Drug Development and Industrial Pharmacy, 23(12), 1191-1200 (1997)

RESEARCH PAPER

In Vitro Stability of Poly(D,L-lactide) and Poly(D,L-lactide)/Poloxamer Nanoparticles in Gastrointestinal Fluids

Gabriele Reich

Institute for Pharmaceutical Technology and Biopharmaceutics, Heidelberg University, Im Neuenheimer Feld 366, 69120 Heidelberg, Federal Republic of Germany

ABSTRACT

Poly(D,L-lactide) (PLA) nanoparticles of various surface and bulk properties were prepared by a nanoprecipitation procedure and evaluated for their physical and chemical in vitro stability in simulated gastrointestinal fluids of 37°C. The influence of polymer characteristics and poloxamer 188 (POL 188) adsorption was studied. Physical stability was followed by visual appearance, particle size, and zeta potential measurements. Molecular weight changes were analyzed by gel permeation chromatography (GPC). Due to a sharp decrease in their negative zeta potential, poloxamer-free nanoparticles flocculated in simulated gastric fluid, irrespective of the polymer properties. Their physical stability in protein-free intestinal fluids increased with an increase in carboxy end group concentration of the PLA and thus, with an increase in their negative zetapotential. Protein effects at pH 7.5 were rather complex indicating a stabilizing effect of negatively charged proteins and a destabilizing effect of positively charged proteins. Poloxamer 188 adsorption sterically stabilized the nanoparticles against flocculation in gastric fluid, irrespective of the PLA characteristics. Physical stability of the PLA/POL 188 nanoparticles in intestinal fluids was affected by the PLA characteristics. Poloxamer 188 increased the physical stability of nanoparticles composed of hydrophobic PLA, irrespective of the proteins present. A gradual particle size increase could, however, be observed for PLA/POL nanoparticles composed of PLA with a high content of carboxy end groups, especially in combination with positively charged proteins. This effect is most likely due to a decrease in PLA/POL interactions resulting from the ionization of the carboxy end groups located on the

nanoparticle surface and leading to conformational changes and/or a distinct desorption of POL 188. The chemical stability of PLA and PLA/POL nanoparticles depended on the glass transition temperature (T_{gH}) of the hydrated polymer matrix. Enzymatic effects could not be detected. Nanoparticles with $T_{gH} > 37°C$ were chemically stable in both gastric and intestinal fluids at 37°C over a time period of more than 48 hr.

INTRODUCTION

Nano- and microparticulates have attracted much attention as oral carriers for the delivery of labile drug molecules such as therapeutic peptides and proteins (1,2). Although the quantitative aspects of the uptake process are still somewhat controversial, many laboratories have repeatedly demonstrated that oral particulate uptake by the Gut Associated Lymphoid Tissue (GALT) or other intestinal sites is, in fact, possible (2–5). Many factors such as particle size (6), nature of polymer (7), surface change and hydrophobicity (3), adsorbed hydrophilic poloxamers (POL) (5,8), and/or covalent attached lectins (5,9) have been reported to affect particle uptake from the gut.

Biodegradable systems based on poly(L-lactide) (PLLA), poly(D,L-lactide) (PLA), and poly(D,L-lactide-co-glycolide) (PLGA) are of special interest for oral immunization purposes (7,9–12), since the rate of antigen delivery and, thus, the onset, duration, and intensity of the desired booster effect can be modulated by the chemical composition, the molecular weight distribution, the microstructure, or the tacticity of the polymer and by the addition of excipients with an adjuvant effect. Although the in vivo factors controlling particle translocation and antigen release are still poorly defined, particle size and matrix degradation have been shown to be critical to the nature, intensity, and duration of the immune response (10,12). In view of this, not only the process and formulation variables during particle preparation, but also the chemical and physical stability of the particles in gastrointestinal fluids are important issues to be addressed.

Recently, a few publications focused on the storage stability of polyester nanoparticles or nanocapsules in aqueous media (13–18). Few data are, however, available on their in vitro and/or in vivo stability in gastrointestinal fluids of 37°C (19). In the present study, PLA and PLA/POL 188 nanoparticles of various surface and bulk characteristics were prepared by a nanoprecipitation process and thoroughly investigated with respect to their physical and chemical in vitro stability in simulated gastric and intestinal fluids of 37°C.

