal of ester moieties. The appearance of smaller peaks and shoulders adjacent to the amide carbonyl

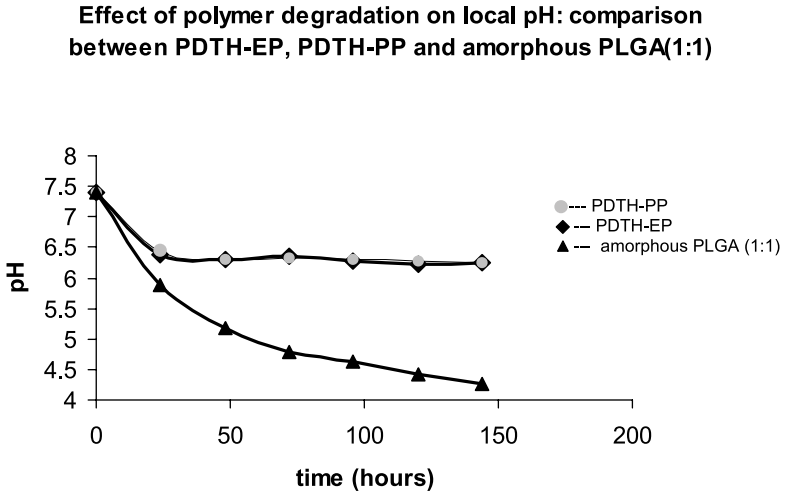


Fig. 6. Effect of L-tyrosine based polyphosphate degradation on pH of surrounding media compared to the effect of similar degradation of PLGA.

(C=O) band at around 1650 cm^{-1} can also be attributed to the formation of acid carbonyl (C=O) due to hydrolysis of the ester. Thus, investigation of the degrading polymer with FTIR techniques and resultant changes in the IR spectra clearly indicate the scission of polymeric chains at the backbone-bonded phosphoester links and progressive scission of the pendant phosphoester and alkyl ester moieties. Fig. 5 also shows a schematic of probable regions of scission in the polymer system for such L-tyrosine based polyphosphate.

3.5. Effect of degradation of L-tyrosine based polyphosphates on local pH

Many biodegradable polymers, on degradation, produce chemical species that can affect the local pH of the environment in which they are degrading. In certain cases, the change in local pH has pronounced effect on the actual performance of the biomaterial device itself, as well as, on the physiological environment and tissue response of the body. For example, it has been reported that the acidic degradation products of the conventional polyester biomaterials like polylactates (PLA), polyglycolates (PGA), polycaprolactones (PCL), etc. and their co-polymers have adverse non-specific inflammatory effects in physiological environment, especially in osseous tissue [14,15]. Such adverse effects have been found to coincide with the progression of polymer degradation and the lowering of pH due to acidic degradation products. In the context of such reports, we considered to study the effect of degradation of L-tyrosine based polyphosphates on local pH, at physiological temperature of 37 °C. To draw a comparison with an already established polymeric biomaterial, a simultaneous study was performed on amorphous PLGA (50:50). Pellets made from L-tyrosine based polyphosphates (poly(DTH–EP) and poly(DTH–PP)) and amorphous PLGA were separately incubated in PBS (pH 7.4), at a concentration of 15 mg/ml, at 37 °C. At specific intervals of time, over a period of 6 days, the pH of the supernatant buffer was measured with a temperature-corrected pH probe. The vials were also weighed periodically to ensure that any loss of water by evaporation was replenished. The pH values were plotted against time and compared. Fig. 6 shows the comparison of the local pH change due to degradation of the respective polymers. As evident from the figure, the polyphosphates apparently did not seem to have a drastic effect on lowering of pH of the surrounding media from the initial value. The minimum value for L-tyrosine based polyphosphates was found to be pH 6.23. This signifies that these polymers do indeed release acidic degradation products, most possibly in form of phosphoric acid derivatives but they do not severely affect the local pH. The degradation of the pendant alkyl ester chain in the DTH part of the polymer, to release carboxylic acid protons in the surrounding media is comparatively less acute compared to the degradation of PLGA. The PLGA showed a pronounced drop in pH over the period of 6 days and the nature of direction of the pH plot with time indicated the possibility of further drop in pH. The value on the sixth day, for PLGA was found to be 4.62. Thus, in the light of investigation of L-tyrosine polyphosphates as biodegradable biomaterials, the apparent inability of these polymers to affect the pH of the surrounding system, can be considered as a positive notion.

4. Conclusion

L-tyrosine based 'pseudo' polyamino acids are being considered as a promising novel group of biomaterials. By incorporating various non-amide linkages in a predominantly L-tyrosine backbone, it has been possible to develop polymeric systems with customizable engineering properties like solubility, degradability, moldability, chemical processability, thermal processability, etc. L-tyrosine based

polyiminocarbonates, polycarbonates and polyarylates have been developed previously by Kohn et al. and are being investigated for a variety of biomaterial applications like bone tissue reconstructive fixtures, tissue engineering scaffold material, etc. Development of a number of degradable polyphosphates and their potential biomaterial applications have been recently reported [12–15,18–22]. In the present article, we have reported investigation of pertinent bio-engineering properties, predominantly degradation characteristics, of L-tyrosine based polyphosphates. The chemical solubility, hydrophilicity, hydrolytic degradability and effect of hydrolytic degradation on local pH were studied for this purpose. The L-tyrosine based polyphosphates were found to be soluble in a variety of common organic solvents, thereby emphasizing their potential for chemical processability. The polymers were also found to be hydrophilic in nature. The polymers were found to be readily hydrolytically degradable in vitro and the degradation products had negligible effect on local pH. All these were considerable improvements compared to pure poly-L-tyrosine, which was mainly insoluble and hardly degradable. Hence, in the light of developing potentially biodegradable novel polymeric biomaterials from naturally obtained amino acids, the L-tyrosine based polyphosphates hold significant promise.

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