FORMULATION OF AN INJECTABLE IMPLANT FOR PEPTIDE DELIVERY AND MECHANISTIC STUDY OF THE EFFECT OF POLYMER MOLECULAR WEIGHT ON ITS RELEASE BEHAVIOR

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ABSTRACT

The effects of polymer molecular weight on drug release from erodible matrices are not well known. It would be more complicated for in-situ forming injectable implants that change gradually from liquid to solid after injection. To investigate this phenomenon, two commercially available PLGA polymers (lactic acid-co-glycolic acid) with molecular weights of 12000 and 48000 Da were used to prepare injectable implants containing leuprolide acetate as a model peptide. The influence of polymer molecular weight on the morphology and erosion of matrices and also on their in-vitro drug release behavior over a period of 28 days was investigated. Results showed that the amount of drug released (32%) over the first 24 hours (burst phase) for 12 kDa PLGA system, was significantly ($P<0.05$) higher than that of the one higher molecular weight (13%). There was no difference between the steady-state release fluxes of drug from the systems. Erosion profiles were also in agreement with those of release behavior in both burst and steady-state phases. Electron microscopy studies showed that the lower molecular weight system is more porous than the higher one, which can explain the difference between burst effects.

Keywords: Injectable implant; Poly (lactide-co-glycolide); Molecular weight; Release; Erosion; Leuprolide acetate

INTRODUCTION

Injectable implants, as novel drug delivery systems, look very promising in protein drugs delivery (1-5). These systems are liquid, which are injected subcutaneously or intramuscularly and deform to semisolid or solid matrices when in contact with aqueous fluids in body or release media and release their drug in a controlled manner (6). Matrices can be prepared by different methods including polymer precipitation by solvent removal. These systems are generally composed of polymer(s) or copolymer(s), organic solvents, drugs and other additives. Polymers and copolymers such as polylactides, polyglycolides or poly (lactide-co-glycolic acid) that are soluble in organic solvents and insoluble in water are able to form solid matrices and thus are very suitable for such systems (7,8).

Release rate of drugs from these systems may be affected by different polymer properties such as the polymer type (9, 10), concentration (9, 11, 12) and molecular weight (9, 10, 13). Besides the diffusion process, degradation also plays an important role in drug release from biodegradable/erodible drug delivery systems. These systems degrade by different mechanisms including heterogeneous, homogeneous, acid-Catalyzed, and polymer degradation via autocatalysis (14-16). The last mechanism is suggested for poly (lactide–co glycolic acid, PLGA), which was used in the present study.

Another important and crucial parameter that may affect the drug release is the morphology of the system. which depends on sol-gel phase inversion induced by polymer precipitation through solvent removal, temperature or other mechanisms.

Conditions such as coagulation bath composition, polymer solution composition, additive composition and polymer type are important in formation of the desirable system after or during the phase inversion (17).

All of the above mentioned mechanisms depend on polymer molecular weight. It is usually supposed that higher molecular weight of polymer leads to slower release rates of drugs. From a study (9) on the effect of polymer molecular weight on the release of tumor necrosis factor receptor incorporated together with bovine serum albumin, it has been found that the higher molecular weight causes faster solidification of the system and therefore, decreases diffusion and release rates of proteins in-vitro. In another study (13) it was found that the amount of naltroxone released from an injectable implant shows a

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parabolic relationship with molecular weight of polymer and it has been suggested that a polymer system can be produced with an optimum polymer molecular weight range for the release of drug over a selected length of time.

Thus, the relationship between polymer molecular weight and release is complicated and requires more investigation. In this study the effect of polymer molecular weight on release of a model peptide, leuprolide acetate which has low oral bioavailability and is a good candidate for parentaral drug delivery was investigated.

MATERIALS AND METHODS

Poly lactide-co-glycolic acid (PLGA) 50:50 copolymers, Resomer® RG 504H (MW= 48 kDa) and Resomer® RG 502H (MW=12 kDa), were obtained from Bohringer Ingelheim, Germany. Leuprolide acetate was purchased from Bachem Inc. (Switzerland). Bio - Rad DC Protein Assay II kit was supplied by Bio-Rad Laboratories (Italy) and 1–methyl-2-pyrrolidone (NMP) was purchased from Merck (Germany). Other chemicals were obtained commercially and all were analytical grade reagents.

