CHARACTERIZATION OF THE ENZYMATIC DEGRADATION OF STARCH/EVOH BLENDS IN A SIMULATED PHYSIOLOGICAL SOLUTION

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Introduction

Following the attempts to introduce starch based blends on biomedical application, this communication reports recent work on the *in-vitro* degradation behaviour of these materials. The study of the mechanisms and of the degradation products involved in the respective degradation process is of major importance, since the biodegradable material must be excreted by the body after been absorbed, leaving no toxic traces. The main products leached to simulated physiological solutions are revealed and reasonable predictions of the materials behaviour inside the organism may be done. Results on the morphological and surfaces changes are also analysed.

Materials and Methods

Samples of a blend of corn starch with poly(ethylene-vinyl alcohol) copolymer (60/40 mol/mol), SEVA-C, were immersed for several ageing periods up to 180 days in a sterile balanced salt solution(HBSS) with a α-amylase concentration similar to the one usually found in human blood plasma (50 unit./l), at 37ºC and pH = 7.4.

- SEVA-C water absorption was determined by thermogravimetric analysis (TGA) in a TGA-50 equipment.
- Contact angle measurements were carried out using the sessile drop technique. Water, formamide and diiodomethane were used to determine the surface free energy components of the material. The plates were dried at 50ºC for 10 days and kept in a exsicator before the analysis.
- All the saccharides, other than glucose leached to the solution were detected by HPLC(high performance liquid chromatography).

Results and Discussion

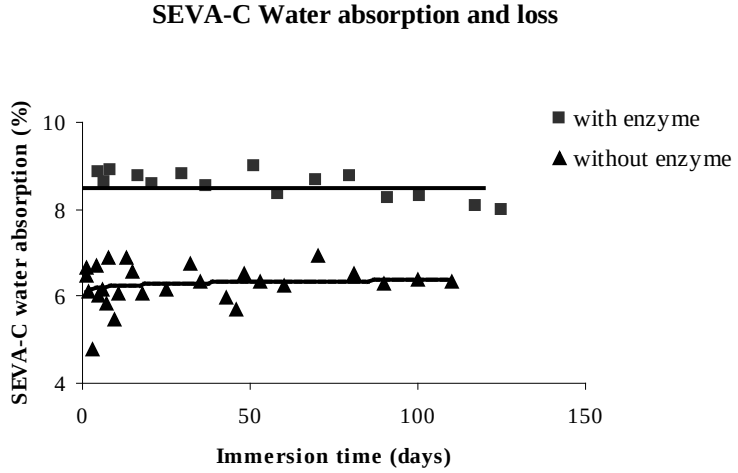

Figure 1 – SEVA-C water absorption (in %), as function of the immersion time.

Keywords: biomaterials, *in vitro* degradation, enzyme(s), surface modification

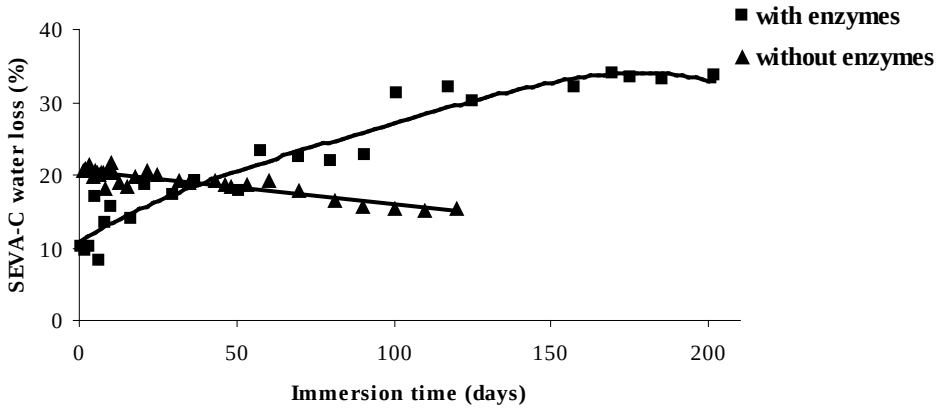

Figure 2 – SEVA-C water loss (in %) during stabilization, as function of the immersion time.

The figures show that:

- SEVA-C water absorption (%) increased in the enzymatic solution as a consequence of the enzymatic action on the amorphous phase. However, the water absorption level remains stable during the test period.
- During the stabilization process (Figure 2), the % of water loss of SEVA-C immersed in a enzymatic solution increased since the beginning to about 35 %. Without enzymes this % remains constant and equal to 20%.

