



## Effect of temperature on the incorporation of Arnica tincture (Arnica Montana) in Polycaprolactone and Chitosan matrices

## Efeito da temperatura na incorporação da tintura de Arnica (Arnica Montana) nas matrizes de Polycaprolactona e Quitosana

DOI: 10.54021/sesv3n3-001

Recebimento dos originais: 05/05/2022  
Aceitação para publicação: 01/07/2022

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**ABSTRACT**

This work aims to produce Polycapactone (PCL) and Chitosan (CTS) matrices in different mass proportions and to analyze the incorporation of Arnica's Tincture and Dr. Humphrey's Curative Wonder under different temperatures. The purpose of this analysis is to make viable the creation of biocompatible matrices capable of absorbing and releasing drugs for future use in the field of Tissue Engineering, more specifically, Bone Tissue Engineering, and in addition, to potentiating the absorption capacity of drugs. The matrices produced were immersed in the cited drugs and placed under conditions of different temperatures. The analyzes were done for Mass Variation and Spectroscopy in the Infrared Region by Fourier Transform (FTIR), and the results showed that, for both drugs, the hot incorporation was better than the room temperature incorporation.

**Keywords:** tissue engineering, biopolymer, drug incorporation.

**RESUMO**

Este trabalho visa produzir matrizes de Polycapactone (PCL) e Chitosan (CTS) em diferentes proporções de massa e analisar a incorporação da Tintura de Arnica e da Maravilha Curativa do Dr. Humphrey sob diferentes temperaturas. O objetivo desta análise é viabilizar a criação de matrizes biocompatíveis capazes de absorver e liberar drogas para uso futuro no campo da Engenharia de Tecido, mais especificamente, Engenharia de Tecido Ósseo, e além disso, potencializar a capacidade de absorção de drogas. As matrizes produzidas foram imersas nas drogas citadas e colocadas sob condições de diferentes temperaturas. As análises foram feitas para Variação de Massa e Espectroscopia na Região de Infravermelho por Fourier Transform (FTIR), e os resultados mostraram que, para ambos os medicamentos, a incorporação a quente foi melhor do que a incorporação à temperatura ambiente.



**Palavras-chave:** engenharia de tecidos, biopolímero, incorporação de fármacos.

## 1 INTRODUCTION

Biomaterial development for various applications has attracted attention in the last years. Different forms, such as films, scaffolds, matrices, membranes, and others, have been made. In this context, polymeric materials, due to their properties, have been studied (ALMEIDA *et al.*, 2021; da SILVA *et al.*, 2021; MENDONÇA, *et al.*, 2012) .

Biomaterial science is so interdisciplinary and allows the design of new materials. Therefore, studies concerning the incorporation of drugs into polymeric devices are increasing (ALMEIDA *et al.*, 2021; da SILVA *et al.* 2021; MENDONÇA, *et al.*, 2013). Thus, the interest in comprehending the absorption process is elementary to modifying polymer device properties.

Polymers have applications as a drug delivery system and for tissue engineering devices. However, for some utilization, their properties have to be improved. Polycaprolactone (PCL) has been used in different biomaterials production techniques due to this biocompatibility.(GUERRA *et al.*, 2018; NAVARRO-BAENA *et al.*, 2016; STEWART *et al.*, 2018). Furthermore, PCL presents excellent mechanical resistance and is easy to form mixtures with other polymers or composites, enabling control of physical properties, such as the structural stability of components during degradation processes. (JUNIOR, 2004; ONDER; YILGOR; YILGOR, 2018; TROVATTI *et al.*, 2016). In addition, PCL can be associated with drugs for the production of biomaterials that serve as drug delivery systems (ALMEIDA *et al.*, 2021; da SILVA *et al.* 2021, OLADAPO *et al.*, 2019).

The *Solidago microglossa* DC (Asteraceae), popularly known as Arnica-do-Brazil, is wide spread plant species used in Brazilian folk medicine. Neto *et al.* studied the systemic use of *Solidago microglossa* DC in the cicatrization of open cutaneous wounds in rats. They observed a significant reduction in the wound area. Although the dose of *Solidago microglossa* DC used in Neto *et al.*, study did not demonstrate hepatotoxicity because it is known that arnica extract has a low therapeutic index making, the systemic utilization of crude aqueous extract of



arnica is dangerous. A good alternative can be to incorporate this drug into polymer systems. (SILVA *et al.*, 2019) Chitosan (Chi), a cationic polysaccharide derived from the deacetylation of the chin, has been combined with polymers to promote drug incorporation through the absorption process. According to the literature, multifunctional matrices consisting of Chi and polyhydroxybutyrate were produced by thermal compression molding. The composition of the matrices affects the fluid (extract of arnica and water) absorption properties (Silva *et al.*, 2019; Mendonça *et al.* 2012).

