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Anti-inflammatory prodrugs as plasticizers for biodegradable implant materials based on poly(3-hydroxybutyrate)

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Salicylic and acetylsalicylic acid esters were tested as plasticizers for biodegradable poly(3-hydroxybutyrate) (PHB). The aim is the combination of plasticizing and anti-inflammatory properties in the fabrication of implant materials. Solution-cast films made of mixtures of PHB and 30% ester showed plasticization accompanied by a decrease of elastic modulus and an increase in elongation at break in comparison with pure PHB films. However, the number of usable plasticizers from the group of the salicylic acid and acetylsalicylic acid esters is limited. Short-chain derivatives are volatile while long-chain compounds tend to crystallize. In both cases PHB films embrittle within short time. Moreover, some derivatives show a fast release in an aqueous environment.

As alternative nonsteroidal anti-inflammatory prodrugs arylpropionic acid esters were tested as plasticizers. The addition of ketoprofen ethyl ester led to PHB films with decreased brittleness.

In summary, various esters of anti-inflammatory drugs show plasticizing effects on solution-cast PHB films comparable with those of commonly used citric acid esters.

1. Introduction

The brittleness of biodegradable polymers led to numerous investigations concerning the addition of plasticizers for applications both in industry and medicine. Citric acid esters were tested as effective plasticizers, for example in poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHB-HV) [1, 2], poly(lactide) (PLA) [3–5] and cellulose derivatives [6–8]. The most important members of citric acid esters are triethyl citrate (TEC), acetyltriethyl citrate (ATEC), tributyl citrate (TBC), acetyltributyl citrate (ATBC) and butyryltrihexyl citrate (BTHC). These plasticizers were classified as biocompatible [9]. For applications in the aqueous environment the water-solubility of the additive has to be considered. TEC and ATEC can be classified as water-soluble citric acid esters [10, 11]. The accelerated enzymatic degradation of PHB-HV [2], PLA [4] and cellulose acetate [8] films was ascribed to the leaching of the plasticizer and the formation of an increased inner surface area. However, leaching is not limited to water-soluble plasticizers. It was shown that water-insoluble citric acid esters were hydrolyzed into water-soluble products [2, 8]. The acid properties of the hydrolysis products such as citric acid, acetic acid (ATBC), or butyric acid (BTHC) can lead to an acid catalysis and consequently to an acceleration of the polymer degradation. In the case of medical implants it has to be considered that water-soluble citrate esters induce a swelling of amorphous polymers which can provoke tissue injuries [5].

Glycerin esters represent another group of plasticizers for biodegradable polymers [12, 13]. The acceleration of the enzymatic hydrolysis of PHB by the addition of glycerin triacetate was attributed to the fast leaching of the plasticizer and increasing of the inner surface of the polymer sample [13]. Water-insoluble plasticizers can lead to the opposite effect. The enzymatic degradation of PHB was decelerated after the addition of 9% glycerin tributyrate to the polymer film [14]. This effect was explained with the formation of a protecting film of the additive on the polymer surface. On the contrary, the polymer degradation was accelerated with a plasticizer content of only 1% [14].

Phthalic acid esters [15, 16], sebacinic acid esters [1, 17] and polyether glycols [1, 6, 17, 18] were also described as plasticizers of biodegradable polymers.

In another study, the decrease of the glass transition temperature of PLA following the addition of salicylic acid was observed [19]. Salicylic acid esters are generally known as plasticizers [20], but their use for biodegradable polymers has not yet been described. It is well-known that acetylsalicylic acid, salicylic acid and their esters have pharmaceutical properties as non-steroidal anti-inflammatory drugs and prodrugs [21].

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Such a combination of plasticizing and anti-inflammatory properties could be very advantageous with regard to the development of biodegradable implant materials which is the aim of our study.

In this paper we describe our screening of salicylic and acetylsalicylic acid esters as potential plasticizers for solution-cast poly(3-hydroxybutyrate) films. As alternative nonsteroidal anti-inflammatory prodrugs arylpropionic acid esters were included into the examinations.

2. Materials and methods

2.1. Materials

Poly(3-hydroxybutyrate) (PHB, $M_w = 800\,\text{kDa}$) was supplied by Thalmann (Muehlhausen, Germany). Triethyl citrate (TEC), butyryltrihexyl citrate (BTHC), salicylic acid ethyl ester (SSOEt) and hexyl ester (SSOHx) were products of Aldrich (Taufkirchen, Germany). Salicylic acid 2-butyloctyl ester was a gift from Condea (Hamburg, Germany).