Preparation of Solutions

Formulations of each polymer were prepared separately. Each formulation was composed of 33% (w/w) polymer and 3% (w/w) leuprolide acetate dissolved in NMP. These formulations were liquid and solidified when came in contact with aqueous media.

Release Studies

Release studies were performed in a home-made diffusion cell at 37°C. In each release study, 0.2g of formulation was placed in donor compartment which was separated from the receptor phase by a mesh. This mesh allows the solvent to exchange (by diffusion) which is necessary for matrix formation. Ten ml of phosphate buffer (0.03 M, pH 7.4) containing 0.01% (w/v) sodium azide and 0.02% (w/v) Tween® 80 was used as the receptor medium according to the published methods (18, 19). The receptor phase was stirring at 100 rpm throughout the experiment.

In predetermined time intervals, 1 ml of the receptor phase was withdrawn using a 0.2 µm Watman filter assisted by 21g needle/2cc plastic syringe assembly. The withdrawn receptor phase was replaced with 1ml fresh receptor medium. The amount of released leuprolide acetate was then determined by Bio-Rad DC Protein Assay II kit, as explained below. Release studies were performed at 37°C for 28 days. Samples were kept frozen until analysis.

Drug Determination by Bio-Rad DC Assay Kit

The Bio-Rad DC-Protein assay is a colorimetric assay based on modified Lowry protein assay method (20) following detergent solubilization. The assay is based on the reaction of peptide with an alkaline copper tartrate solution and Folin reagent. As with Lowry assay (18), there are two steps which lead to color development: the reaction between peptide and copper in an alkaline medium, and subsequent reduction of Folin reagent by the copper-treated peptide (21).

The amount of drug in samples was determined by this method using a calibration curve over the range of 10-200 µg/ml of peptide solution. The linearity, accuracy, precision, detection limit and quantitation limits of this method was also determined in the present study.

PLGA Erosion Studies

The polymer erosion was studied using two different methods as follows. (a): L-lactic acid detection (22) was studied with Randox kit which is based on a colorimetric method for enzymatic determination of L-lactate using a Perestige 24i at 546 nm. (b): pH change study (23) which was performed using Metler pH-meter (Switzerland) with an attached microelectrode.

Scanning Electron Microscopy

Samples of each formulation were injected into aqueous medium and held at 37°C for 3 days. The solidified matrices were then fractured in liquid nitrogen and vacuum-dried at 25°C for 1 day. The morphology of the matrices was then analyzed by scanning electron microscope (Cambridge S360) after gold coating of sample (12).

Statistical Analysis

A Student t-test for unpaired samples supported by SPSS 10 for Windows (SPSS, Inc, USA) was used for comparing the burst release in two formulations. Also nonlinear regression was used to analyze statistically the release rates (the slope of the amount of drug released versus time profiles), and lactic acid formation.

RESULTS AND DISCUSSION

Validation of the Assay Method

The data of assessment of the validity of the Bio-Rad DC assay method are shown in Table 1. Linearity was evaluated by determining five working standard solutions containing 10-200 µg/ml of leuprolide acetate in triplicates. The validation of Bio-Rad DC assay method was confirmed. The regression data, as shown in Table 1, showed a good linear relationship over the concentration range of 10-200 µg/ml. The limit of detection (LOD) and limit of quantitation (LOQ)

were determined based on standard deviation on the blank with 3:1 and 10:1 ratios respectively. The LOD and LOQ were found to be 17.37 and 52.65 µg/ml, respectively. The accuracy of this method was in the range of 80 to 120% of target concentration. The repeatability of sample application were expressed in terms of relative standard deviation (R.S.D.) and found to be 2.78%.

Scanning Electron Microscopy Studies

Figure 1 shows scanning electron micrographs (SEM) of surface of two solidified formulations, 3 days after injection into release media. As it is shown, the morphological structures of two systems are different and the lower molecular weight polymer formulation shows higher porosity and pore diameter than the one with higher molecular weight.

