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Research paper

Copolymers of pharmaceutical grade lactic acid and sebacic acid: Drug release behavior and biocompatibility

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Abstract

Pharmaceutical grade D,L-lactic acid, which is rather an economic source in comparison to lactide monomer, was utilized for synthesis of a series of copolymers with sebacic acid. Polymers were characterized by GPC, FTIR, NMR and DSC techniques, and formulated into blank and methotrexate (MTX) loaded microspheres by emulsion-solvent evaporation method. *In vitro* degradation of blank microspheres was studied by FTIR, GPC and SEM analysis. MTX loaded microspheres showed the encapsulation efficiency of 44–64% and were in the size range of 40–60 μm. These were used to study the release profile of the encapsulated drug. The release was found to be affected by the pH and buffer concentration of the release medium which was in turn revealed by solubility studies of MTX. The overall study demonstrates significance of drug as well as polymer properties on release. Biocompatibility of polymer was evaluated by injecting microspheres subcutaneously into Sprague–Dawley (SD) rat and no local histopathological abnormalities were found.

Keywords: PLA–PSA; Microspheres; Biodegradable polymers; Drug delivery; Biocompatibility; Drug release kinetics; Profile modeling

1. Introduction

Microspheres are a useful type of delivery system for administration of drugs, since at one fell swoop they can be used to encapsulate, protect and control the release of a wide variety of drugs [1,2]. By delivering the drug at a controlled rate over a prolonged time in the localized area, such devices can maintain optimal drug concentrations and aid patient compliance by reducing the frequency of administration. The main advantage of localized drug delivery is high locoregional concentration of therapeutic agents with prolonged retention and hence, chances of various adverse effects are reduced or completely eliminated due to the escape of high systemic dose to achieve the therapeutic concentration at diseased site [3]. In addition, biodegradable microspheres are easily administered by injection, and they do not require surgical removal after drug exhaustion. Since the drug loaded in a microsphere remains separated from that in other microspheres, a further advantage is the potential to administer multiple drugs in a single injection by mixing different drug loaded microspheres, which for compatibility reasons would otherwise need to be separated.

Besides, the drug release rates can be controlled by manipulation of the particle size, the polymer degradation and/or erosion rates, the type of polymer and polymer erosion mechanism (bulk vs. surface erosion), among other factors. Surface eroding polymers, such as polyanhydrides, may simplify the drug release kinetics because water penetration into the microsphere interior is minimized, and the drug release rate becomes dependent predominantly on the polymer erosion rate [4].

Foremost important criterion for any polymer used for drug delivery is biocompatibility and because the surface of the material is in immediate contact with the biological medium, the interfacial characteristics are often more

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significant for impermeable solid devices. In such cases, the surface structure governs the biological response. This is initially determined by cell and protein interactions. However, in case of biodegradable polymers used for drug delivery overall bulk properties are also important contributing factor [5]. Biocompatibility of the degrading polymer can be checked by histopathology at different time intervals of the tissue near implantation site in the animals.

Poly(ester-anhydride) copolymers have been synthesized to combine the individual properties of the two widely used biodegradable polymer classes. Once these are formulated into microspheres, bulk erosion would be expected for polyesters whereas polyanhydrides exhibit a surface erosion profile controlled by the hydrophobic nature of the polymer backbone and the lability of the anhydride unit. Depending on the poly(ester-anhydride) copolymer ratio, contrasts in degradation behavior can be achieved to influence controlled release profiles of encapsulated bioactive moiety [6]. In addition to adding versatility to poly(ester-anhydride) degradation and release behavior, the polyanhydrides content of formulated delivery devices provides a simple method for covalent surface modification. The lability of the anhydride bond allows for the addition of amine and acid containing compounds. This property would become especially meaningful in the context of poly(ester-anhydride) microspheres by extending the delivery potential for these devices [2,4,7,8]. Thus, by varying the polymer chemistry, a suitable degradation time can be achieved to meet delivery needs.

In the present study, PLA–PSA in varying ratios has been synthesized and formulated into microspheres with and without MTX. Their *in vitro* and *in vivo* degradation and drug release behavior have been studied. Biocompatibility study of the microspheres prepared from 50:50 PLA–PSA was carried out subcutaneously in SD rats.

2. Materials and methods

2.1. Materials

Sebacic acid (for synthesis) was purchased from Lobachemie (Mumbai, India). D,L-Lactic acid (90% aqueous solution, Merck, Mumbai, India) was the starting monomer for PLA synthesis. Acetic anhydride LR (Qualigens, Mumbai, India), chloroform GR (Merck, Mumbai, India), petroleum ether LR (Qualigens, Mumbai, India), diethyl ether stabilized (Lobachemie, Mumbai, India), dichloromethane (Lobachemie, Mumbai, India) and poly(vinyl alcohol) (Sigma, Germany) were used as received. Methotrexate was received as gift sample from Astron Pharmaceuticals (Ahmedabad, India).

2.2. Methods

2.2.1. Polymer synthesis

Poly(lactic acid) (PLA) was synthesized from pharmaceutical grade D,L-lactic acid by melt-polycondensation method. PLA was activated by refluxing with acetic anhydride at 150 °C for 30 min. Similarly, sebacic acid was prepolymerized by refluxing with acetic anhydride at 150 °C for 30 min. PLA–PSA copolymer was synthesized by melt-condensation of the two prepolymers at 150 °C for 1 h, using varying ratios of the two components; nomenclature of which is given in Table 1 (detailed procedure is described elsewhere) [9].

