THE POTENTIAL OF BIOPOLYMERS IN CONTROLLING THE *IN VITRO* DISSOLUTION OF A HYDROPHOBIC DRUG FROM FILAMENTS

Maryana B. da Silva[1], Layde T. de Carvalho[2,3], Gizelda M. Alves[1 *] & Simone F. Medeiros[1*]

[1] *Department of Chemical Engineering, University of São Paulo, School of Engineering of Lorena, Lorena, Brazil.*
[2] *Biotechnology Department, University of São Paulo, School of Engineering of Lorena, Lorena, Brazil.*
[3] *IMT Mines Albi, RAPODEE UMR CNRS 5302, Albi, France*
** Corresponding author's email address: simonemedeiros@usp.br*

ABSTRACT

The use of biodegradable polymers in filament manufacturing for bone regeneration for future application in 3D printing has been growing. Among the materials used, poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) has been considered promising for the fabrication of biomedical scaffolds due to its properties. However, to overcome its low degradability and hydrophobicity, PHBV is blended with other materials, such as pullulan, a biocompatible and hydrophilic polysaccharide declared safe by the Food and Drug Administration. In this study, PHBV filaments loaded with 10 and 20 %w/w pullulan and 5 %w/w ketoprofen were prepared through hot extrusion (HME) in a mini extruder. The influence of the amount of charge present in the samples was evidenced by Differential Scanning Calorimetry (DSC), X-ray Diffraction (XRD) and the in vitro ketoprofen release profile from the filaments. Filaments containing 20 %w/w pullulan (relative to PHBV) showed a release of 10.8% over 240 minutes of dissolution testing, compared to the pure drug, which presented a release of 71.4% in 10 minutes. In addition, they exhibited a lower crystallinity compared to pure filament. These results highlight the impact of adding hydrophilic polysaccharides to the filament matrix for future biomedical applications, such as bone regeneration.

Keywords: PHBV. Pullulan. HME. FDM. Scaffold.

1 INTRODUCTION

Tissue engineering (TE) scaffolds represent a promising strategy for the repair and regeneration of injured tissues, notably in the context of bone tissue reconstruction. The design and fabrication of scaffolds capable of mimicking the intricate microenvironment of native bone tissue while facilitating cellular adhesion and proliferation represent a critical milestone in advancing regenerative medicine[1].

Deposition Modeling (FDM) is one of the most economical and popular methods for manufacturing filaments used in subsequent 3D printing [2]. Over the past few years, there has been an increasing interest in renewable and biodegradable polymers, driven by growing concerns about sustainable development. Among these, polymers naturally obtained from microbial fermentation have gained significant attention [3].

Polyhydroxyalkanoates (PHAs) are environmentally friendly polyesters that can be synthesized by various bacterial species under nutrient-limiting conditions and carbon excess. Among them, polyhydroxybutyrate (PHB) shows promise for creating scaffolds due to its good tensile strength, flexibility, and excellent biocompatibility and biodegradability properties, which are crucial for biomedical applications [4]. However, due to its hydrophobicity, low degradation rate, and fragility, it often requires reinforcement with other polymers [5], such as pullulan.

Pullulan (PUL) is a highly biocompatible, biodegradable polysaccharide produced by strains of the polymorphic fungus *Aureobasidium pullulans*. Declared safe by the Food and Drug Administration (FDA) in the United States, pullulan added to other biopolymers can enhance properties such as water solubility, flexibility and high hydrophilicity [6]. In this context, the study aimed to produce PHBV filaments blended with pullulan and charged with ketoprofen, a non-steroidal anti-inflammatory drug (NSAID) used for pain relief and inflammation reduction[7], for the development of an efficient material for future application as scaffolds for bone tissue regeneration.

2 MATERIAL & METHODS

The materials used were commercial pullulan (PUL, Mn=120 kDa, cosmetic grade, Hayashibara Inc., Okayama, Japan), poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV, 8.0% HV by mass, Mn = 159,710 Da, Sigma-Aldrich), and ketoprofen, which was supplied by Sanofi (Sanofi-Aventis, France).