MATERIALS AND METHODS

Materials

Aliphatic polyesters based on poly(D,L-lactide) (PLA:R 206, M_W = 130 kDa, PD = 1.8, [COOH] = 5 μeq/100 mg, T_g = 58.9°C; R 202, M_W = 14.5 kDa, PD = 1.3, [COOH] = 12 μeq/100 mg, T_g = 48.2°C; R 104, M_W = 3.6 kDa, PD = 2.4, [COOH] = 68 μeq/100 mg, T_g = 34.0°C where M_W is weight average molecular weight, and PD is polydispersity index.) were obtained from Boehringer Ingelheim, Ingelheim, Germany. To study the effect of free carboxy end groups, R 206 was modified by a standardized degradation process according to H. von Büren (20). The polymer obtained by this procedure is further designated as R 206* with the following characteristics: M_W = 16.3 kDa, PD = 1.9, [COOH] = 27 μeq/100 mg, T_g = 46.9°C. Poloxamer 188 (POL 188) was purchased from Erbslöh, Düsseldorf, Germany. Bovine serum albumin (BSA, pI = 4.7), pepsin, pancreatin, and bovine pancreas trypsin (pI = 10.8) were from Sigma Chemical Co., Deisenhofen, Germany. Acetone and tetrahydrofuran (THF) were from Merck, Darmstadt, Germany. All other chemicals were of analytical grade or better.

Preparation of Nanoparticles

Nanoparticles of various composition (for details see Table 1) were prepared by nanoprecipitation of the polymer according to the method originally described by Fessi et al. (21). Briefly, 20 ml of double-distilled water or an aqueous solution of poloxamer 188 (1%, w/v) was added to 10 ml of an acetonic solution of PLA (1%, w/v) under moderate stirring using a magnetic bar. The formation of nanoparticles occurred spontaneously upon polymer precipitation. Acetone was removed un-

Table 1

Characteristics of Nanoparticles

No.	Polymer	Aqueous Stabilizer	Mean Particle Size (nm)	PD	Zeta Potential (mV)	M_W (kDa)	M_W/M_n	[COOH] (μeq/100 mg)
I	R 206	—	185.7	0.07	-42.2	129.8	1.8	5
I	R 202	—	163.6	0.06	-46.8	14.0	1.3	12
I	R 206*	—	190.2	0.05	-50.4	15.9	1.9	27
I	R 202/R 104 (1/1)	—	175.5	0.08	-54.8	9.0	4.0	40
II	R 206	POL 188	183.2	0.06	-14.8	103.1	1.8	5
II	R 202	POL 188	190.0	0.06	-18.6	14.1	1.3	12
II	R 206*	POL 188	201.0	0.07	-24.3	15.8	1.9	27
II	R 202/R104 (1/1)	POL 188	192.2	0.10	-28.1	9.1	4.1	41

der reduced pressure and the aqueous nanosuspension was concentrated to a final volume of 5 ml.

Preparation of Films

Films (ca. 50 μm in thickness) composed of the same polymers as the nanoparticles, both without and with 10 wt% poloxamer 188, were cast on siliconized paper from organic solutions using a film forming device type 509/1 from Erichson, Wuppertal, Germany. After 12 hr under room conditions, the films were cut into disks, diameter 2 cm, rigorously dried in a vacuum oven to constant weight, and characterized with respect to their glass transition temperature (T_{g0}) in the dry state and after 24 hr in simulated gastric and intestinal fluids at 37°C (T_{gH}).

Stability Studies

Stability studies were carried out in simulated gastric and intestinal fluids of 37°C. To distinguish pH and salt effects from enzymatic effects, test fluids with the following compositions were used: *a*) Gastric fluid composed of hydrochloric acid pH 1.2 with pepsin (USP XXII); *b*) gastric fluid pH 1.2/USP XXII without pepsin; *c*) intestinal fluid composed of 0.05 M phosphate buffer pH 7.5 with pancreatin (USP XXII); and *d*) intestinal fluid pH 7.5/USP XXII without pancreatin. To evaluate protein effects other than enzymatic effects such as protein adsorption and/or bridging flocculation, and to overcome the problem of turbidity inhibiting particle size measurements in intestinal fluid with pancreatin, two intestinal fluids based on 0.05 M phosphate buffer pH 7.5/USP XXII without pancreatin, but with 1% w/v of either BSA or trypsin, were used additionally. These two proteins were chosen to simulate soluble proteins of the intestinal fluid with a negative and positive charge at pH 7.5, e.g., with $7.5 < pI < 7.5$, respectively.

Aqueous nanosuspensions (1 ml) various composition (for details see Table 1) were incubated with 10 ml of simulated gastric or intestinal fluids and stored at 37°C in an incubator under slight horizontal agitation. The study was conducted over a time period of at least 24 hr since nanoparticles might stick to gastrointestinal surfaces, thus prolonging the passage time. Every 30 min samples were withdrawn and monitored with respect to their macroscopic and microscopic appearance (sedimentation, flocculation, coalescence), their particle size distribution, their zeta potential, and the molecular weight distribution (M_W; M_W/M_n) of the polymer.