2.2.2. Characterization of polymers

The polymers were characterized by ^{1}H NMR (300 MHz spectrometer, Bruker Avane, Germany), FTIR (Perkin Elmer, USA), DSC (Mettler Toledo, Switzerland), SEM (Leo Electron Microscopy LTD, Cambridge, England) and GPC. GPC was carried out with Waters Styragel HR3 column and chloroform as the mobile phase. Molecular weight was determined with reference to polystyrene standards in the range of 682–28,000 Da. The system consisted of Shimadzu LC-10AT VP HPLC pump (Shimadzu Corporation, Kyoto, Japan), Shimadzu SIL-10AD VP autoinjector, and SIL-10AD VP refractive index detector. Dried microspheres were gold coated for electron microscopy.

2.2.3. Microsphere preparation

The synthesized polymers were formulated into blank and drug loaded microspheres using emulsion-solvent evaporation method [10–13].

2.2.3.1. Blank microspheres. Briefly, polymer was dissolved in methylene chloride (5%, w/v) (4 ml) and was added to 100 ml of 1% PVA solution at room temperature with stirring at 1200 rpm until all the methylene chloride evaporated (about 4 h). The solidified microspheres were collected by filtration using 0.45 μm filter paper (mdi, India) and air dried overnight.

2.2.3.2. MTX loaded microspheres. Polymer was dissolved in methylene chloride (5%, w/v). MTX (20% by weight) was added to polymer solution and probe sonicated (50% amplitude, Dr. Heilscher GmbH, Germany) for 1 min. After suspending the drug in polymer solution, the organic phase was added to aqueous phase of 1% PVA solution and magnetically stirred at 1200 rpm at room temperature to evaporate methylene chloride (about 4 h). The solidified microspheres were collected and dried in manner similar to blank microspheres.

Table 1
Nomenclature of synthesized polymers

Polymer	Composition
1	PLA–PSA; 100:0
2	PLA–PSA; 75:25
3	PLA–PSA; 50:50
4	PLA–PSA; 25:75
5	PLA–PSA; 0:100

2.2.4. In vitro degradation of polymers

For this, blank microspheres were used. The degradation of the polymers was evaluated by placing 10 mg blank microspheres in 1.5 ml of 0.01 M phosphate buffer, pH 6.5, in 1.5 ml Eppendorf tube, placed in shaker water bath maintained at 37 °C and 100 rpm. Eppendorfs were taken out at 1, 2, 4, 8, 12 and 15 days interval, centrifuged at 5000*g* for 4 min, decanted and dried overnight under vacuum at room temperature. The degradation of the polymer was monitored by molecular weight loss, decrease in anhydride content and SEM analysis for morphological changes.

2.2.5. In vitro MTX release

Accurately weighed amount (around 10 mg) of MTX loaded microspheres containing known quantity of drug was suspended in 1.5 ml of release medium in Eppendorfs. The Eppendorfs were placed in shaker water bath maintained at 37 °C and 100 rpm. At various time intervals, the Eppendorfs were centrifuged at 5000*g* for 4 min and aliquots of 1 ml withdrawn from the supernatant, filtered and analyzed for MTX content at 303 nm using HPLC method. To maintain constant volume of the release medium, an equal amount (1 ml) was replaced immediately and vortexed slightly to resuspend the particles.

2.2.6. Drug release kinetics

Kinetics of drug release from microspheres is described using zero-order, first-order, Higuchian and Hixson–Crowell's models. Zero-order kinetics show linear relationship between amount released and the time (Eq. (1)). Constant release rates can be achieved with systems following this model. First-order kinetics (Eq. (2)) describes release kinetics of a CR system when the release of drug is proportional to the amount of drug remaining to be released. Higuchi described drug dissolution from matrix based modified release systems as a diffusion process and based on Ficks's law, square root time dependent expression was derived (Eq. (3)) [14,15]. Hixson–Crowell described a model (Eq. (4)), assuming the release rate to be limited by the drug particles dissolution rate and not by the diffusion that might occur through the polymeric matrix [16]. This model recognizes that the particle regular area is proportional to the cubic root of its volume and can be applied where surface diminishes during the dissolution.

$$Q_t = K_0 t \tag{1}$$

$$\ln Q_t = \ln Q_\infty + K_1 t \tag{2}$$

$$Q_t = K_H t^{1/2} \tag{3}$$

$$Q_\infty^{1/3} - Q_t^{1/3} = K_{HC} t \tag{4}$$

where Q_t is the amount of drug released in time t, Q_∞ is the initial amount of drug and K_0, K_1, K_H and K_{HC} are release rate constants for zero-order, first-order, Higuchi and Hixson–Crowell equation, respectively. Dissolution data were fitted to these models and regression analysis was carried out. The criterion for selecting the most appropriate model was based on best goodness-of-fit.

2.2.7. Comparison of profiles

Differences in the release profiles between batches and with reference profile were evaluated using similarity factor, f_2 value (Eq. 5). f_2 value is a logarithmic reciprocal square root transformation of one plus the average mean squared differences in percentage dissolved between the test (T_i) and reference (R_i) products over time points (n).

$$f_2 = 50 \times \log\left\{\left[1 + (1/n)\sum_{j=1}^{n}|R_j - T_j|^2\right]^{-0.5} \times 100\right\} \tag{5}$$

The Center for Drug Evaluation and Research (FDA) suggests that two dissolution profiles can be declared similar if f_2 is between 50 and 100 [17].