The composites based on PHBV, Pullulan and Ketoprofen were mixed manually and subsequently fed into a mini extruder (Weellzoom, model B Desktop, Guangdong Province, China) in an extrusion process at a temperature of 162 ± 2 °C. Three filaments were prepared with the following composition: the first, pure PHBV, second PHBV with 10 %w/w pullulan and 5 %w/w ketoprofen (PHBV-PUL10%-KETO5%) and the third PHBV with 20 %w/w of pullulan and 5 %w/w of ketoprofen (PHBV-PUL20%-KETO5%).

To assess the thermal transformation in the samples, measurements were carried out using a differential scanning calorimeter (DSC) (TA Instruments Q20, USA), in which the samples were heated at a rate of 10 degrees Celsius per minute from -20 to 200° W. Two heating profiles were carried out, the first with the aim of eliminating the influence of water on the samples and the second, used to obtain thermograms. All experiments were performed under a nitrogen flow of 50 mL/min and an empty aluminum pan was used as a reference.

The degree of crystallinity of the extruded filaments (X_c) was calculated based on the reduction in the enthalpy of crystallization of the polymer after hot extrusion, according to the following equation 1, where w_t is the weight fraction of reinforcement, ΔH is the fusion enthalpy, ΔH° is the fusion enthalpy of 100% crystalline polymer (crystalline PHBV = 146.6 J/g)[8].

$$X_c = \frac{\Delta H}{\Delta H^\circ (1 - w_t)} \quad (1)$$

The material's crystallinity was evaluated by X-ray diffractometer (XRD) (Shimadzu model XDR-6000) with CuKα radiation source, voltage of 40 kV, current of 30 mA, sweep 0.05 (2θ/ 5s) for values of 2θ between 10 and 90º, with increments of 0.05° and a counting time of 1 second.

The in vitro release tests were conducted in PBS solution (pH = 7.4) at 37°C in a manner analogous to the procedures delineated in the scholarly work (Naranjo et al., 2021)[9]. To construct the cumulative drug release curve over time, 10.0 mg of pure ketoprofen was placed inside gelatin capsules submerged in 200 ml of the buffer solution, and the capsules were continuously agitated for 4 hours. The same procedure was repeated to obtain the cumulative release profile for PHBV-PUL10%-KETO5% and PHBV-PUL20%-KETO5% filament, using the proportional amount of ketoprofen, around 200 mg of the total cryoground filament were submitted to dissolution test. The drug dissolution profile was assessed at various time points (5, 10, 15, 20, 30, 45, 60, 90, 120, 180, and 240 minutes) at a temperature of 37 ± 0.5°C, and the aliquots taken were analyzed at 258 nm using the Shimadzu UV-1800 UV/Visible spectrophotometer.

3 RESULTS & DISCUSSION

Differential Scanning Calorimetry (DSC) analysis was conducted for the purpose of examining the thermal behavior and crystallinity of Polyhydroxybutyrate-co-valerate (PHBV) composite filaments. Table 1 summarizes the melting temperature and enthalpy results for each sample. Figure 1a illustrates two distinct melting temperatures observed for the PHBV-based filaments: the first corresponding to the HV crystalline structure and the second to the HB structure[10]. Filaments containing pullulan displayed higher melting peak values compared to pure PHBV. Specifically, the filament with 10 wt% pullulan exhibited a more prominent peak, indicating a significant interaction with the HV crystal structure. However, the graph of the PHBV-PULL10%-KETO5% filament showed a slight leftward shift. Conversely, the filament containing 20% pullulan demonstrated a profile closer to the polymer and a more evident second melting peak, suggesting increased interaction with the HB structure. Regarding crystallinity, the addition of pullulan resulted in reduced crystallinity in the filaments, likely due to its impediment on the movement and orientation of the matrix chains[11].

