Chemical Interactions Between Drugs Containing Reactive Amines with Hydrolyzable Insoluble Biopolymers in Aqueous Solutions

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Chemical reactions between drugs containing reactive amines with hydrolyzable polymers in buffer solutions were investigated. Phenylalkylamines with increasing nucleophilic reactivity were used as model drugs. Solutions of phenylalkylamines were reacted heterogeneously with representative biodegradable polyanhydride and polyester powders in various pH solutions, and the recovery of the amines from the solutions was determined. Poly(sebacic acid), a reactive polyanhydride, reacted by amide formation with the tested amines and their respective HCl salts when exposed to physiologic pH (pH 7.4). However, at pH 5.0 no interaction occurred. The aromatic polyanhydride, PCPP, and the polyesters based on lactic acid and caprolactone did react with the amine derivatives at pH 7.4, but at a slower rate. The reaction can be avoided with appropriate salt derivatives of the amines.

KEY WORDS: polyanhydrides; poly(lactic acid); amidation; drug interactions.

Introduction

Biodegradable polymers have been used for the last three decades as drug carriers in implantable devices [1–3]. Various drugs have been incorporated in hydrolyzable polyesters and polyanhydrides to achieve extended release under physiological conditions (pH 7.4 at 37°C). Some of these drugs contain reactive amines that may compete with the hydrolysis process of the polymer carrier and react with the polymer to form undesired amide drug derivatives. Drug - matrix interaction may occur either 1) during drug incorporation in the polymer matrix by injection or compression molding fabrication processes, where high temperature and pressure are applied, 2) during long term storage and 3) during the process of biodegradation and drug release. The polymer drug interaction should depend on both drug and polymer characteristics.

Many polyanhydrides have been synthesized for the past two decades for use as bioerodible carriers for controlled delivery of drugs [1,2]. Polyanhydrides have been extensively explored and are among the few degradable polymers in clinical use [1,2]. The degradation and drug release processes of these polymers have been extensively studied [3].

Polyanhydrides and substituted anilines react during compression molding under anhydrous conditions to form amide bonds only at temperatures above 150°C [4]. This study did not address the potential interactions in aqueous solutions. The interaction of drugs containing reactive functional groups with bioerodible polymers has not been systematically studied. Potential interactions were speculated to account for poor protein release from PLA matrices [5]. The addition of n-decylamine to poly(caprolactone) (PCL) significantly enhanced polymer degradation in buffer which was probably a result of aminolysis [6].

We report here the interaction between primary phenylalkylamines and their hydrochloride salts with several degradable polymers under physiological condition *in vitro*. The selected polymers represent both fast and slow degrading biopolymers. The phenylalkylamine hydrochloride salts were studied to minimize chemical interaction because of their reduced nucleophilicity.

Experimental

Materials: The following compounds (98% purity or higher) were purchased from Aldrich (Milwaukee, WI): aniline, benzylamine, 2-phenylethylamine, benzyl alcohol, 2-phenylethanol, cyclohexanol, 3-phenylpropylamine, sebacoyl chloride and 4-phenylbutylamine. Poly(sebacic acid) (PSA) [Mw = 20,000], and poly(1,3-bis-p-carboxyphenoxypropane anhydride) (PCPP) were synthesized as previously described [7]. Poly(DL-lactic acid) (PLA) (MW = 1,750) and poly(caprolactone) (PCL) (MW = 2,000) were purchased from Polysciences (PA).

Instrumentation

High Pressure Liquid Chromatography (HPLC) apparatus: a Spectra Physics (Darmstadt, Germany) modular system composed of a Spec1000 pump, a UV-detector (220 nm) and a Data Jet integrator. A Rheodyne (Cotati, CA) injection valve equipped with a 20 µl loop was used. The samples (20 µl in buffer solution) were eluted through a C8 column (Lichospher 100 RP-8, 5 microns, Merck). The pH was measured using a PHM62 standard pH meter (Radiometer, Copenhagen). The melting points were determined on an Electrothermal melting point apparatus (Electrothermal, England).

Infrared (IR) spectroscopy was performed on a Jasco A - 200 spectrophotometer (Japan Spectroscopic Co.). Polymer samples were prepared in dichloromethane and cast onto NaCl plates for recording the spectra. Insoluble polymer degradation products were either pressed into KBr pellets or dispersed in Nujol onto NaCl plates. ^{1}H-NMR spectra were obtained on a Varian 300 MHz Spectrometer using 1% w/v polymer solutions in deuterated chloroform containing tetramethylsilane (TMS) as internal reference.