2.2.8. Histopathology studies

2.2.8.1. Animals and legal prerequisites. Female SD rats (250–270 g) were used to examine the biocompatibility of the polymer. Anesthesia, surgical and injection procedures were justified in detail and were approved by Institutional Animal Ethics Committee (IAEC, NIPER). All the animals were housed individually in plastic cages in a controlled environment (22–24 °C and 12:12 light/dark cycle) with free access to food and water. The study complied with local and federal requirements for animal studies.

2.2.8.2. Biocompatibility of polymers. Biocompatibility of polymers in the form of blank microspheres (of representative polymer 3) was studied in female SD rats. Rats were divided into two groups, one group acts as vehicle treated and the other as polymer treated. Blank microspheres (50 mg) suspended in 0.5 ml of normal saline was injected in the subcutaneous tissue of the upper site of the shaved rat back with the help of 22 gauge needle. Vehicle treated group was injected with equal volume of normal saline. At 1, 3, 7 and 21 days following injection, the animals were sacrificed. Incision was made at injection area to remove the tissue near the site which was then stored in 10% formalin solution in phosphate-buffered saline (pH 7.4) for about 48 h. The tissues were dehydrated by placing them in gradiently increasing concentration of absolute alcohol and xylene. The anhydrous tissue samples were then embedded in the paraffin blocks. Thick sections (5 μm) of the tissue were cut with the help of microtome. Sections were then processed for hydration and finally staining was performed with hematoxylin–eosin dye. Sections of tissue samples were then observed under microscope [18,19] and evaluated for different histological changes.

3. Results and discussion

3.1. Polymer synthesis

The polymers were synthesized with different ratios of SA and LA, the details of which are described elsewhere [9]. Table 2 summarizes the physical properties of the synthesized copolymers. The yield, calculated on the basis of starting material, of the copolymers was between 77% and 93% which increases with the increase in the sebacic acid content. The difference in yield may be due to the difference in molecular weight of the monomers. Molecular weight of lactic acid is 90 Da while that of sebacic acid is 202 Da. For condensation of every two lactic acid molecules, there is 10% reduction in the molecular weight of the diad while for condensation between every two sebacic acid molecules; there is only 4% reduction in the molecular weight of the diad. Hence, with increase in the sebacic acid component, the yield of the synthesis increases.

Table 2
Physical properties of the synthesized polymers and copolymers with varying ratios of PLA and PSA

Polymer	Yield (%)	M_w (10^3 Da)	M_n (10^3 Da)	T_g (°C)	Melting point (°C)
1	77.1	3.1	1.6	21	–
2	80.3	6.0	3.3	–	60–64
3	85.3	9.6	4.4	–	78–82
4	89.1	15.5	9.0	–	80–84
5	93.2	9.0	5.8	–	80–84

Table 3
Physical properties of MTX loaded microspheres

Polymer	Yield (%)	Encapsulation efficiency	Mean diameter (μm ± SD)
1	53.7	44.3	39.6 ± 15.3
2	71.6	64.1	37.0 ± 23.9
3	82.9	57.4	44.1 ± 12.4
4	84.1	46.4	71.6 ± 19.1
5	89.5	64.5	43.3 ± 8.4

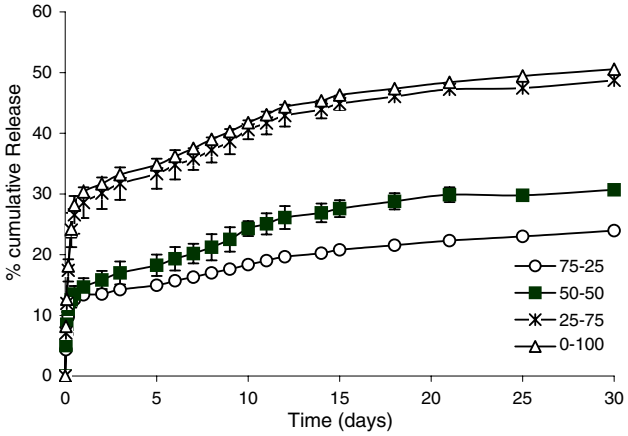

Fig. 2. Drug release profiles of methotrexate in water from microspheres of copolymers obtained from different ratios of LA and SA monomers. Ultra pure water (obtained by reverse osmosis; SG, Germany) was used without any pH adjustments for dissolution studies. Microspheres were prepared from copolymers obtained by polymerization of 75:25, 50:50, 25:75 and 0:100 ratios of LA:SA monomers. Dissolution studies were carried out at 37 °C under shaking. Each data point indicates mean ± SD of three replicates.