In the X-ray diffraction (XRD) spectrum of Polyhydroxybutyrate-co-valerate (PHBV), broad peaks are observed at 25.52°, 20.14° and 30.82°, which correspond to the semi-crystalline structure of the polymer[12]. It is also noted that the composites containing 10% and 20% by weight of pullulan have a structure similar to that of the matrix, in contrast to ketoprofen, which exhibits two sharp peaks at 18.35° and 22.85°, corresponding to its crystalline structure[13], due to its low concentration in the mixture. Furthermore, the incorporation of greater amounts of pullulan into the composites results in a more significant reduction in peak intensity at 30.82°, indicating a decrease in the crystallinity of the samples due to the presence of the amorphous polysaccharide. This finding is in accordance with the results of the Differential Scanning Calorimetry (DSC) analysis. Additionally, the filament containing 10% by weight of pullulan exhibits an initial profile of around 13.2°, similar to that of the drug, compared to the filament containing 20% by weight of pullulan, which reinforces previous findings from the analysis by DSC.

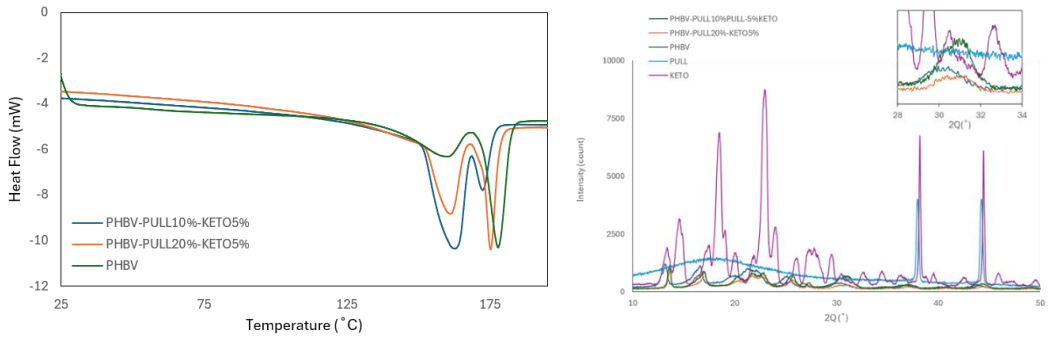

Figure 1 a) DSC curves of PHBV, PHBV-PUL10%-KETO5% AND PHBV-PUL20%-KETO5% filaments b) XRD pattern of PUL, PHBV, KETO, PHBV-PUL10%-KETO5% AND PHBV-PUL20%-KETO5% filaments.

Table 1 DSC thermal parameters of PHBV, PHBV-PUL10%-KETO5% and PHBV-PUL20%-KETO5% filaments.

Samples	T_{m1} (ºC)	$\Delta H_1 (J\ g^{-1})$	T_{m2} (ºC)	$\Delta H_2 (J\ g^{-1})$	Xc^a (%)
PHBV	159.35	21.14	177.81	38.23	40
PHBV-PULL10%-KETO5%	162.32	32	172,7	6,22	22
PHBV-PULL20%-KETO5%	161,08	20,17	175,16	16,79	19

The controlled release of the drug was evaluated by comparing the release profile of free ketoprofen with its release from PHBV-PULL10%-KETO5% and PHBV-PULL-20%-KETO5% filaments in PBS (pH 7.4) and at 37° W. Free ketoprofen demonstrated rapid release, reaching 71.4% of the drug in 10 minutes, remaining constant with a release rate of 74±1%. In contrast, filaments containing pullulan in the matrix showed a more controlled release. The PHBV-PULL10%-KETO5% filament exhibited a gradual release of the drug, reaching 5.6% in 240 minutes. The filament containing 20% by weight of pullulan and 5% by weight of drug revealed a higher release rate, reaching 10.8% in 240 minutes, attributable to the higher concentration of the polysaccharide. However, when comparing the release rates between the composite filaments, it was observed that the filament with 10% by weight of pullulan presented a release more similar to the rapid profile of the pure drug, corroborating previous findings. Thus, the inclusion of a hydrophilic polysaccharide in the filament matrix improved the delivery of ketoprofen, without compromising the controlled release system.