Analytical Methods

To study the physical stability of the nanoparticles in gastrointestinal fluids, the nanosuspensions were centrifuged (1200 rpm) to observe flocculation in the form of sedimentation. Sediments were investigated under a light microscope to clearly distinguish between flocculation and coalescence. In non-flocculated nanosuspensions

changes in particle size distribution were determined by photon correlation spectroscopy using an Autosizer IIc (Malvern Instruments, Malvern, UK). Data used for comparison are a mean particle size (z-average) and a polydispersity index ($0 < PD > 1$). Each value is the average of four measurements.

Zeta potential measurements ($n = 3$) were carried out on a Zetasizer 4 (Malvern Instruments, Malvern, UK).

The initial molecular weight distribution (M_W; M_n; $PD = M_W/M_n$) of the polymer raw materials and nanoparticles and the corresponding changes upon incubation with simulated gastric and intestinal fluids were analyzed by gel permeation chromatography (GPC) in tetrahydrofuran (THF) as mobile phase (flow rate: 1 ml/min; $T = 35°C$). Polymer solutions in THF (10 µl of a 0.5 wt% solution) were injected into a set of two 10-µm PL-gel columns (Polymer Laboratories, Shropshire, UK) with nominal pore sizes of 10^3 Å and 10^5 Å, respectively. Polymer elution was traced refractometrically (detector type 198.00, Knauer, Berlin, Germany) and analyzed by a GPC integrator from Shimadzu, Kyoto, Japan. Calibration was carried out with monodisperse polystyrene standards ranging from 1 kDa to 1400 kDa (Merck, Darmstadt, Germany).

To evaluate the concentration of free carboxy end groups associated with the different polymers ([COOH] = µeq/100 mg), a known amount of polymer was dissolved in 10 ml dichlorethan, diluted with 20 ml ethanol, and titrated with a 0.1 N sodium methylate solution in methanol using phenolphtalein as a indicator. The sodium methylate solution was previously titrated with a standard benzoic acid solution.

The glass transition temperature (T_g) of the polymer raw materials and films and corresponding changes upon hydration in gastrointestinal fluids were determined by differential scanning calorimetry (DSC) using a DSC system TA 3000 from Mettler, Greifensee, Switzerland, equipped with a DSC 30 low-temperature cell and a TC 10A processing unit. Calibration of heat of fusion and temperature scale were carried out with indium and indium, lead and tin, respectively. Each sample was scanned from −120°C to 200°C in a sealed aluminum pan with a heating rate of 10°C/min. Glass transition temperatures of the dried and hydrated samples (T_{gD} and T_{gH}) were taken as the midpoints of the baseline shifts associated with c_p changes. T_{gH} values were taken from the first heating cycle to reflect the actual physical state. T_{gD} values of the raw materials were taken from a second heating cycle, thus reflecting an equilibrium state.

RESULTS AND DISCUSSION

Nanoparticles based on PLA of different M_W, polydispersity, and free carboxy end group concentration were prepared with and without poloxamer 188 as aqueous phase stabilizer and evaluated for their chemical and physical stability in simulated gastric and intestinal fluids of 37°C. A detailed characterization of all the samples with respect to polymer characteristics, particle size distribution, zeta potential, and designations used is given in Table 1.

Physical Stability of Poloxamer-Free PLA Nanoparticles

Poloxamer-free PLA nanoparticles might be of great interest for oral immunization purposes since it has recently been demonstrated (8) that adsorbed hydrophilic poloxamers can inhibit oral uptake of nanoparticles in the small intestine. To study the effect of polymer characteristics on their physical and chemical in vitro stability in simulated gastric and intestinal fluids of 37°C, nanoparticles have been prepared from R 206, R 202, R 206*, and R202/R104 = 1/1, respectively. Their physical stability in simulated gastric fluid pH 1.2 of 37°C with and without pepsin is given in Table 2.

Nanoparticles immersed in simulated gastric fluid pH 1.2 without pepsin (series A in Table 2) were found to flocculate spontaneously, irrespective of the polymer characteristics. This phenomenon can clearly be attributed to a sharp decrease of the initially highly negative zeta potential of these electrically stabilized nanoparticles to values of ±0.5 mV upon pH reduction, e.g., due to a spontaneous deionization of free carboxy end groups on the particle surface.

Microscopic inspection of the flocculates after 2 hr of incubation revealed aggregates composed of several individual nanoparticles with particle sizes in the range of 0.8–20 µm. For flocculates composed of R 206, R 202, and R 206* nanoparticles, no time-dependent size changes or coalescence could be detected. R 202/R 104 (1/1) flocculates showed, however, a time-dependent coalescence. The reason for this is the significantly lower glass transition temperature ($T_{gH} < 37°C$) of the particles compared to hydrated R 206, R 202, and R 206* nanoparticles.