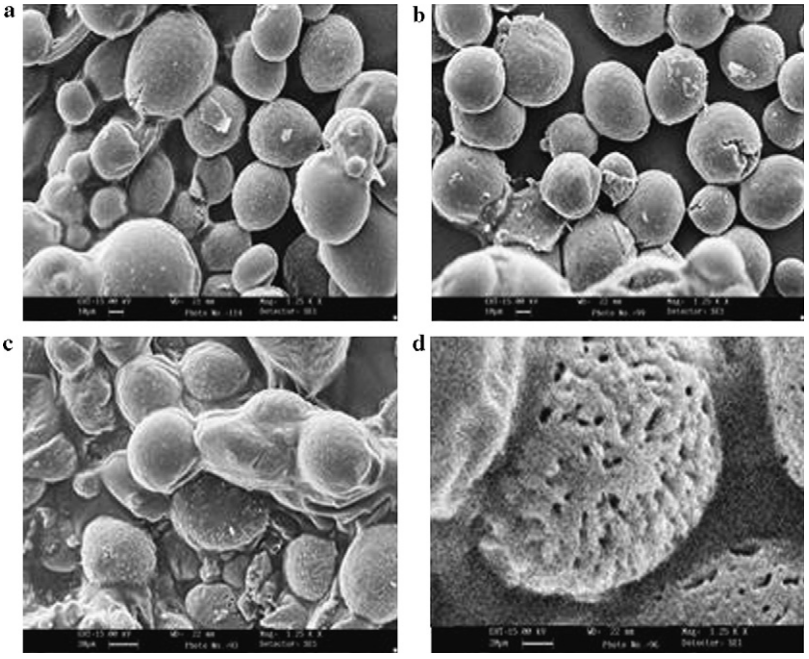

Fig. 1. The surface morphology of microspheres of PLA–PSA, 50:50 following degradation in phosphate buffer (pH 6.5) at 37 °C. (a) day 1; (b) day 2; (c) day 4; (d) day 8.

3.2. Polymer characterization

The polymers were synthesized in the molecular weight range of 3000–15,000 Da. However, the molecular weight of the copolymers was seen to increase with the increase in PSA ratio with the exception of polymer 5, with polymer 4 showing the highest average molecular weight of 15,500 Da. This was due to chain terminating action of the activated OH end group of PLA. IR spectroscopy provides a convenient means of appraising the effectiveness of the condensation reaction. FTIR spectra showed the presence of both, ester as well as anhydride peaks, in the region of 1810–1740 cm^{-1} for all the copolymers, the intensities corresponding to the ratio of the respective components. The ^1H NMR ($CDCl_3$, δ) showed the peaks of the equivalent protons from the repeating units of lactic acid and sebacic acid. In DSC, polymer 1 showed a glass transition temperature of 26 °C with no other peak observed due to the fact that PLA, obtained from pharmaceutical grade D,L-lactic acid, is amorphous and its melting point cannot be defined [20]. Polymer 2 showed a broad melting peak (due to the high content of amorphous substance) ranges from 53 to 70 °C with peak at 66 °C. Polymers 3–5 were relatively high melting copolymers with melting point in the range of 80–84 °C due to the semicrystalline nature of the polymer due to PSA component.

3.3. Microsphere preparation

MTX loaded microspheres were prepared by emulsion-solvent evaporation method. The physical properties of MTX loaded microspheres are given in Table 3. The yield was in the range of 50–90% and increased with increase in the molecular weight of the polymer with the exception of polymer 5. Polymer 1 gives a very low yield of the microspheres and hence could be included only in few of the further studies. The low yield of polymer 1 microspheres may be due to the amorphous nature and low molecular weight of the polymer which is about 3000 Da. Similarly, the yield of the microspheres increases with an increase in molecular weight of other polymers. Polymer 2 (M_w 6000 Da) microspheres show the yield of 71% while polymer 3 (M_w 9600 Da) gives the yield of 82.9%.

The particles were in the size range of 40–60 μm obtained from particle size analyzer and it was also evident from SEM scale bar at 5 KX and 1.25 KX magnifications. Average particle size increased with increase in molecular weight, with polymer 4 having highest M_w of 15,500 Da giving the largest particle size of 71.64 μm. The increase in mean particle sizes of the microspheres with increasing polymer molecular weight may be due to the difference in the viscosity of polymer solutions in methylene chloride. For a given rate of stirring, higher viscosity of the organic phase of polymer solution would result in an increased resistance of the organic phase droplets to shear stress and break up, resulting in larger microspheres. Liang et al. [12] have reported the similar trend in particle size with increase in molecular weight.

Encapsulation efficiency of MTX loaded microspheres was found to be between 45% and 65% and was independent of different copolymer ratios. Encapsulation efficiency for all the microspheres was not very high because of the relatively lower molecular weight of the polymers

Table 5
Comparison of the drug release form microspheres prepared with copolymers obtained by polymerization of monomers LA and SA at different ratios

Copolymer	f_2 value
2	34.08
3	39.43
4	87.82
5	100.00

f_2 value were calculated taking polymer 5 (100% SA) as reference. Drug release was found to increase with increase in the ratio of monomer SA.

Table 4
Drug release kinetics of methotrexate from microspheres formulated from LA and SA copolymers in water

Release duration	Zero-order		First-order		Higuchi	
	K_0 (day^{-1})	R^2	K_1 (day^{-1})	R^2	K_H ($day^{-1/2}$)	R^2
Polymer 2						
0–0.5 day	16.91	0.8291	16.97	0.9902	1.24	0.9214
0.5–30 days	0.40	0.9369	0.341	0.9815	0.10	0.9452
Polymer 3						
0–0.5 day	15.95	0.7849	25.32	0.9772	1.46	0.9390
0.5–30 days	0.60	0.8736	0.232	0.9631	0.13	0.9490
Polymer 4						
0–0.5 day	40.85	0.9121	38.16	0.9954	1.38	0.9529
0.5–30 days	0.74	0.8741	0.291	0.9810	0.10	0.9576
Polymer 5						
0–0.5 day	41.66	0.9407	48.57	0.9997	1.31	0.9657
0.5–30 days	0.74	0.8879	0.287	0.9893	0.10	0.9610

(3000–15,000 Da) and therefore there was not much of a difference found in the encapsulation efficiency among the different polymers.