Acetylsalicylic acid ethyl ester (ASSOEt) and hexyl ester (ASSOHx) were prepared by acetylation of the corresponding salicylic acid esters with acetic anhydride in pyridine (molar ratio 1 : 1.2 : 2.4) according to standard procedures. Acetylsalicylic acid decyl ester (ASSODc) was prepared by reaction of acetylsalicoyl chloride with 1-decanol (molar ratio 1 : 1) in tetrahydrofuran/pyridine according to a method described in Banerjee and Amidon [22], and salicylic acid decyl ester (SSODc) by deacetylation of the acetylated compound in the presence of sodium methoxide (1% in methanol).

Ibuprofen ethyl ester and ketoprofen ethyl ester were prepared from ethanolic solutions of the free acids in the presence of thionyl chloride according to Hernaiz *et al.* [23].

2.2. Film fabrication

Films of PHB and plasticizer (70/30) were fabricated by dipping a metal core into a PHB/chloroform (5% w/v) solution. On removal from the solution and drying for a few minutes, the core was inverted and re-dipped into the solution. This process was repeated to produce film thicknesses up to about 100 μm. The film was stripped from the core and dried at room temperature and normal pressure to achieve a chloroform content of less than 0.2% as determined by elemental analysis.

2.3. Mechanical testing

Tensile testing on the films was carried out on a Zwick (Type BZ 2.5/TN1S) at room temperature. The gauge length of all samples was 15 mm and the width was 5 mm. The thickness was measured before testing. The crosshead speed was maintained at 5 mm/min. Tensile properties were calculated from the stress–strain curves as means of five measurements.

Dynamic mechanical properties were measured on a DMA 2980 dynamic mechanical analyzer (TA Instruments) at a frequency of 1 Hz with a force track of 200% and amplitude of 15 μm with a film tension clamp. The heating rate was 2 °C/min.

2.4. Plasticizer migration

For plasticizer leaching in aqueous environment, the films were stored in distilled water at 37 °C. For analysis of the plasticizer content, ^{1}H NMR measurements of sample films dissolved in chloroform-d$_1$ were performed on a Bruker AC 250 NMR spectrometer (250 MHz). The plasticizer content was calculated from the ratio of the intensities of polymer and plasticizer signals.

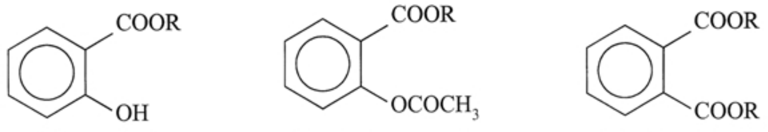
3. Results and discussion

In the development of a drug delivery system by incorporation of salicylic acid or acetylsalicylic acid into PHB films we have found that these anti-inflammatory drugs crystallize within the polymer matrix leading to embrittlement of the films shortly after their fabrication. Consequently, alternative materials to salicylic acid and acetylsalicylic acid esters, which are also of pharmacological interest [21, 22], were included into the examinations. Solution-cast films made of mixtures of PHB and 30% ester showed plasticization accompanied by a decrease of elastic modulus and an increase in elongation at break in comparison with pure PHB films (Table I).

The plasticizing effect of salicylic acid and acetylsalicylic acid esters is not unexpected considering the similar chemical structure in comparison with phthalic acid esters (Scheme 1) successfully tested in PHB [15] but problematic with regard to the biocompatibility [9].

The dynamic mechanical analysis enables the assessment of the mechanical behavior of polymer samples at 37 °C which is of particular interest for the adjustment of the material properties to the medical requirements. In this temperature region pure PHB shows a high storage modulus E' of about 4700 MPa and a stiff and brittle behavior. The cause is the high degree in crystallinity. The glass transition temperature T_g of 10 °C was determined from tan δ (Table I, Fig. 1).

In the dynamic mechanical spectra of the plasticized PHB films shifts of T_g and E' to lower values typical for plasticization were observed (Fig. 1). By analyzing tan δ, two transition regions below 37 °C were found. These regions could be attributed to the melting temperature of the plasticizer and the glass transition temperature of PHB. In the case of SSODc an overlapping of both