In the presence of pepsin (series B in Table 2), the flocculation rate of R 206, R 202, and R 206* nanoparticles was slightly retarded for 1 hr, 2 hr, and 4 hr, respectively. Partial pepsin adsorption on the

Table 2

Physical Stability of PLA Nanoparticles in Simulated Gastric Fluid pH 1.2 of 37°C: (A) Without Pepsin, (B) with Pepsin

		Time (hr)				
No.	Polymer	<0.5	1	2	4	8
A	R 206	+	+	+	+	+
A	R 202	+	+	+	+	+
A	R 206*	+	+	+	+	+
A	R 202/R 104 (1/1)	+	+	+	++	++
B	R 206	-	+	+	+	+
B	R 202	-	-	+	+	+
B	R 206*	-	-	-	+	+
B	R 202/R 104 (1/1)	+	+	+	+	+

+ : Phase separation, flocculation.
++ : Coalescence.
- : Stable nanosuspension.

nanoparticles' surface inducing a certain sterical stabilization and/or a certain electrical stabilization due to the slight reversal of the zeta potential to values between +3 mV and +8 mV might be responsible for these phenomena. Nanoparticles prepared from R 202/R 104 (1/1) were, however, found to flocculate again spontaneously. Interestingly, coalescence did not occur in this case, suggesting that pepsin might partly adsorb even on this rather hydrophilic particle surface, thus avoiding at least coalescence.

The physical stability of PLA nanoparticles in 0.05 M phosphate buffer (pH 7.5) simulating protein-free intestinal fluid depended on the PLA end group characteristics. As illustrated in Fig. 1, mean particle size of R 206* and R 202/R 104 (1/1) nanoparticles with a high concentration of free carboxy end groups (see Table 1) remain unchanged over a time period of more than 24 hr. A slight increase in mean particle size within the first hours after buffer incubation was observed with R 202 nanoparticles. R 206 nanoparticles with the lowest concentration of free carboxy end groups showed a pronounced increase in mean particle size from 185.7 nm to 218 nm within the first 4 hr and up to 248 nm after 24 hr, respectively. The observed effects can be attributed to significant differences in the zeta potential resulting from differences in negative charge density due to differences in the concentration of ionized carboxy end groups on the particle surface (Fig. 2).

The effects of negatively and positively charged proteins which might be present in intestinal fluids are rather complex. As illustrated in Figs. 3a and 3b for R 206 and R202/R 104 (1/1) nanoparticles, respectively, the negatively charged BSA was found to have a stabilizing effect, irrespective of the polymer end group characteristics. The positively charged model protein trypsin had, however, a destabilizing effect which led to

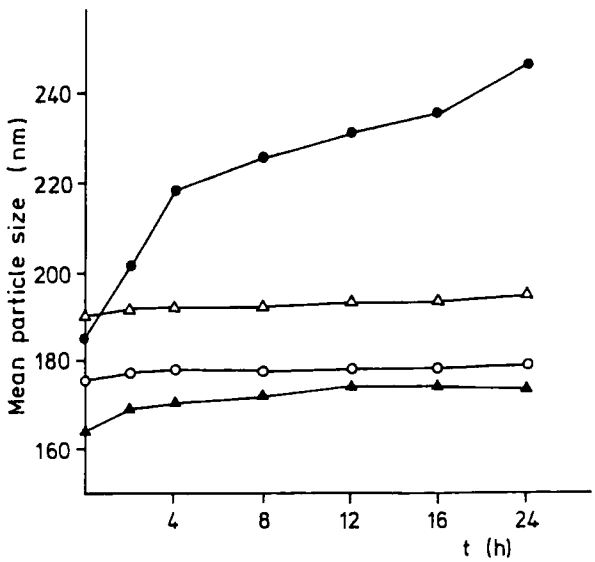


Figure 1. Time-dependent changes of mean particle size of PLA nanoparticles in simulated intestinal fluid without pancreatin at 37°C: (●) R 206, (▲) R 202, (Δ) R 206*, and (○) R 202/R 104 (1/1); $n = 4$.