HPLC analysis

Amines were quantitatively analyzed by HPLC using a 20:80 v/v mixture of pH 6.80 buffer containing 0.05M and 0.1M KCl and methanol as mobile phase at a flow rate of 1 ml/min and UV detection at 220 nm. The peak areas correlated linearly with the amine concentrations. Typical reten-

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tion times (min) for benzylamine, 2-phenylethylamine, aniline, phenylpropylamine, and 4-phenylbutylamine were: 4.65, 6.92, 8.50, 14.29, and 31.67, respectively. Alcohols were determined under similar conditions.

Preparation of amine hydrochlorides

Solutions of the amines in dry ether (about 40% w/v) were added dropwise with stirring to an anhydrous 0.1 M HCl solution in ether. The precipitated salt was filtered under anhydrous conditions, stirred with a new portion of ether for 1 hour and washed once with dry ether and packed. All amine salts were pure and did not contain free amines.

Preparation of sebacamides

Sebacamides were prepared by mixing equivalent amounts of phenylalkylamine and sebacoyl chloride in dichloromethane. The white precipitate was isolated by filtration and washed with water (>90% yield). The product was mostly the monosebacamide derivative as determined by ^{1}H NMR and elemental analysis. The products were eluted by HPLC at retention times between 20 and 35 minutes using similar HPLC method described above for the analysis of the amines.

Reaction between polyanhydrides and primary amines

The heterogeneous reaction of PSA and PCPP with amines and their hydrochloride salts was studied at 37°C in different pH buffer solutions. The following primary amines were used: aniline, benzylamine, 2-phenylethylamine, 3-phenylpropylamine, 4-phenylbutylamine and their hydrochloride salts. All amine hydrochlorides were used without additional purification. The reaction kinetics of polymer with amines and their hydrochlorides were followed by the decrease in amine content in the reaction mixture as determined by HPLC analysis. Alcohols reacted similarly with PSA.

In a typical experiment, PSA or PCPP powders (50 mg) were placed into a narrow test tube and 1 ml of pH 7.40 0.1 M phosphate buffer containing 20 mg of the tested amine or its hydrochloride were added. The pH of the amine solution was adjusted to 7.40 with 1N HCl prior to the addition of polymer powder. The reaction mixture was stirred using a vortex and kept at 37°C. Samples were withdrawn from the buffer supernatant and analyzed for amine content. Calibration tables were determined for all amines and their hydrochloride salts. The reactions of the amines with sebacic acid monomer, PLA and PCL were similarly determined. The reaction of PSA with benzylamine hydrochloride at various pH solutions was conducted in 0.1 M phosphate buffer solutions containing 5 mg/ml of benzylamine: HCl with the pH adjusted to the desired pH using 1N HCl or 1N NaOH solutions. Samples withdrawn from the reaction were analyzed by HPLC.

The remaining solid polymer degradation products were separated from the buffer by centrifugation and decantation, washed twice with water and dried overnight at room air. The dry residue was analyzed by HPLC for sebacamide identification, IR for amide group detection and for nitrogen content by nitrogen analysis.

Results and Discussion

The reaction between amines and erodible polyanhydrides and polyesters in buffer solutions is reported. The structures of the polymers are shown in Fig. 1. The experimental system consists of a solution of the model drug-phenylalkylamine in buffer solutions containing the polymer powder.

PSA was reacted with representative primary amines and their hydrochloride salts. The reaction was monitored by following the amine content in the buffer solution as a function of time. The polymer was analyzed for potential amide formation by nitrogen analysis and IR spectroscopy. The amide derivatives of the amine with PSA, if produced, are sebacamides which are insoluble in water and thus nitrogen content in the remaining polymer should correlate with the degree of the amidation side reaction. The formation of amide bonds was confirmed by Infrared spectroscopy by the appearance of typical absorption peaks for amides at 3400 (N-H), and 1630 (C=O) cm^{-1}. In addition, the existence of sebacamide derivatives in the polymer mass was revealed by HPLC analysis showing peaks at retention times identical to the corresponding sebacamides. Under physiologic pH (pH 7.4) all of the tested amines and their respective HCl salts did react with PSA (Table 1). About 30% of the amines were lost within 1 hour of exposure to PSA and about 70% of the amines were lost after 24 hours. The unrecovered amines had reacted with the anhydride groups in the polymer backbone to form amide derivatives as confirmed by IR and nitrogen analysis. However, under acidic pH (pH 5.0 and 1.0) no reaction occurred for both the free amines and their hydrochloride salts (Table 2) as indicated by the high benzylamine recovery (over 90%) and the absence of detectable amide by-products in the polymer residue.