Release Studies

The release profiles of leuprolide acetate from both systems are shown in Figure 2. Both systems showed an initial burst phase (Phase I) followed by slower drug release patterns. The amount of the drug released in the initial burst phase was decreased by about 2.5 times by increase in polymer molecular weight. The cumulative amount of drug released (32%± 0.48%) over the first 24 hours (burst phase) for the system which was prepared with 12 kDa PLGA, (mean ± SD, n=3), was significantly ($P<0.05$) higher than that of the polymer with higher molecular weight (48 kDa), 13% ± 0.078% (mean ± SD, n=3).

As it is seen in Figure 2, the release profile after the burst phase (phase I) can be divided into three phases with different release rates. Phase II starts with formation of the polymeric matrix (setting) that covers the system initially as a film and grows toward the center of the system. As it is shown in erosion studies, release of drug from the system in this phase should be mainly controlled by passive diffusion of drug through the formed matrix. Therefore, this phase can be called diffusion phase.

The next phase (phase III) is related to polymer degradation, based on erosion results that will be discussed later, drug release in this phase is apparently controlled by erosion. Non-linear analysis of phase III for both systems is shown in Table 2. Results show that there are no significant differences between the phase III release rates (slopes) of high and low molecular weight polymer-containing systems (K = 0.85, Table 2). This phase starts with glycolic acid formation accompanied with lactic acid formation in later stage, based on our observation in erosion study (see Figure 4). Phase IV which is a depletion phase, shows similar behavior in both systems.

As it is shown by SEM (Figure 1), the lower MW polymer showed higher porosity than the other system. This could be the reason behind higher burst release of lower molecular weight system. The membrane formation and also solidification time vary between systems and might be considered as an influencing factor on the morphology of systems. Our observations revealed that the lower molecular weight system solidifies faster than the higher molecular weight, which is in agreement with that has been reported for PLGA 50:50 implant (24).

It has been reported that addition of polyvinyl-pyrollidone as a hydrophilic polymer has a great influence on release rate and gelation rate (12). This mechanism also applies for two different molecular weight polymers used in this study. The lower molecular weight polymer is more hydrophilic than the other one, thus it has increased gelation rate and higher initial burst.

Viscosity of the solution is another important factor. The higher molecular-weight polymers in the solution will tend to give higher solution viscosity, thus decreasing the diffusion rate of peptide into the medium during the burst phase (8, 9). However the release rate from both systems in the steady-state phases (Phase III) are similar. The drug is water soluble. If we consider that a part of drug is dissolved in the liquid phase in the pores and the rest is embedded in the polymeric matrix, it is possible to explain the release behavior to some extent. After solidification of the systems, the drug dissolved in the pores releases (burst phase, Phase I). After this initial phase, which covers 30% of the total drug in the lower MW polymer and 13% in the higher MW system, diffusion of drug from the polymeric matrix (phase II) followed by degradation of the matrix (Phase III) become the rate-determining steps. Considering the amount of drug released in the burst and diffusion phases, drug concentration after phases I and II in 48 kDa molecular weight system is about 20% higher than that of 12 kDa polymer (see Figure 2). However the degradation of the higher Mw polymer is slower than the other one (as discussed later, Figure 3) and as result equal release rates (product of degradation rate and drug concentration) are observed.

Erosion Studies

Polyesters such as PLGA show sigmoidal degradation curve especially with higher molecular weight polymers. The product of degradation (oligomers or monomers) affect acid catalyzed hydrolysis of the ester linkages (14). Here, the difference in the PLGA erosion between two polymers is determined by monitoring the pH changes in pH and lactic acid formation.