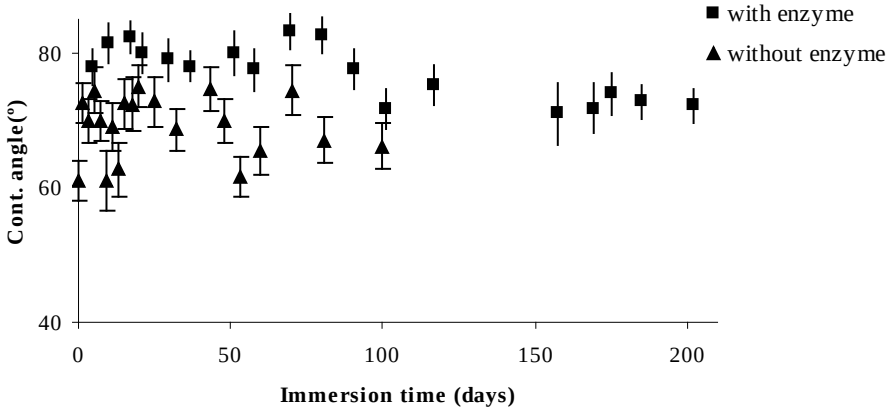

Figure 3 – Mean contact angles measurements(º) of water on SEVA-C surface, immersed in solution with and without enzymes, as a function of immersion time(measurements in dried samples).

Table 1 – Surface tension components of SEVA-C surface, determined by contact angles measurements, in mJm^{-2}, for different immersion times.

Immersion time(days)	γ_S^{LW}	γ_S^-	γ_S^+	γ_S^{AB}	γ_S^{TOT}
5	22.02	14.19	0.39	4.7	26.72
37	26.62	13.69	0.55	5.5	32.17
80	22.07	6.46	1.29	5.78	27.85
101	23.89	12.33	0.4	4.43	28.32

Table 2 – Interfacial free energy of interaction(ΔG_{sws}) between the surface(s) and water(w), as calculated from the surface free energy components listed in table 1, in in mJm^{-2}, for different immersion times.

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Immersion time(days)	ΔG_{sws}^{LW}	ΔG_{sws}^{AB}	ΔG_{sws}^{TOT}
5	-0.001	-22.71	-22.71
37	-0.48	-23.25	-23.73
80	-0.002	-39.26	-39.26
101	-0.096	-27.19	-27.29

• Concerning the contact angle measurements with water, no significant differences were observed in the surface immersed in the enzymatic solution with time. This suggests that no significant alteration of surface hidrophobicity was observed after enzyme immersion.

• The values of the free energy of the hydrophobic interaction are in the same magnitude (Table 2), evidencing a negligible variation with the immersion time. These values also confirm the surface hydrophobic nature ($_{\Delta G_{sup}^{TOT}} < 0$).

• The surface free energy components of SEVA-C surfaces revealed that the apolar component (γ_S^{LW} = 24 mJm^{-2}) remains constant during the immersion time, being predominantly electron donating ($\gamma_S^- > \gamma_S^+$) and hydrophobic ($\gamma_S^- < 28$ mJm^{-2}).

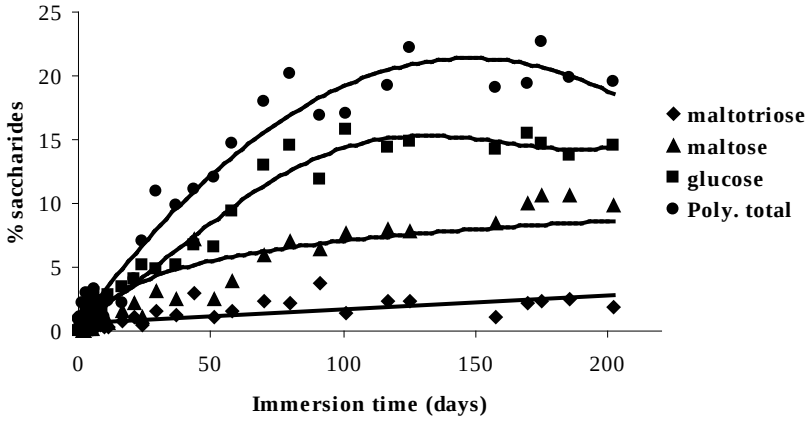

Figure 4 – Mass of maltose, maltotriose, glucose and polysaccharides in the solution per initial specimen mass (in %), as a function of immersion time. The amount of solution is 50 ml and specimen mass 1.61 g.