The formation of sebacamide by-products was evident from IR analysis (peaks at 3300, and 1630 cm^{-1}) and confirmed by TLC and HPLC chromatography. Elemental analysis of the remaining polymer at the end of the reaction showed the presence of bound nitrogen (Table 1). The changes in pH with time of the hydrolysis medium of PSA and in the presence of benzylamine:HCl are shown in Figure 2. A significant pH change, from pH 7.4 to about 3.0 was observed for PSA hydrolysis in the presence of benzylamine

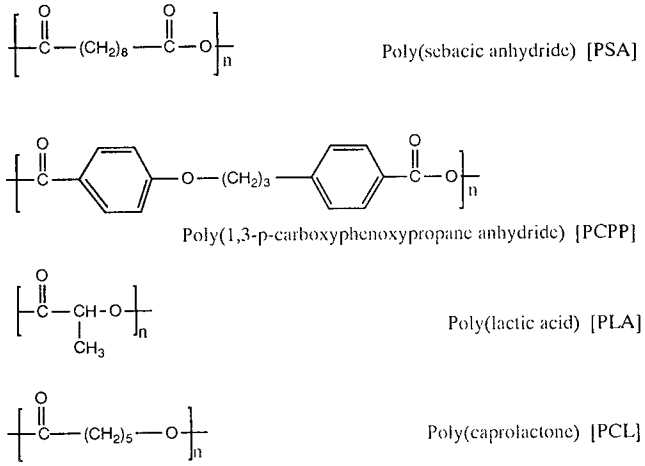

Figure 1: Structures of polymers used in this study.

Table 1. Reactions Between Phenlalkylamines with PSA and SA in Buffer pH 7.4[a]

Reaction between:	Amine analysis	Amide Formation	
	Nitrogen (%)	Recovery (%)	(by IR)
$C_6H_5NH_2$ + PSA	2.88	26	+ +
$C_6H_5CH_2NH_2$ + PSA	2.84	33	+ +
$C_6H_5NH_2$ + SA	0	105	–
$C_6H_5CH_2NH_2$ + SA	0	100	–
$C_6H_5NH_2$:HCl + PSA	1.24	41	+
$C_6H_5NH_2$:HCl + SA	0	104	–
$C_6H_5CH_2NH_2$:HCl + PSA	1.40	31	+
$C_6H_5CH_2NH_2$:HCl + SA	0	96	–
$C_6H_5(CH_2)_2NH_2$:HCl + PSA	1.40	25	+
$C_6H_5(CH_2)_2NH_2$:HCl + SA	0	106	–
$C_6H_5(CH_2)_3NH_2$:HCl + PSA	1.86	22	+

a. Solution of the amine in 0.1M phosphate buffer solution pH 7.4 (1ml, 20 mg/ml) reacted with sebacic acid or PSA powder (50 mg) at 37°C for 24 hours. Nitrogen content in polymer residue was determined by elemental analysis. Amine % recovery was determined by HPLC. IR absorption peak at 3400 cm^{-1} (amide N-H), + + strong peak, + small peak, – no peak.

as compared with PSA hydrolysis alone which decreased only to pH 5.5. This pH change was attributed to the rapid aminolysis which releases acidic products to the reaction medium.

Sebacic acid was reacted with the amines and their hydrochloride salts under the same conditions used for the reaction with PSA. As seen in Table 1, the initial concentrations of amines and their salts remain constant throughout the duration of the study (48 hours). The solid mass did not contain bound nitrogen after the reaction as determined by nitrogen analysis. IR and ^1H NMR spectra analysis confirmed that the solid residue was pure sebacic acid. This study indicates that sebacic acid degradation product was not involved with PSA - amine interaction.

The effect of pH on the amine-polyanhydride reaction was studied using benzylamine hydrochloride as representative amine (Table 2). No interaction occurred at pH 5.0 or below. However, at pH 7.4 amidation reactions proceed at considerable rates. This decrease in the reaction rates between amines and polymers at acidic pH correlates with the free amine concentrations available for reaction at the various pH solutions.

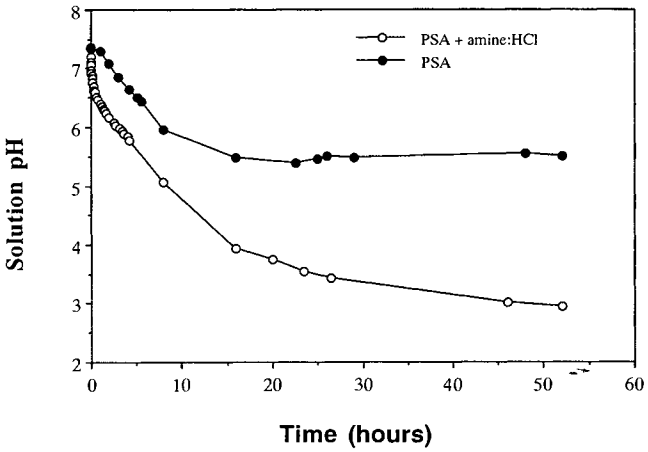

Figure 2: pH change during the reaction between benzylamine and PSA. Reaction at 37°C, pH was monitored using a pH meter.