salicylic acid ester acetylsalicylic acid ester phthalic acid ester

TABLE I Properties of the plasticized PHB films

Plasticizer	Water soluble	Volatile on air[a]	Elastic modulus (MPa)	Tensile strength (MPa)	Elongation at break (%)	Glass transition temperature (°C)
None			3000 ± 120	33.0 ± 1.8	2 ± 0	10
Salicylic acid esters						
Salicylic acid ethyl ester	+	+	[b]			
Salicylic acid hexyl ester	−	+	[b]			
Salicylic acid decyl ester	−	−	850 ± 80	11.8 ± 1.1	104 ± 27	−28
Salicylic acid 2-butyloctyl ester	−	−	810 ± 20	13.5 ± 0.6	22 ± 7	−28
Acetylsalicylic acid esters						
Acetylsalicylic acid ethyl ester	+	+	[b]			
Acetylsalicylic acid hexyl ester	−	−	680 ± 20	11.8 ± 0.4	126 ± 33	−14
Acetylsalicylic acid decyl ester	−	−	830 ± 80	10.6 ± 0.3	34 ± 15	−7
Arylpropionic acid esters						
Ibuprofen ethyl ester	−	+	[b]			
Ketoprofen ethyl ester	−	−	600 ± 10	11.8 ± 0.2	58 ± 8	−14
Citric acid esters						
Triethyl citrate	+	−	960 ± 70	18.6 ± 1.2	21 ± 4	−24
Butyryltrihexyl citrate	−	−	800 ± 60	9.4 ± 0.4	32 ± 2	−23

[a] Volatility was assessed according to the plasticizer content (determined by NMR, see Section 2.4.) of the polymer films stored 4 weeks at room temperature and normal pressure. A plasticizer loss exceeding 1% (w/w, refered to the overall sample weight) was classified as volatility.
[b] Mechanical data were not analyzed in the case of volatile compounds.

regions was observed. In the case of ASSODc, which has a melting point (33 °C) above T_g of PHB/ASSODc (7 °C), a strong decrease of E' due to increasing interactions between the plasticizer and the polymer was observed in the melting region.

Some of the examined plasticizers were readily volatile thereby limiting their applicability. The volatility was assessed by the NMR spectroscopical determination of the plasticizer content of the stored polymer films (Table I). SSOEt evaporated almost completely from the sample films within few days of storage at room temperature and normal pressure. ASSOEt was released more slowly. However, this compound is subjected to a fast hydrolysis of the acetyl group forming the volatile SSOEt. Among the examined long-chain ester derivatives SSOHx was found to be volatile, too. The acetylated form was stable at storage under exclusion of moisture and prevention of hydrolysis. SSODc and ASSODc are also non-volatile compounds. The latter is a solid which crystallized within the PHB film and deteriorated the properties in comparison with those of the non-acetylated compound. The addition of other long-chain, solid salicylic acid derivatives, like stearoylsalicylic acid ethyl ester, acetylsalicylic acid stearyl ester and the dimer of salicylic acid ethyl ester, led to brittle polymer films (data not shown).

The plasticizers examined in this study can also be classified according to their water-solubility (Table I). For example, a fast release of ASSOEt in distilled water was found, whereas ASSOHx was not released within the observation period (Fig. 2). In the case of the acetylated compounds a cleaving-off of the acetyl group in aqueous environment has to be considered. Therefore, a deterioration of the mechanical properties of PHB/ASSOHx films can be expected because of the hydrolysis of ASSOHx and formation of the volatile non-acetylated derivative SSOHx. Moreover, the cleaving of the second ester bond and formation of free salicylic acid could lead to an embrittlement of the material as described above.

The migration behavior of the components has to be considered in the choice of an implant system with the desired mechanical properties and a limited host response. On the one hand, a water-soluble anti-inflammatory prodrug will be released quickly from the implant matrix and can reduce initial tissue reactions in the implant region. Ideally, the hydrolysis and conversion of the prodrug into the pharmaceutical agent should accompany the release process. However, an embrittle-