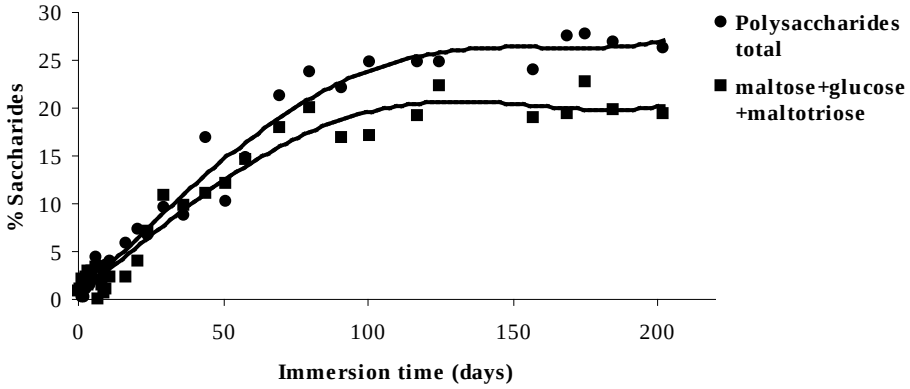

Figure 5 – Mass of the sum of all the components and polysaccharides in solution per initial specimen mass (in %), as a function of immersion time. The amount of solution is 50 ml and specimen mass 1.61 g.

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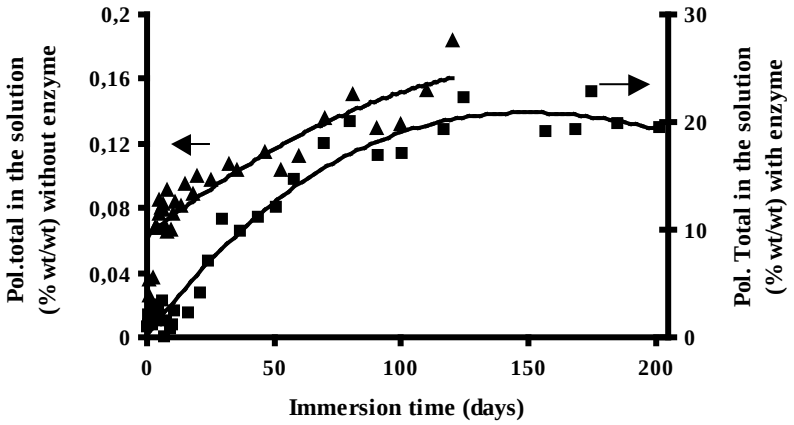

Figure 6 – Mass of polysaccharides in solution, with and without enzymes, per initial specimen mass (in %) as function of the immersion time. The amount of solution is 50 ml and specimen mass 1.61 g.

• Other sugars than glucose, such as maltose, maltotriose and maltotetraose appeared in the solution in quantities up to 10% (w/w of initial specimen mass). No oligosaccharides of higher molecular weight were detected. This suggests that either high molecular weight cannot be evacuated from the prosthetic matrix, or that the activity of α-amylase over the material is not significant to release other polysaccharides.
• Almost all the saccharides detected by HPLC in the degradation solutions, correspond to the total organic components leached to the solution, as their weight match almost exactly the total polysaccharides in solution.
• Comparing the same assay with and without enzyme activity (Figure 6), the % of polysaccharides leached to the solution increased more than 100% in the case of the enzymatic assay.

Conclusions

• The work confirmed the significant effect of the α-amylase on the degradation rate of the SEVA-C blend and evidenced that almost all the starch released to the solution was converted into glucose and other saccharides. It must be underlined that α-amylase is a natural enzyme always present in the body plasma.
• SEVA-C water absorption (in %) increased with enzymatic activity, comparison with the same assay without enzymes.
• The values of the free energy of the hydrophobic interaction of SEVA-C surface, revealed a hydrophobic surface nature (ΔG_{sws}^{TOT} <0), predominantly electron donating ($\gamma_S^- > \gamma_S^+$), without significant alteration of surface hydrophobicity with the immersion time.

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