The reaction between PCPP, an hydrophobic slow degrading polyanhydride [1,2], and benzylamine hydrochloride is shown in Figure 3. The rate of the decrease in benzylamine concentration with time is slower in comparison to the reaction with PSA. These results agree with the relative hydrolysis rate of these polymers, with PCPP hydrolyzing at a significantly lower rate than PSA [1,2]. The decrease in amine content in the presence of PLA and PCL is shown in Figure 3. About 15% of the benzylamine was lost from both polymers after one week at 37°C. Elemental and IR analyses of the remaining polymer indicated the presence of amide bonds and nitrogen.

The reactions between benzyl alcohol, 2-phenylethanol, cyclohexanol and PSA were investigated by monitoring alcohol content in mixtures with the polymer in buffer pH 7.4. The alcohol content did not change with time which indicates that no interaction occurred.

The potential for polymer-drug interaction exists for hydrolyzable polymers when a nucleophile stronger than water is present during polymer hydrolysis. A 70 and 15% amine loss was found for polyanhydrides and polyesters in buffer pH 7.4, with evidence for amine-polymer interactions. The reaction rate is dependent on the reactivity of the hydrolyzable polymer, the nucleophilicity of the incorporated drug, the type of salt of the drug, the relative concentration of

Table 2. Reaction of PSA with Benzylamine in Various pH Solutions[a]

Amine	Reaction pH	Amine Recovery (%) 1 hour	24 hours	48 hours	Amide formation (by IR)
Benzylamine	7.4	66	33	–	+ +
Benzylamine	5.0	92	94	90	–
Benzylamine:HCl	7.4	70	31	30	+
Benzylamine:HCl	5.0	9.4	–	94	–
Benzylamine:HCl	1.0	105	94	94	–

a. Solution of the amine in 0.1M phosphate buffer solution (1ml, 20 mg/ml) was reacted with PSA powder (50 mg) at 37°C for 48 hours. pH of benzylamine solutions was adjusted to pH 5.0 or pH 7.4 with 2N HCl or NaOH solutions prior to polymer addition. Benzylamine recovery was determined by HPLC. IR absorption peak at 3400 cm^{-1} (amide N-H), + + strong peak, + small peak, – no peak.

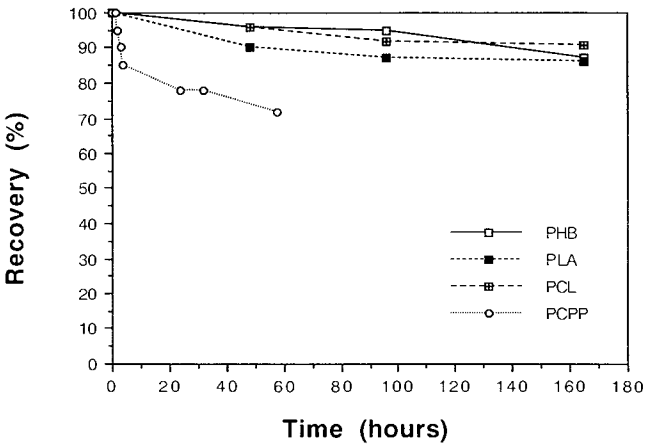

Figure 3: The reaction between PCPP, PLA and PCL and benzylamine in pH 7.4. Solutions of benzylamine: HCl in 0.1M phosphate buffer pH 7.4 (1ml, 20 mg/ml) were reacted with polymer powders (50 mg) at 37°C. Amine concentrations were determined by HPLC.

water and drug within the polymer matrix, and the pH of the reaction medium.

The in vitro hydrolysis of PSA matrix is characterized by a rapid anhydride hydrolysis during the first 24 hours and then a slow and steady degradation for two weeks [1–3]. The acidic degradation products remaining in the matrix, monomer and oligomers of sebacic acid, decrease the pH near and within the matrix to about 5 [3,9]. Under this acidic environment no interaction occurs (Table 2).

The possibility for a chemical interaction between a hydrolyzable polymer and reactive body proteins is unlikely because of their limited accessibility within the protein structure for a heterogeneous reaction with a solid polymer. Several studies conducted on polyanhydride elimination and drug release indicates complete drug release and polymer elimination from animals [1,9,10]. The in vitro and in vivo release of sebacic acid comonomer and various amine containing drugs from various polyanhydrides showed a complete drug and SA elimination [1,2,10]. These published studies support the claim that polyanhydrides are safe, biocompatible and biodegradable in the human body, and that there is no evidence for polymer-drug interactions. However, this study presents a concern regarding the interaction between amine containing drugs and biodegradable polymers. Thus, attention should be given to assure that no interactions occur.

Acknowledgments

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