3.4. In vitro degradation of polymers

The degradation of the polymers was qualitatively evaluated using FTIR, GPC and SEM. FTIR showed the presence of acid peak on degradation which was shown to intensify with time of degradation while anhydride peak was observed to decrease. GPC showed the molecular weight loss during degradation. M_w decreased rapidly during first two days which was more sharp in case of polymer containing higher ratio of PSA, i.e., polymer 5. After 2 days, the decrease in molecular weight was very slow and almost constant for about 15 days, where the molecular weight was reduced to 1000–1500 Da. M_w of polymer 2 decreased after a lag time of 1 day which may be due to the relative stability of the ester bonds compared to anhydride bonds. The morphological change after 1, 2, 4 and 8 day of degradation was seen from SEM analysis as shown in Fig. 1. After day 1, there was not much signs of degradation. Day 2 picture showed the development of cracks on the surface. Day 4 picture showed the presence of crystals on the surface which could be small oligomers eroding during degradation. After 8 days of degradation, polymer showed a porous surface indicating polymer erosion.

3.5. In vitro MTX release

In vitro release retarding performance of microspheres prepared by solvent evaporation method using copolymers obtained from different ratios of LA and SA was studied in water. MTX release profile for 30 days dissolution is given in Fig. 2, and the rate kinetics obtained by fitting release profiles in various kinetic models is given in Table 4. Methotrexate release was found to be consistently retarded and not more than 50% of the drug was released from all the copolymeric microspheres in a span of 30 days. It is obvious from the results that microspheres prepared from copolymer containing increasing proportion of SA are relatively fast releasing. No change in the drug release rate was found when the SA proportion was more than 75%. Copolymer with predominant (75%) LA proportion provided high retardation. This could be again due to relative stability of the ester bonds compared to anhydride bonds. It is interesting to note that all the formulations provided a biphasic release with initial burst effect until 0.5 day, and the release rates drastically reduced after 1 day of study initiation. This effect can be ascribed to the drug release from the surface before the polymer erosion started. An initial burst of drug followed by constant release is desirable to reach a therapeutic concentration and maintain the level by compensating for metabolic loss thereafter.

In order to provide better understanding of kinetics, release data were split as 0–0.5 day and 1–30 days (Table 4). Kinetic profiling indicated that drug release followed first-order kinetics in both the phases of 0–0.5 day and 1–30 days. The difference in release rates of initial burst and constant release (1–30 days) was found to be about 50-fold in case of polymer 2 and about 169-fold for polymer 5. Considering, first-order release rate from 0 to 0.5 day time, the rate and extent of release was also found to be dependent on the monomer ratio used for copolymer synthesis.

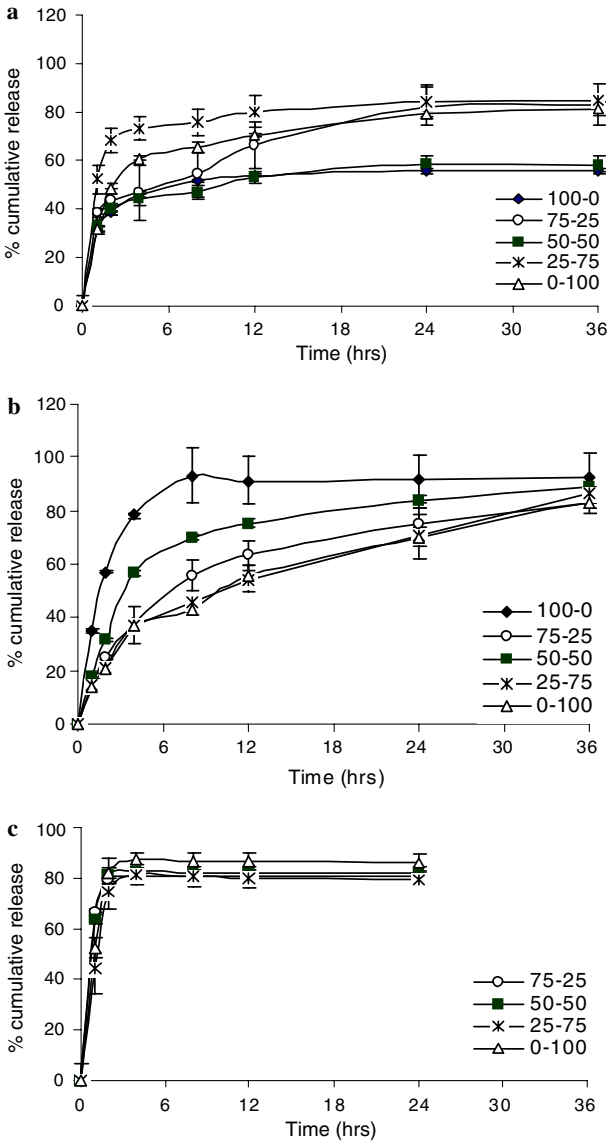

Fig. 3. Methotrexate release in: (a) pH 7.4, 0.01 M phosphate buffer; (b) pH 6.5, 0.01 M phosphate buffer; and (c) pH 6.5, 0.1 M phosphate buffer, from microspheres of copolymers obtained from different ratios of LA and SA monomers. Microspheres were prepared from copolymers obtained by polymerization of 100:0, 75:25, 50:50, 25:75 and 0:100 ratios of LA:SA monomers. Dissolution studies were carried out at 37 °C under shaking. Release studies were stopped at 36 h since the drug is completely released in all the formulations. Each data point indicates mean ± SD of three replicates.