This work aimed to produce amphiphilic PCL/Chi matrices with potential application in tissue engineering. Accordingly, the following specific aims of this work were: to evaluate and predict the swelling behavior of PCL/Chi in arnica alcoholic extract at room temperature and to evaluate the effect of temperatures on arnica absorption.

## 2 MATERIALS AND METHODS

### 2.1 PREPARATION OF PCL AND CHITOSAN SAMPLES

The following materials were applied to produce the matrices: food-grade chitosan (Chi) with a size distribution of particles expressed as equivalent volume diameters at 10% ( $d_{0.1} = 56.133 \mu\text{m}$ ), 50% ( $d_{0.5} = 200.297 \mu\text{m}$ ), and 90% ( $d_{0.9} = 1132.420 \mu\text{m}$ ) of the cumulative volume, determined by laser diffraction using a Mastersizer 2000 particle analyzer (Malvern Instruments); 22% degree of acetylation purchased from Polymar SA (Ceará powder, low molecular weight, and PCL (Capa 6500, average molecular weight of 50,000 g/mol), kindly donated by Perstorp; *Solidago microglossa* DC (Asteraceae), *Arnica-do-Brasil*, the concentrated extract purchased from Seven Phyto (Minas Gerais, Brazil) and

Table 2 shows the mass proportions of Chi and PCL used to produce the matrices. The production consisted of adding PCL, Chi, and 10 ml of water to a glass recipient. Then, the resulting mixture was heated in the microwave for 20 seconds to melt the PCL. in sequence. The hot mixtures were molded on a glass surface to obtain a film.



Table 1: Mass/Mass Ratios of Chitosan and PCL

Sample code	Chitosan (%)	PCL (%)	Mass of PCL (g)	Mass of chitosan (g)
PCL	-	100	1,06	0
PCL_Chi_25	25	75	1,06	0,35
PCL_Chi_40	40	60	1,06	0,71
PCL_Chi_50	50	50	1,06	1,06

## 2.2 SWELLING DEGREE ARNICA-DO-BRASIL

The swelling degree (Sw) of the PCL/Chi matrices was determined in Arnica-do-Brasil (arnica (glycolic extract at 10wt %). To a recipient containing the glycolic extract, added PCL/Chi matrices for 8 hours at room temperature. %). Sw is calculated by Equation 1. The swelling degree of samples in arnica extract was also performed, at 50°C, for 1 hour.

$$S_w = \left( \frac{W_t - W_0}{W_0} \right) \times 100 \quad (1)$$

Where Sw is the swelling degree, Wt mass of the sample in the instant t, and W<sub>0</sub> is the initial mass of the sample.

## 2.3 THE THIN PLATE SPLINE INTERPOLATION METHOD (TPSIM) AND POLYNOMIAL FITTING

Interpolation is a technique for adding new data points within a range of a set of known data points. Interpolation can be used for making predictions. Thus, in this work, this method<sup>20</sup> was used to predict the swelling behavior of PCL/Chi matrix, as a function of the time and composition, in arnica extract. For it, computational software, Scilab, was used to apply the TPSIM and generate a matrix with the interpolated point (time, composition and S<sub>w</sub>). The experimental data used to obtain the interpolated data using TPSIM were obtained by Equation 1 as described in da Silva et al.(2019).

## 2.4 INFRARED SPECTROPHOTOMETRY - FTIR

Infrared spectrophotometry was applied to investigate possible interactions between PCL and Chi. The analysis occurred in an infrared spectrometer (Nicolet,

model 6700) under attenuated total reflectance (ATR) by recording measurements from 4500 to 600  $\text{cm}^{-1}$ .

### 3 RESULTS AND DISCUSSION

PCL and chitosan were mixed in different mass proportions, according to Table 2. Figure 1 shows matrices photography. It is possible to observe the difference between samples. According to Figure 1, the color of the samples changes with the increase of Chi amount.

Figure 1- Photography of A) pure PCL; (B) 50% PCL/50% Chitosan (C) 60% PCL/40% Chitosan; (D) 75% PCL/25% Chitosan.