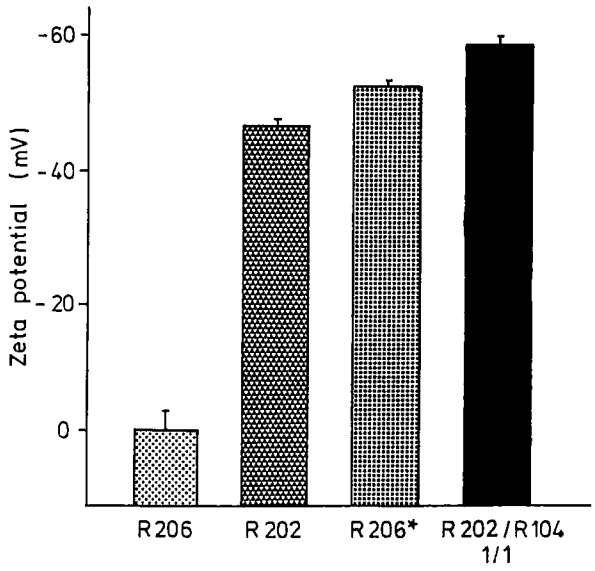
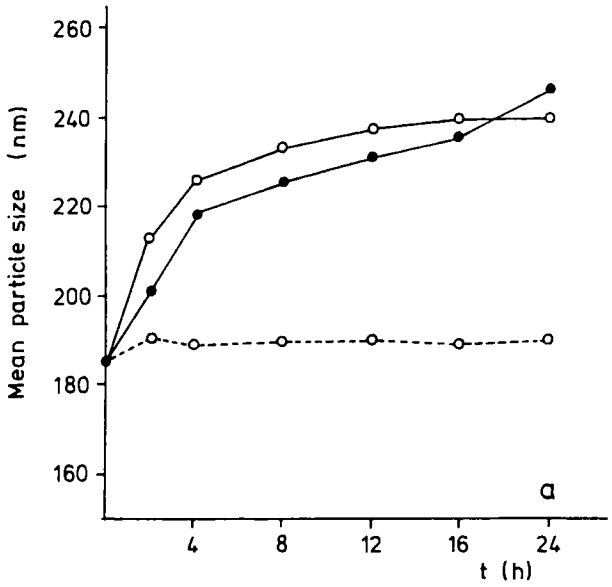


Figure 2. Zeta potential of PLA nanoparticles in simulated intestinal fluid without pancreatin at 37°C (n = 3).



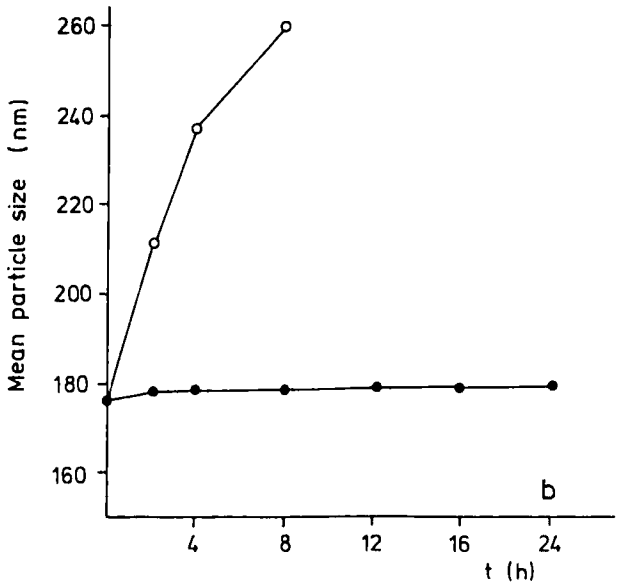


Figure 3. Time-dependent changes of mean particle size of (a) R 206 and (b) R 202/R 104 (1/1) nanoparticles in simulated intestinal fluids at 37°C: (●—●) protein-free, (○---○) with 1% w/v BSA (pI = 4.7), and (○—○) with 1% w/v trypsin (pI = 10.8); n = 4.

an accelerated aggregation of R 206 nanoparticles (Fig. 3a) and even induced aggregation of R 202/R 104 (1/1) nanoparticles (Fig. 3b). This last feature might be induced by electrostatic attractions of positively and negatively charged patches on the particle surface due to an incomplete adsorption of trypsin and/or a bridging flocculation mechanism.

Physical Stability of PLA/POL 188 Nanoparticles

Due to the more or less spontaneous flocculation of all poloxamer-free PLA nanoparticles in gastric fluid ± pepsin of 37°C, it was interesting to evaluate the effect of adsorbed poloxamer 188 on their physical stability. Fig. 4 illustrates the time-dependent changes in mean particle size observed for PLA/POL 188 nanoparticles composed of various PLA (see Table 1). Compared to poloxamer-free nanoparticles the stability was significantly increased, irrespective of the PLA characteristics. This can be attributed to a steric stabilization of the nanoparticles by the adsorbed poloxamer 188. Interestingly, a slight increase in mean particle size was observed for nanoparticles composed of the more hydrophilic R 206* and R 202/R 104 (1/1) nanoparticles.