To compare the release profiles similarity factor (f_2 value) was calculated taking release from microspheres prepared using polymer 5 (100% SA) (Table 5) as reference. Results substantiate the effect of monomer ratio in determining the efficiency of copolymer in retarding the drug release. Release profiles matched when the microspheres were formulated from copolymers predominantly containing SA (i.e., >75%). As the LA proportion increased, release was statistically dissimilar with the reference. Overall, release studies in water indicated that drug release can be manipulated to achieve clinical requirements, based on the optimization of monomer ratio.

3.5.1. Effect of dissolution medium on the drug release

Methotrexate release from microspheres obtained from different copolymers was found to be highly influenced by the dissolution medium composition. In pH 7.4 (0.01 M phosphate buffer) medium, the rate of drug release was found to increase with increase in SA component of the copolymer. However, inverse was observed when the dissolution studies were carried out at pH 6.5 (0.01 M phosphate buffer) medium (Fig. 3). Microspheres prepared from polymer containing 100% of LA monomer showed complete release (about 93%) by 8 h in pH 6.5 (0.01 M phosphate buffer) medium, but only about 42% was released from microspheres prepared from polymer containing 100% of SA monomer (Fig. 3). Further, except for polymer 3, drug release significantly reduced with increase in SA at pH 6.5. Release profiles in buffers reasonably fitted to only Higuchi and first-order models (Table 6). Higuchi and first-order release rate constants also showed a similar decrease with increase in SA ratio in pH 6.5 (0.01 M phosphate buffer). Moreover drug release study in phosphate buffer at pH 7.4 had shown similar trend in release profile as in water, although the release rate was faster. It confirms the effect of pH on MTX release trend for the polymers with different ratios of SA and LA.

The effect of increased dissolution with change in proportions of monomers in copolymer can be attributed to the difference in their ionization nature at different pH values. SA has a relatively high solubility at alkaline pH and thus the release from copolymer is faster under more alkaline conditions, which could contribute to the

Table 7
Effect of buffer concentration on equilibrium solubility: methotrexate solubility in three different media

Media	Solubility (μg/ml)
Water	0.0593
0.01 M phosphate buffer (pH 6.5)	0.4018
0.1 M phosphate buffer (pH 6.5)	1.1837

Solubility study was carried out in ultra pure water and phosphate buffer (pH 6.5) of different buffer concentration. Excess MTX was added to the media and allowed to equilibrate, centrifuged and supernatant analyzed for drug concentration. The readings were taken in triplicate.

Table 6
Effect of pH and ionic strength of the dissolution media on the drug release kinetics of methotrexate from microspheres formulated from LA and SA copolymers

Dissolution medium	Zero-order		First-order		Higuchi		Hixson–Crowell	
	K_0 (h^{-1})	R^2	K_1 (h^{-1})	R^2	K_H ($h^{-1/2}$)	R^2	K_{HC} (h^{-1})	R^2
Polymer 1								
pH 7.4, buffer (0.01 M)	0.9391	0.4042	0.5962	0.9578	0.1374	0.6708	0.02	0.9078
pH 6.5, buffer (0.01 M)	1.7065	0.4068	1.081	0.9998	0.4284	0.9947	0.0218	0.8442
pH 6.5, buffer (0.1 M)	–	–	–	–	–	–	–	–
Polymer 2								
pH 7.4, buffer (0.01 M)	1.7279	0.6801	0.279	0.9950	0.1175	0.9309	0.0146	0.9815
pH 6.5, buffer (0.01 M)	2.6597	0.7564	0.369	0.9397	0.2303	0.9563	0.0349	0.9913
pH 6.5, buffer (0.1 M)	–	–	–	–	–	–	–	–
Polymer 3								
pH 7.4, buffer (0.01 M)	0.9345	0.7100	0.427	0.9877	0.1194	0.9967	0.023	0.8778
pH 6.5, buffer (0.01 M)	13.315	0.9341	0.795	0.9764	0.3026	0.9269	0.0469	0.9502
pH 6.5, buffer (0.1 M)	–	–	–	–	–	–	–	–
Polymer 4								
pH 7.4, buffer (0.01 M)	–	–	–	–	–	–	–	–
pH 6.5, buffer (0.01 M)	4.3288	0.9215	0.234	0.9258	0.1794	0.9884	0.0156	0.9628
pH 6.5, buffer (0.1 M)	–	–	–	–	–	–	–	–
Polymer 5								
pH 7.4, buffer (0.01 M)	8.148	0.8363	0.660	0.9132	0.1622	0.9420	0.0353	0.8235
pH 6.5, buffer (0.01 M)	4.4042	0.9331	0.1067	0.9743	0.1494	0.9901	0.0171	0.9670
pH 6.5, buffer (0.1 M)	–	–	–	–	–	–	–	–

R^2, is the correlation coefficient; K, is the release rate constant for respective models. '–' Drug release kinetics were not calculated when more then 60% drug released before 4 h.