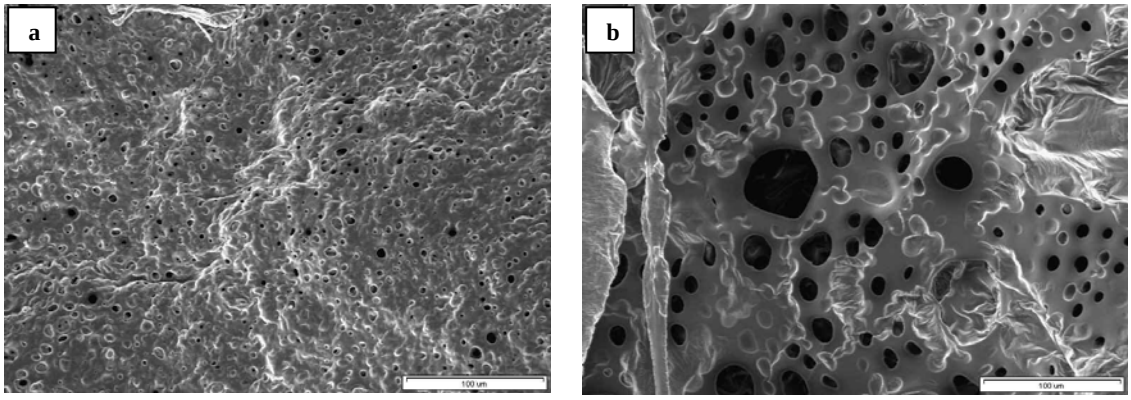

Figure 1. Surface morphologies of solidified in-situ gel-forming PLGA formulations 3 days after incubation in release medium as studied by SEM. (a) Resomer® RG 504H (Mw = 48 kDa) and (b) Resomer® RG 502H (Mw = 12 kDa). Magnifications are equal.

Table 1. Summary of Bio-Rad DC assay method validation parameters evaluated over the range of 10-200 µg/ml leuprolide acetate.

Parameter	Obtained Data
Specificity	Specific
Linearity range (µg/ml)	5-250
Correlation coefficient (r ± S.D.)	0.9994 ± 0.000294
Slope ± S.D.	0.00145 ± 0.000191
Intercept ± S.D.	0.008925 ± 0.004556
Limit of detection (µg/ml)	17.37
Limit of quantitation (µg/ml)	52.65
Recovery (n = 6)	94.36 ± 2.62
Precision (RSD %)	2.78

Table 2. Segmental regression analysis of phase III of leuprolide acetate release from systems made of two different PLGA polymers.

Polymer	Slope (release rate, % hr^{-1})		
	Estimate (mean)	95% confidence intervals	
		Lower	Upper
Slope of RG 502H	0.1527	0.1011	0.2043
Slope of RG 504H	0.1786	0.1270	0.2303
K=Slope of RG 502H/ Slope of RG 504H	0.8539	0.4747	1.2350

Table 3. Segmental regression analysis of lactic acid release from systems made of two different PLGA polymers.

Polymer	Slope (release rate, mg dL^{-1} hr^{-1})		
	Estimate (mean)	95% confidence intervals	
		Lower	Upper
Slope of RG 502H	0.1584	0.1044	0.2124
Slope of RG 504H	0.1884	0.1344	0.2424
K= Slope of RG 502H/Slope of RG 504H	0.8406	0.4662	1.2150

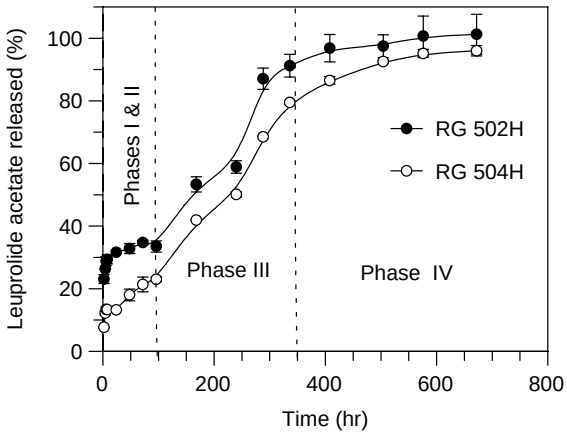

Figure 2. Profiles of release of the leuprolide acetate from systems prepared by two PLGA polymers of Resomer® RG 502H and Resomer® RG 504H, showing different release phases. The initial burst phase (Phase I) covers approximately the first 24 hours. Data are mean ± SD, n = 3