Physical stability of PLA/POL 188 nanoparticles in 0.05 M phosphate buffer pH 7.5, simulating protein-free intestinal fluid, depended on the PLA characteristics, namely on the end group characteristics. As illustrated in Fig. 5, mean particle size remained nearly unchanged over a time period of 7 days for R 206/POL 188 nanoparticles with a low carboxy end group concentration, indicating a stabilization by the adsorbed poloxamer compared to poloxamer-free R 206

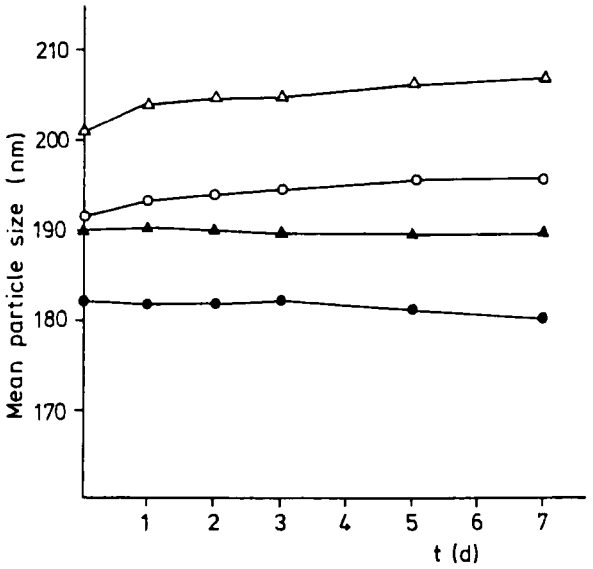


Figure 4. Time-dependent changes of mean particle size of PLA/POL 188 nanoparticles in simulated gastric fluid of 37°C: (●) R 206, (▲) R 202, (Δ) R 206*, and (○) R 202/R 104 (1/1); $n = 4$.

nanoparticles (Fig. 2). The same was valid for R 202 nanoparticles. In the case of R 206* and R202/R104 nanoparticles, however, poloxamer adsorption resulted in a slight decrease in the physical stability. Mean particle size gradually increased from 195 nm and 200 nm to 215 and 218 nm within the first day of incubation and even up to 225 nm and 246 nm within one week. This last feature was significantly increased in the presence of the positively charged model protein trypsin and slightly decreased in the presence of the negatively charged BSA (Fig. 6). For the more hydrophobic polymers such as R 206, protein effects were less dramatic since only a slight destabilization was observed with the positively charged trypsin (Fig. 6).

The observed carboxy end group effects on the physical stability of PLA/POL 188 nanoparticles in simulated intestinal fluids of various composition are most likely the result of differences in the poloxamer conformation on the surface of these nanoparticles resulting either from a different adsorption pattern during the particle preparation procedure and/or from rearrangements due to the ionization and salt formation of the carboxy groups upon incubation in intestinal fluids. On a hydrophobic particle surface such as R 206, the poloxamer 188 obviously adsorbed mainly with the PPO part of the molecule, thus leading to a high PEO surface density in contact with the medium. On more hydrophilic particle surfaces with a high density of carboxy end groups on the particle surface, the attachment of PEO segments is favored, resulting in a flatter adsorption pattern with more attachment points and thus a re-

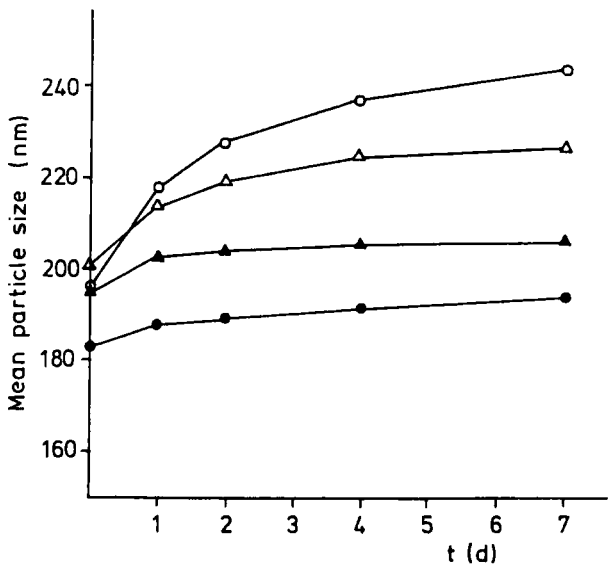


Figure 5. Time-dependent changes of mean particle size of PLA/POL 188 nanoparticles in simulated intestinal fluid without pancreatin at 37°C: (●) R 206, (▲) R 202, (Δ) R 206*, and (○) R 202/R 104 (1/1); $n = 4$.

