ENZYMATIC DEGRADATION OF STARCH BASED THERMOPLASTIC COMPOUNDS USED IN PROTHESES

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Abstract

The present work presents the degradation behaviour of blends of corn starch with poly(ethylene-vinyl alcohol) copolymer(SEVA-C). The characterization included long term degradation trials on simulated physiological solution with α-amylase up to 200 days, and the degradation solutions were analysed by several techniques. High performance liquid chromatography (HPLC) and colorimetric methods were used to monitor the liberation of carbohydrate as a consequence of starch hydrolysis by α-amylase from the starch hydrolysates. It was possible to identify several degradation products such as carbohydrates ranging from C_6 to C_{18}. The action of α-amylase solely led to the degradation of starch, in contrast with other assays without enzymes where no carbohydrates were found in the degradation solutions.

Introduction

Natural origin and synthetic polymers are currently extensively used as biomaterials and in biodegradable applications, because their properties can be tailored to meet specific demands. Starch-based blends present an enormous potential to be widely used in the biomedical and environmental fields, as they are biodegradable, inexpensive and an almost unfailing source of raw material [1-2].

In the orthopaedic field, the aim is to develop systems that will be able to sustain their integrity and mechanical performance in the presence of aqueous media in the first implantation stages and start to degrade thereafter. The material must be designed with a degradation rate that assures that the strength of the scaffold is retained until the newly grown tissue takes over the synthetic support [3].

Starch is totally biodegradable in a wide variety of environments and could permit the development of totally degradable products for specific demands. It consists of two major components: amylose, a mostly linear alpha-D-(1-4)-glucan and amylopectin, an alpha-D-(1-4)-glucan which has alpha-D-(1-6) linkages at the branch point, but it contains only a single type of carbohydrate glucose[4]. Both fractions are readily hydrolysed at the acetal link by enzymes. The α-1-4-link in both components of starch is attacked by amylase, and α-1-6-link in amylopectin is attacked by glucosidases.

The endoamylases generally hydrolyse only the main chain acetal bonds in either amylose or amylopectin and are not active on the branch points of the latter, but many exoamylases can cleave either main chain on branch bonds. The exoamylases can generate either glucose or the dimer (maltose) or the trimer (maltotriose) by attacking the non-reducing end of the starch molecules.

The addition of poly(ethylene-vinylalcohol), EVOH, to starch blends seems to improve the processability, increase the toughness, and ameliorate the moisture instability of the product, but will also decrease the overall rate of biodegradability of the system (EVOH biodegrades, although on a time scale much larger than that of starch) [5]. One of the major problems in the use of

synthetic degradable polymers as biomaterials is to make sure that they are biocompatible by themselves, and the use of particular additives and/or processing technologies required to obtain different properties and or configurations, will not interfere with the biocompatible behaviour [6].
Apart from favourable physico-chemical and mechanical properties, the most important requirement for a biodegradable polymer to be used in medical applications is its biocompatiliby in a specific environment, together with the non-cytotoxicity of its degradation products [6]. Therefore, the knowledge of the degradation process of biodegradable polymeric biomaterials and of the effects that their degradation products might have on the body is crucial for long-term success of a biomaterial [7].
This paper presents some experimental evidence of the complex interactions occurring between starch and vinyl-alcohol copolymers, able to influence the degradation behaviour when in simulated physiological solution with enzymatic activity.

Materials and Methods

The material studied was a thermoplastic blend of corn starch with poly(ethylene-vinyl alcohol) copolymer (60/40 mol/mol), SEVA-C. Injection moulded square plates 30 mm wide and 2 mm thick were used for the assays.
The SEVA-C samples were weighed and immersed for several pre-fixed ageing periods as long as 6 months at pH 7.4 and 37ºC in individual containers with a Hank´s balanced salt solution(HBSS) with a α-amylase concentration similar to the one usually found in human blood plasma (50 unit./l). The total amount of polysaccharides in the degradation solutions was quantified using the Dubois method [8].
The amount of starch present in the SEVA-C specimens was obtained by acid hydrolysis and quantified by HPLC and Dubois methods. A sample of about 5 mg of dried SEVA-C powder was dissolved in 250 μl of H_2SO_4 78% and incubated at 25ºC during 30 minutes in a sealed tube. After that, 1.2 ml of distilled water and 0.5 ml of glucose (0.05 g/l) and sorbitol (1 g/l) used as internal standards for Dubois method and HPLC detection, respectively, were added to the mixture. The tubes were incubated at 100ºC for 120 min. After cooling the samples for 15-30 min, 0.6 ml ammonia solution 25% (NH_3) were added and cooled again for 10 min.
For each method (HPLC and Dubois) three duplicates were performed, and the average of all the results of each method was considered, as the final % of starch present in the material.
High performance liquid chromatography (HPLC) with 830-RI (Jasco, Japan) refraction index detection and a 880-PU pump (Jasco) was used to separate the sugar derivatives from the starch hydrolysates of the degradation solutions.
Commercial standards were used for the calibration of the Chrompack carbohydrates Ca column and a Chrompack guard column at 90ºC with ultra-pure water as eluent (0.5 ml/min). Sorbitol (1 g/l) was used as the internal standard. Three duplicates of each sample were performed.