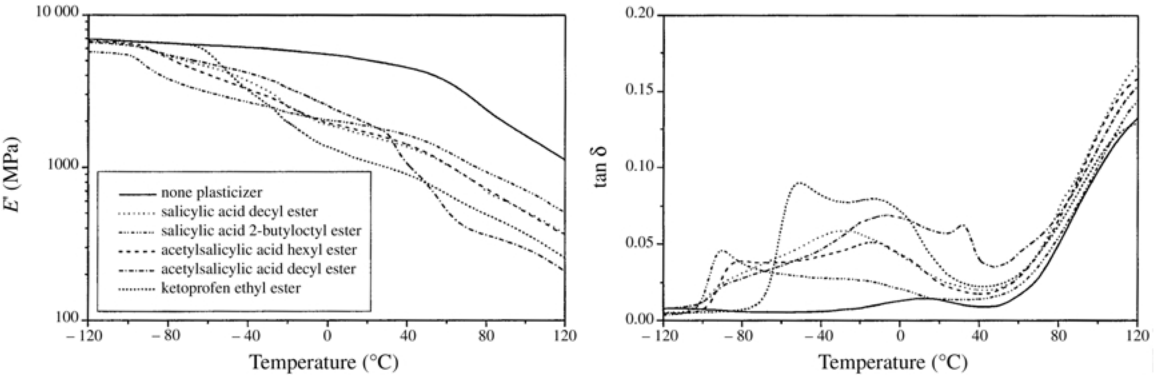


Figure 1 Dynamic mechanical spectra of plasticized PHB films.

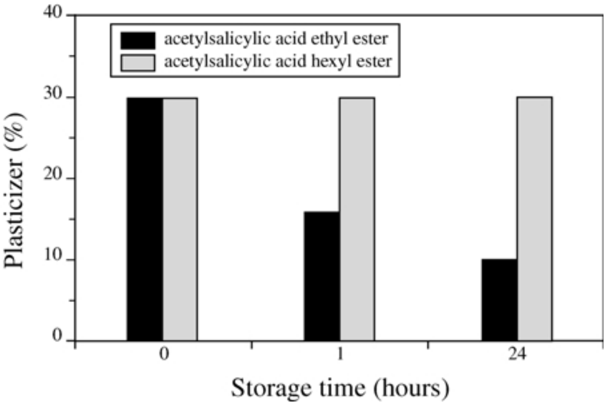


Figure 2 Plasticizer content (in %) of PHB films stored in distilled water at 37 °C.

ment of the implant material will be associated with the leaching. On the other hand, an insoluble additive will be released mostly at the final stage of the polymer degradation (mass loss) and therefore reduce tissue reactions caused by degradation products. In this case the plasticizing effect would remain over a longer period. Since an absolute water insolubility of a drug is normally not the real case, also a poorly soluble additive will be gradually released. Interactions with the physiological environment leading to the formation of water-soluble products may also play a role in this case. The addition of a mixture of a water-soluble and insoluble anti-inflammatory plasticizer could influence both the initial host response after implantation and the tissue reaction at the final stage of the degradation and could nevertheless maintain the mechanical properties of the implant. This case is possibly the most interesting one for a biomedical application. Ultimately, the selection of the modification method depends on the requirements of the intended application.

Because of their volatility at storage on air, the short-chain, water-soluble plasticizers tested in this study have a limited applicability for implant systems. From the long-chain derivatives, salicylic acid decyl ester and salicylic acid 2-butyloctyl ester can be considered as plasticizers for PHB implants. Both compounds are migration stable and do not crystallize within the polymer matrix, thus maintaining the mechanical properties of the implant material.

PHB films with an addition of 30% of the nonsteroidal anti-inflammatory drugs ibuprofene and ketoprofene were flexible and showed a high elongation at break after their fabrication. However, a progressive embrittlement of the samples caused by the crystallization of the drugs was observed. For that reason the ethyl esters which are liquids at room temperature were synthesized and tested as plasticizers. The volatile ibuprofen ethyl ester evaporated readily from the polymer film. Non-volatile ibuprofene esters with longer side chains were not examined in this study. Ketoprofen ethyl ester was migration stable both at storage on air and in water. The addition of this compound led to PHB films with decreased brittleness as indicated both in the static tensile test (Table I) and in the dynamic mechanical spectrum (Fig. 1).

The plasticizing effects of the anti-inflammatory prodrugs examined in this study are comparable with those of commonly used citric acid esters as shown on the example of TEC and BTHC (Table I).

4. Conclusions

The addition of esters of salicylic and acetylsalicylic acid as well as arylpropionic acid lead to plasticization of solution-cast PHB films. These esters are anti-inflammatory prodrugs so that an interesting combination of plasticization and antiinflammation in the fabrication of implant materials could be possible.

The number of usable plasticizers from the group of the salicylic acid and acetylsalicylic acid esters is limited, though. Short-chain derivatives readily evaporate while long-chain compounds tend to crystallize. In both cases PHB films embrittle within short time. Moreover, short-chain derivatives show a fast release in aqueous environment. On the basis of these observations, salicylic acid decyl ester and salicylic acid 2-butyloctyl ester can be considered as plasticizers for PHB in medical applications.

The addition of ketoprofen ethyl ester leads to PHB films with improved mechanical properties. In the case of ibuprofen esters a longer side-chain than ethyl is necessary to decrease the volatility.

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