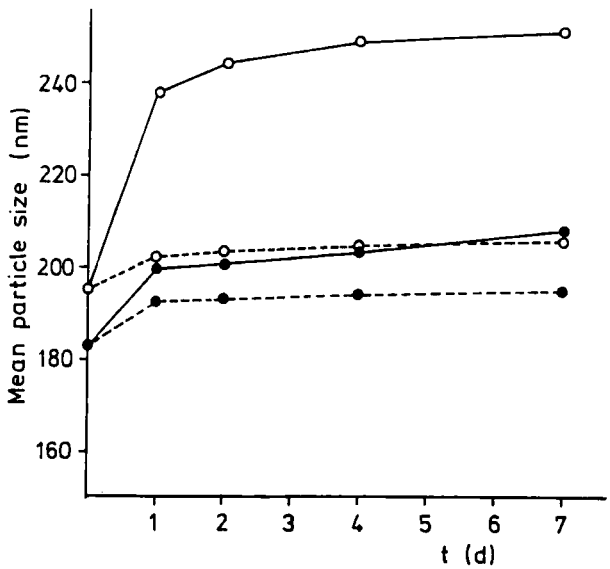


Figure 6. Time-dependent changes of mean particle size of PLA/POL 188 nanoparticles in simulated intestinal fluids containing (---) 1% w/v BSA and (——) 1% w/v trypsin: (●) R 206, (○) R 202/R 104 (1/1); $n = 4$.

duced PEO surface density or, in other words, a thinner adsorption layer. This last feature is reflected by an increase in the negative zeta potential of PLA/POL 188 nanoparticles in water with an increase in carboxy end group concentration of the PLA (see Table 1). As it can be assumed that hydrophilic interactions between PEO units of the poloxamer 188 and carboxy end groups of the PLA are sensitive to changes in pH and ionic strength, it is obvious that incubation with gastric or intestinal fluids might lead to conformational changes or even a partial desorption of the adsorbed poloxamer 188 from the more hydrophilic surfaces of R 206* and R 202/R 104 (1/1) nanoparticles with a high carboxy end group density. Differences in the POL 188 adsorption layer thickness resulting from the aforementioned effects can clearly explain the physical stability differences of PLA/POL 188 nanoparticles with different end group characteristics which have not only been observed in intestinal fluids (Figs. 5 and 6), but to a certain extent also in gastric fluid of 37°C (Fig. 4).

Chemical Stability of PLA and PLA/POL 188 Nanoparticles

Chemical stability measurements were only carried out with non-flocculated nanosuspensions. To clearly distinguish between pH effects and enzymatic effects, gastric fluid/USP XXII ± pepsin and intestinal fluid/USP XXII ± pancreatin of 37°C were used, respectively.

As evidenced by the M_W data listed in Table 3, the chemical stability of PLA (I) and PLA/POL 188 (II) nanoparticles incubated in the aforementioned simulated gastric and intestinal fluids of 37°C depended strongly on the polymer and on the medium pH. It was only slightly affected by the adsorbed poloxamer 188 and/or the presence of enzymes.

Particles prepared from R 206, R 202, and R 206* remained stable over a time period of more than 48 hr, irrespective of the fluid composition. A tremendous M_W decrease was, however, observed for R 202/R 104 (1/1) nanoparticles with adsorbed poloxamer 188 within the first hours of incubation in gastric fluid ± pepsin (see also Fig. 7). The effect was less dramatic, but still significant when R 202/R 104 (1/1) nanoparticles without (I) or with adsorbed POL 188 (II) were incubated in intestinal fluids ± pancreatin. This clearly indicates that pH differences are responsible for this behavior.

As evidenced by the glass transition temperatures (T_{gH}) determined from 50 μm films of all four polymers, both without (I) and with 10 wt% poloxamer 188 (II), after 24 hr in gastric fluid/USP XXII and intestinal fluid/USP XXII of 37°C (Fig. 8), the high chemical stability of R 206, R 202, and R 206* nanoparticles, both without and with adsorbed poloxamer 188, can clearly be explained by their high T_{gH} well above 37°C, reflecting nanoparticles in a glassy state. A rather low T_{gH} well below 37°C, obviously resulting from the plasticizing effects of the low molecular weight R 104 fraction within the particle bulk and the poloxamer 188 (22)

Table 3

Weight Average Molecular Weight (M_W) of PLA and PLA/POL 188 Nanoparticles After 48 hr in Simulated Gastric and Intestinal Fluids of 37°C (n = 4)

	M_W (kDa)							
	R 206		R 202		R 206*		R 202/R 104 (1/1)	
Type of Fluid	I	II	I	II	I	II	I	II
Gastric fluid without pepsin	nd	129.8	nd	14.1	nd	15.7	nd	6.9
Gastric fluid with pepsin	nd	129.7	nd	14.0	nd	15.9	nd	6.8
Intestinal fluid without pancreatin	130.1	129.9	14.0	14.1	16.1	16.0	8.7	8.9
Intestinal fluid with pancreatin	130.2	130.1	13.9	13.9	16.0	15.9	8.5	8.9

nd: Not determined.