Results and Discussion

Analysing the Fig.1 the most abundant compounds identified is glucose, followed by maltose and finally maltotriose. This is expected, since α-amylase random hydrolyse α-1,4-glucosidic bonds of starch into dextrins. Values up to 20% were measured, as demonstrated by polysaccharides curve which rise is mainly due to oligomers of glucose. The enzyme increases the release of long chain oligosaccharide substrates, difficult to obtain during hydrolysis.
All the oligosaccharides in solution tend to increase for longer immersion times, from monosaccharides to trisaccharides, being a sign of degradation of SEVA-C material degradation.
The low mass percentage released after 100 days can be attributed to the nature of the blend, in which starch and the ethylene-vinylalcohol are combined as an interpenetrating network. The presence of ethylene also reduced the mass loss percentages as less accessible hydroxyl groups are present in the blend.

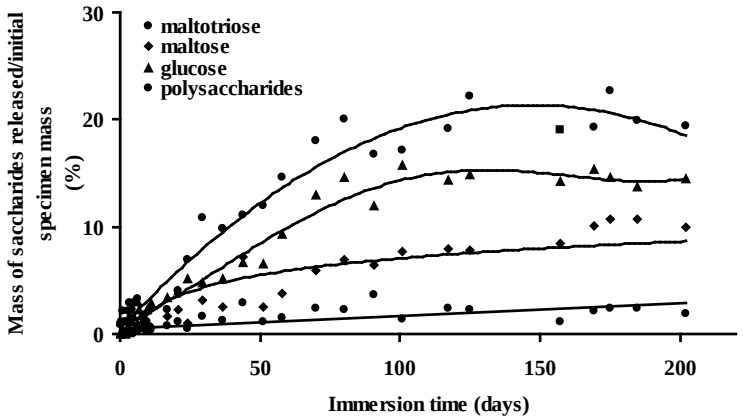

Figure 1 – Mass of saccharides of glucose released to the solution per initial specimen mass (1.61 g) in 50 ml of solution as function of immersion time.

Table 1 indicates the results obtained for the two quantified methods used: HPLC and Dubois method. From 5 mg of dried powder it was possible to detected 40% and 43% (w/w) for the Dubois and HPLC methods, respectively, of the starch amount (w/w). An average of the results gave a value of 42%, significantly different from the expected theoretically, 60% (w/w).
The percentage of saccharides found in the degradation solutions (25%) are almost half from the mass of starch in the material structure (42%). Not all the starch was degraded by enzymatic activity, remaining nearly 17% in the inner part of the material. This difference may be due to the difficulty of the enzymes to reach starch molecules strongly interpenetrated with the synthetic insoluble component. Moreover, the low porosity of the material makes the polysaccharides not completely accessible to the enzymatic attack.

Table 1 – Mass of saccharides released from acid hydrolysis obtained for HPLC and Dubois methods, from 5 mg of SEVA-C dried power.

	HPLC	**Dubois**
Mass of saccharides released/ initial specimen mass(%)	**43**	**40**

Conclusions
The biodegradability of starch blends with α-amylase increased until nearly 100 days of immersion, being the total mass loss percentage of 35%. The main saccharides in the degradation solutions were glucose, maltose and maltotriose. For the time used in the experiments, only part of amorphous of SEVA-C blend was degraded to the solution, the remainder stayed embedded in the network structure.
The model used in the scope of our study is a simple one, where the starch may be dispersed by chains of vinyl-alcohol copolymer, increasing the difficulty for enzymes to reach starch molecules strongly interpenetrated with the synthetic insoluble component.
The present experimentation demonstrated the major role of enzymes on SEVA-C degradation. However, the residual non-degraded starch, after 100 days degradation, leads us to raise the hypothesis that the remaining starch molecules are not degraded because they are surrounded by the non-degraded EVOH chains, blocking the enzyme access.

References

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