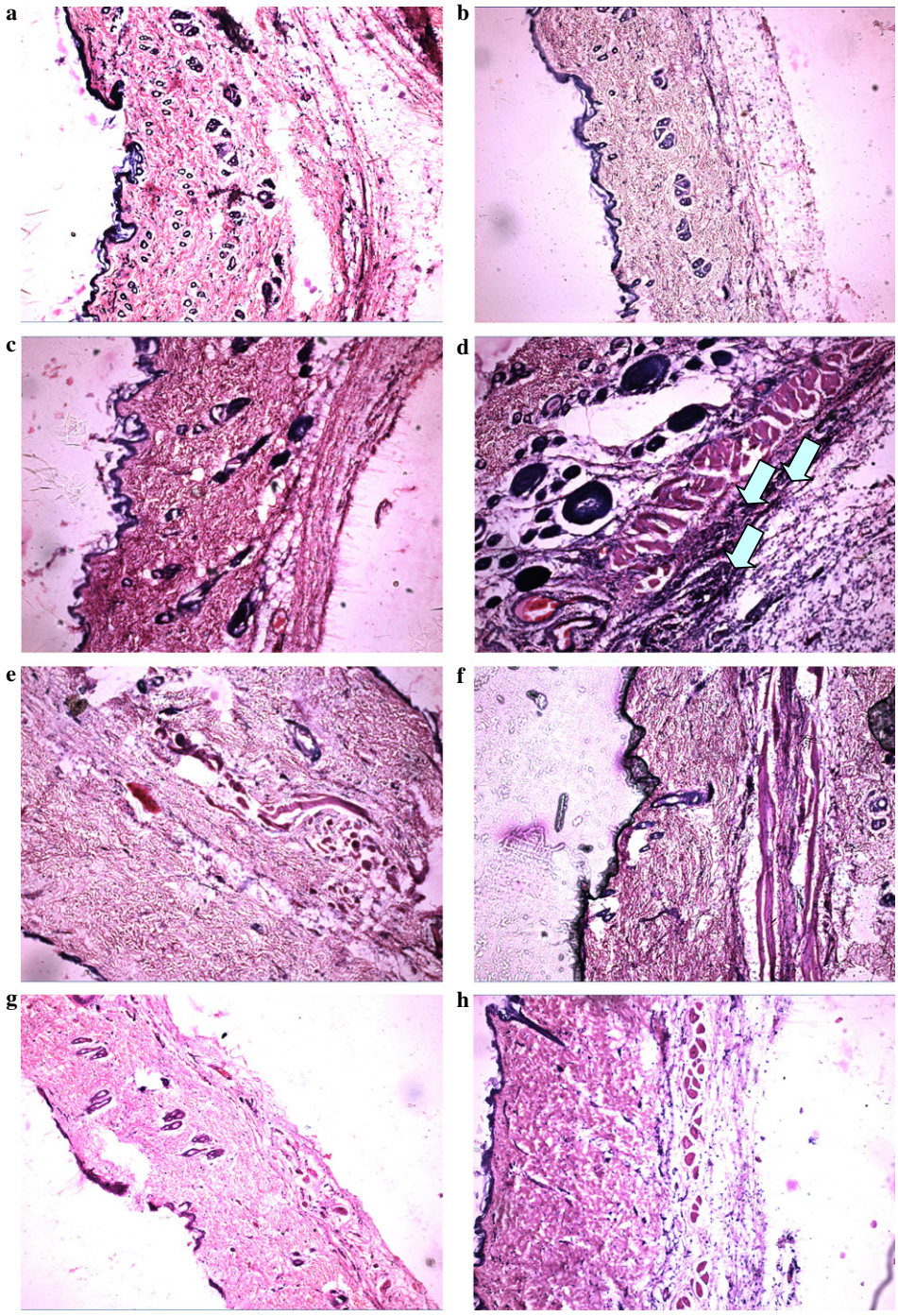

Fig. 4. Representative histopathological sections noted of the tissue at the site of injection in vehicle and polymer treated group. (a) 1 Day vehicle treated; (b) 1 day polymer treated; (c) 3 days vehicle treated; (d) 3 days polymer treated; (e) 7 days vehicle treated; (f) 7 days polymer treated; (g) 21 days vehicle treated; (h) 21 days polymer treated. Minimal reaction after one day but subacute reaction sets in and maximum inflammation can be seen after 3 days of injection, indicated by arrows. The reaction subsided with time and disappeared after 21 days; compare (g) and (h).

increasing release rate and extent at pH 7.4 over pH 6.5 from polymer 5 [21]. Further, the higher dissolution rate of methotrexate from polymer 1 microspheres in pH 6.5 medium over pH 7.4 can be ascribed to the establishment of ionic interactions between the ionized carboxyls of the polymer. Similar pH effect was also reported for poly(methylvinyl ether-alt-maleic anhydride) and methotrexate complexation [22]. Although the release was slow in the medium of lower pH (water), the observed difference is too high to quantitatively attribute to the effect of pH on disintegration and release of SA from the copolymer matrix. This may further be substantiated by the high difference in dissolution for polymer 1, which as such has no SA.