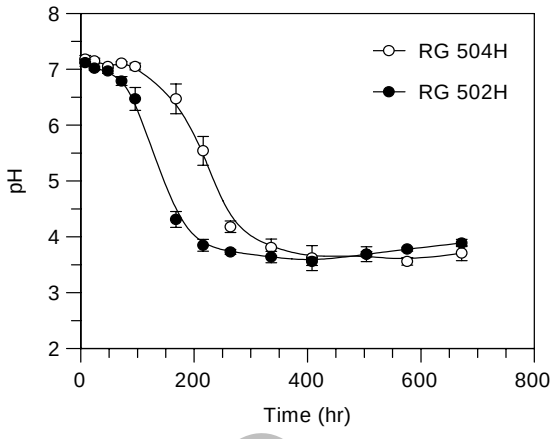

Figure 3. pH changes due to degradation of PLGA polymers in systems containing Resomer® RG 502H and Resomer® RG 504H. Data are mean ± SD, n = 3.

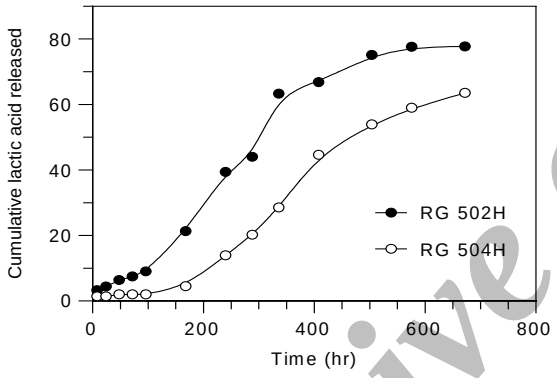

Figure 4. Profiles of lactic acid release due to degradation of PLGA polymers of Resomer® RG 502H and Resomer® RG 504H.

Figure 3 shows the changes in pH of 0.03M, pH 7.4 aqueous phosphate buffer as a function of incubation time. The pH remained almost constant over the first 100 hours for Resomer® RG 502H (MW = 12kDa) and over 110 hours for Resomer® RG 504H (MW = 48 kDa). In this phase, pH is constant and might be considered as an indication for the absence of degradation, as is confirmed by lactic acid release study. This phase is also in correlation with the first 100 hours of release profile. After this phase, both formulations showed marked decrease in pH of phosphate buffer solution which is probably related to formation of acidic monomers and oligomers such as lactic and glycolic acids. The pH dropped about 3 units over 5 days to a value of around 4 for both systems (Figure 3). As it is observed, the main difference between two systems is their onset of pH drop, which is shorter for lower MW polymers, which again could be due to its faster degradation and therefore higher concentration of degradation products (lactic and glycolic acid) (see Figure 4). This is in correlation with lactic acid release as discussed in the following paragraph.

As it is shown in Figure 4, formation of lactic acid is also different in two formulations. Both systems show lag-phases in lactic acid release which is again related to phase transition and setting of injectable implant, in which, the lactic acid release from lower MW system is about 7 times higher than that of the higher molecular weight system. After this initial lag-phase, both systems reach a steady state phase. The steady-state slopes, although slightly higher in the lower MW polymer (1.2 times), are statistically similar (Table 3). The presence of more ending groups in low molecular weight polymer induces higher hydrophilicity and hence water permeation. Water permeation is one of the main factors that affect polymer degradation, hence higher amount of lactic acid release from the lower Mw system. The pH drop behavior (Figure 3) and lactic acid release profile (Figure 4) are in good agreement with drug release profile (Figure 2).

CONCLUSION

Results of this study showed that molecular weight affects morphology and degradation of biodegradable polymer matrices and also release of drugs from such systems. A polymer with a higher molecular weight might provide a stronger matrix with lower porosity, lower degradation rate and lower diffusivity than that of a lower molecular weight polymer. These prosperities may result in differences in release behavior, especially

the burst effect, which is a very crucial point in efficacy and safety of in-situ gel-forming injectable systems. These are believed to be the results of chain entanglements in the higher molecular weight polymer. It also seems that increase in the polymer molecular weight results in slower water influx rate and therefore lower polymer degradation, a crucial rate-determining step in drug release from biodegradable systems.

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