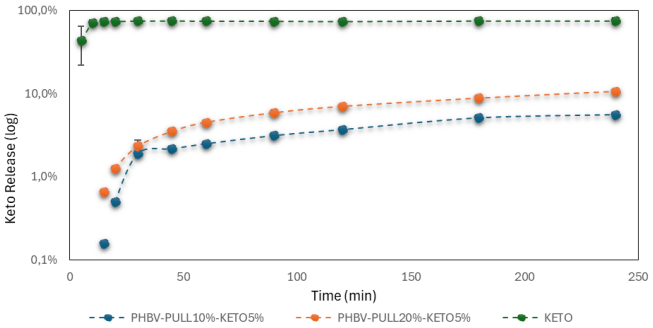

Figure 2 Dissolution profiles of ketoprofen from free form, PHBV-PUL10%-KETO5% and PHBV-PULL-20%-KETO5% at 37°C in PBS medium at pH 7.2, using concentration in log scale.

4 CONCLUSION

In this work, filaments of PHBV were prepared with different amounts of pullulan, 10 and 20 %w/w and both with 5% (w/w) of ketoprofen. The filaments were successfully by hot-melt extrusion, and it was possible to observe that the presence of pullulan promoted a reduction in the PHBV crystallinity, and the amount of pullulan can impact the drug dissolution profile, which was aimed with the introduce of the hydrophilic polysaccharide. Therefore, the filaments can now be applied in future applications in the biomedical area, especially tissue engineering, such as bone regeneration.

REFERENCES

[1] TOMMASINO, C., AURIEMMA, G., SARDO, C., ALVAREZ-LORENZO, C., GAROFALO, E., MORELLO, S., FALCONE, G., AQUINO, R. P. 2023. Int J Pharm. 641. 123093.

[2] DEB, D., JAFFERSON, J. M. 2021. Mater. 46. 1308-1318.

[3] MEHRPOUYA, M., VAHABI, H., BARLETTA, M., LAHEURTE, P., LANGLOIS, V. 2021. Mater. Sci. Eng. C. 127. 112216.

[4] LEZCANO, M. F., ÁLVARES, G., CHUHUAICURA, P., GODOY, K., ALARCÓN, J., ACEVEDO, F., GAREIS, I., DIAS, F. J. 2022. Biology. 11. (5). 706.

[5] BAKHTIARI, S. S. E., KARBASI, S., TOLOUE, E. B. 2021. Int. J. Biol. Macromol. 166. 986-998.

[6] COLTELLI, M. B., DANTI, S., DE CLERCK, K., LAZZERI, A., MORGANTI, P. 2020. J. Funct. Biomater. 11. (1). 20.

[7] SANTOS-ROSALES, V., ARDAO, I., GOIMIL, L., GOMEZ-AMOZA, J. L., GARCÍA-GONZÁLEZ, C. A. 2021. Polymers (Basel). 13. (1). 159.

[8] BRDLÍK, P., BORŮVKA, M., BĚHÁLEK, L., LENFELD, P. 2022. Polymers (Basel). 14. (4). 838.

[9] NARANJO, L. V., BASTANTE, C. C., CARDOSO, L. C., SERRANO, C. M., DE LA OSSA, E. J. M. 2021. Polymers (Basel). 13. (12).

[10] NASEEM, R., MONTALBANO, G., GERMAN, M. J., FERREIRA, A. M., GENTILE, P., DALGARNO, K. 2022. Molecules. 21. (21). 7633.

[11] MARTINS, L. S., CORTAT, L. I., ZANINI, N. C., BARBOSA, R. F., SOUZA, A. G., MEDEIROS, S. F., ROSA, D. S., MULINARI, D. R. 2021. Mater. Today Commun. 28. 102690.

[12] MARTINAUD, E., HIERRO-IGLESIAS, C., HAMMERTON, J., HADAD, B., EVANS, R., SACHARCZUK, J., LESTER, D., DERRY, M. J., TOPHAM, P. D., FERNANDEZ-CASTANE, A. 2024. J Polym Environ. 1-13.

[13] SHAHZAD, Y., SAEED, S., GHORI, M. U., MAHMOOD, T., YOUSAF, A. M., JAMSHAID, M., SHEIKH, R., RIZVI, S. A. A. 2018. Int. J. Biol. Macromol. 109. 963-970.

ACKNOWLEDGEMENTS

Laboratory for Research and Applications of Biopolymers in Biomedicine and Sustainable Development (LABPBio), School of Engineering of Lorena (EEL), University of São Paulo (USP), Unified Scholarship Program (PUB).