Increase in buffer concentration showed a marked difference in the drug release characteristics from all the copolymers (Fig. 3) [23,24]. Methotrexate release was complete within 2 h in pH 6.5 (0.1 M phosphate buffer) medium. Considering the results of dissolution studies in all the media, it is obvious that both pH and buffer concentration of the medium have a direct effect on the system's performance. Differences in release could be due to the effect of these factors on the solubility of methotrexate or on the physicochemical properties of the polymer that regulate the drug release.

To estimate the role of methotrexate solubility on the drug release in different media, equilibrium solubility studies were carried out in water and buffers with salt concentration of 0.1 and 0.01 M, keeping the pH constant. Solubility of methotrexate in water was found to be 0.059 mg/ml, but increased to 1.183 mg/ml in 0.1 M phosphate buffer (Table 7), indicating that the observed effect of drug release is in part a result of increase in methotrexate solubility with increase in buffer concentration. The rapid release of methotrexate in 0.1 M phosphate buffer (pH 6.5) can be explained by their release mainly due to diffusion through pores and channels generated by the erosion of the polymer resulting from the attack of water. Also, this rapid release can further create more pores and channels for release [25–28]. On the other hand, due to poor solubility of methotrexate in water, it is less likely to dissolve in water and diffuse through the pores and channels which may become the rate limiting step for their release from the device. The aqueous diffusion boundary layer offers resistance to dissolution and hence their release is controlled by polymer erosion to a minor extent. Thus, it can be said that diffusion and dissolution properties of loaded drug along with polymer erosion play a significant role in overall release, implying PLA–PSA undergoes surface as well as bulk erosion. Purely surface eroding polymer would enable one to obtain release independent of the properties of the loaded compounds [29]. Solubility in addition to erosion rate was found to influence the release rate as a function of pH and the nature of the encapsulated drug. However, based on the quantitative differences in the dissolution rates in media with different buffer concentration, it may be inferred that drug as well as polymer characteristics affect the release behavior.

3.6. Biocompatibility of polymers

All animals were healthy throughout the experiments, as judged from the body weight of the treated rats during the course of experiments and no gross pathological changes at the site of injection. Histological evaluation indicated that in the animals injected with blank polymer, the degree of inflammation was generally not higher than the control group, suggesting no significant adverse effects upon injection of the polymer (Fig. 4). Different components of tissue reactivity like density of lymphocytes, indicating the state of inflammation (acute and chronic), fibroblastic proliferation, collagen formation, presence of foreign body, abscess, necrosis or any other were compared at four different time periods and were arbitrarily given the grades. The mean score of histological findings and representative photographs are shown in Table 8 and Fig. 4, respectively.

The histopathological examination of the excised tissues surrounding the injection site revealed weak acute inflammatory response, evident by presence of eosinophils and polymorphs (Fig. 4b). The tissue slides of animal treated with blank microspheres showed the presence of some spherical shiny foreign particles in the subcutaneous tissue which were discerned as microspheres. The inflammatory response observed was highest after 3 days (subacute response) (Fig. 4d) of injection could be considered as a typical reaction produced by a foreign body, which is resolved after a short time (Figs. 4f and h) owing to the microsphere biodegradation. Moreover, no adverse reactions (calcification, necrosis, tumorigenesis, and infection) were observed at the injection site designate the biocompatibility of the polymer samples. These findings clearly indicate that PLA–PSA microspheres are well tolerated *in vivo* at the site of injection.

Table 8
Mean score of histopathological findings

Type of reaction	After 1 day		After 3 days		After 7 days		After 21 days	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Inflammation								
Acute	Nil	+	Nil	+++	Nil	Nil	Nil	Nil
Chronic	Nil	++	Nil	+	Nil	Nil	Nil	Nil
Fibroblastic proliferation	Nil	+	++	++	Nil	++	Nil	++
Collagen formation	Nil	Nil	+	+	Nil	++	Nil	Nil
Foreign body	Nil	+	Nil	+	Nil	Nil	Nil	Nil
Any other	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Animals were sacrificed 1, 3, 7 and 21 days following injection. The severity was determined by designating severity score to the control and treated animal. The condition was predefined but not observed in any of the tissue section. The reactive inflammatory changes were assigned severity grade: Nil, no reaction observed; +, mild inflammation; ++, diffused moderate; +++, high; ++++, necrosis along with inflammation.

4. Conclusions

Synthesis of pure low molecular weight poly(lactic acid) from cheap resource, free from catalysts and solvents, was achieved which is highly desirable for medical applications. Copolymers were synthesized in the molecular weight range of 3000–15,000 Da with low polydispersity index between 1 and 2. The synthesis was supported by GPC, FTIR and ^{1}H NMR characterization. Degradation was trailed by FTIR, GPC and SEM, for morphological changes during degradation using. The synthesized polymers were formulated into microspheres by solvent evaporation method and MTX encapsulated efficiency of 45–65% was obtained. Particles were in the size range of 40–60 μm, size observed to increase with increase in molecular weight. MTX release from microspheres was studied with kinetic modeling and found to be affected by the pH and buffer concentration of the release medium. The histopathological studies in animals showed no adverse reaction at the local site of injection. The study offers the number of biodegradable materials that may be used for tailored drug release in various applications focusing on the localized delivery.

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