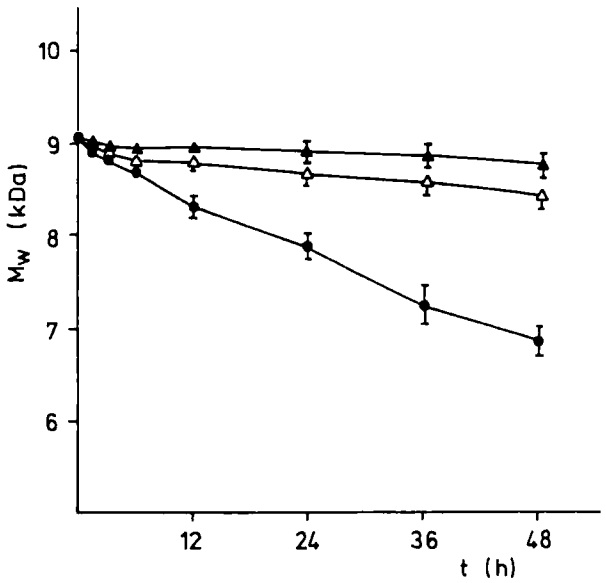


Figure 7. Time-dependent M_W-changes of R 202/R 104 (1/1) nanoparticles in (●) simulated gastric fluid, (▲,Δ) simulated intestinal fluid: (●,▲) with POL 188 and (Δ) without POL 188; $n = 4$.

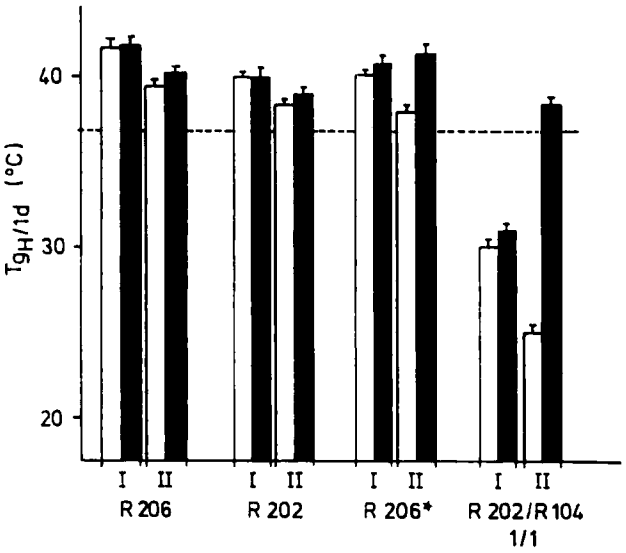


Figure 8. Glass transition temperature (T_{gH}) of hydrated (I) PLA and (II) PLA/POL 188 (9/1) films after 24 hr in (□) simulated gastric fluid and (■) simulated intestinal fluid of 37°C; $n = 3$.

on the particle surface, is, however, likely to be responsible for the instability of R 202/R 104 (1/1)/POL 188 nanoparticles in gastric fluid/USP XXII of 37°C. Moreover, it can be assumed that the high amount of the low molecular weight acidic R 104 fraction additionally catalyzes and thus accelerates the ester chain cleavage under the acidic conditions in simulated gastric fluid.

The degradation behavior of R 202/R 104 (1/1) nanoparticles, both without (I) and with adsorbed poloxamer 188 (II), in intestinal fluids ± pancreatin (Fig. 7 and Table 3) is, however, difficult to solely explain on the basis of T_{gH} differences. As will be demonstrated mechanistically in another paper (23) the overall higher stability of R 202/R 104 (1/1) nanoparticles in intestinal fluids compared to gastric fluids, irrespective of POL 188 being adsorbed or not, is mainly the result of a combination of pH and buffer salt effects on the ionization and solubility, and thus, on the phase behavior of the low molecular weight R 104 fraction within the polymer blend. The slight, but significant differences in the degradation rate of R 202/R 104 (1/1) nanoparticles without (I) and with adsorbed POL 188 (II) in intestinal fluids ± pancreatin (Fig. 7 and Table 3) are most likely due to additional pH and salt effects on the PLA/poloxamer 188 interactions, which dramatically reduce the plasticizing effect of POL 188 on the particle surface (Fig. 8).

CONCLUSIONS

The study revealed that many factors have to be controlled to enhance the physical and chemical stability of PLA nanoparticles in simulated gastric and intestinal fluids of 37°C. Steric stabilization by poloxamer 188 turned out to be essential for a sufficient physical stability of PLA nanoparticles under the acidic conditions in the stomach. Physical stability of PLA and PLA/POL nanoparticles in simulated intestinal fluid pH 7.5 is difficult to predict, since protein adsorption effects are likely to play a critical role. Moreover, PLA end group characteristics affecting not only the zeta potential, but also PLA/POL interactions have to be considered upon adjusting physical stability in the intestinal tract. To maintain chemical stability of PLA and PLA/POL nanoparticles in gastric and intestinal fluids for at least 24 hr, PLA characteristics have to be carefully chosen with respect to their glass transition temperature (T_{gH}) in the hydrated state, since a glassy state ($T_{gH} >$ 37°C) is essential.

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