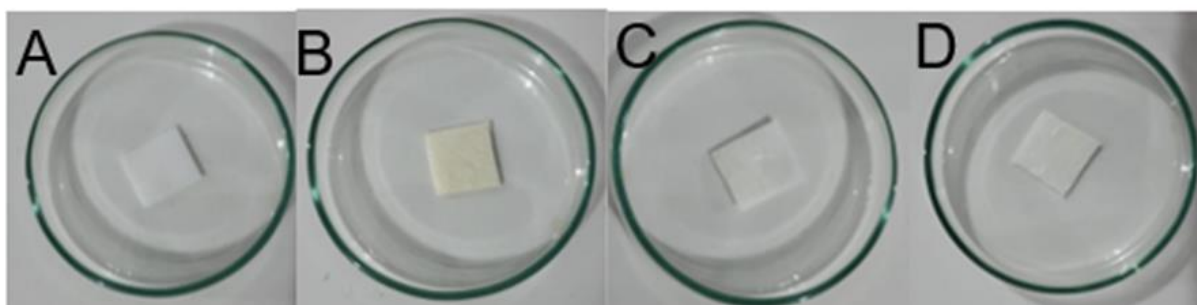
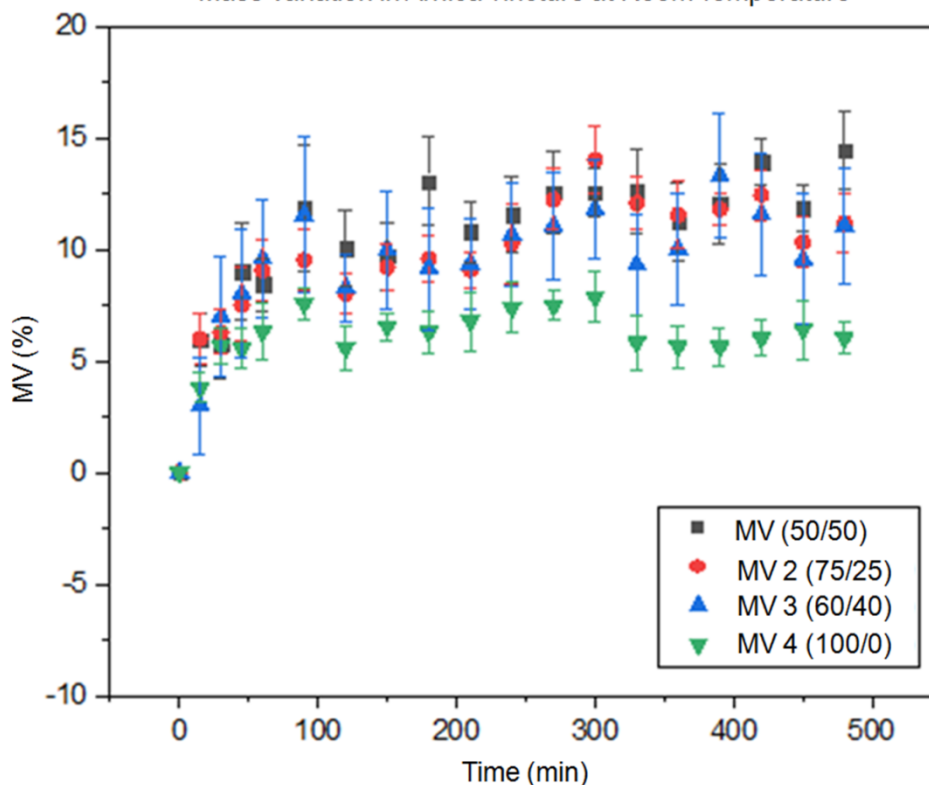


Figure 2 shows the Sw kinetic of samples. The PCL/Chi matrices show better Sw results than the pure PCL sample. The high absorption characteristic of Chi can justify this fact. Chi is a pseudocationic polymer and can swell in different fluids. Similar behavior was observed by Mendonça et al., 2012 and Silva et al., 2019 that studied matrices of polyhydroxybutyrate and Chi.

The matrix PCL\_Chi\_50 has the more significant Sw value, corroborating that Chi improves the absorption process. In this study, for 8 hours, the maximum Sw was approximately 13.9% for PCL\_Chi\_50. Although, the values obtained for the other matrices contained Chi were close. da Silva et al. (2019) produced a matrix of polyhydroxybutyrate with 50% wt of chitosan. They found an Sw in arnica, at room temperature around 30%. This study by da Silva et al. (2019) was performed for 8 hours. Thus, we state that the polymer also affects the swelling behaviour.



Figure 2 - Swelling degree of matrices  
Mass Variation in Arnica Tincture at Room Temperature



Power model fitted The Sw data (Equation 2), and Table 2 shows the values of the coefficients. The PCL may absorb arnica due to the presence of pores. According to Figure 2, for PCL, Sw decreases after 300 minutes. On the other hand, Chi increases arnica absorption. However, decreases in Sw also occurred. It occurred more intensely in samples with less Chi. It is because the behavior of Chi in fluids is different from that observed by PCL.

$$Y = A(t - t_c)^P \quad (2)$$

Where Y is the swelling degree, A is a constant that depends on sample composition, t is the time and  $t_c$  is a critical value, that was equal to zero for all compositions studied.

Table 2: Equation 2 coefficient's values

Sample	A	p	R <sup>2</sup>
PCL	4,05133	0,0827	0,72236
PCL_Chi_25	3,41124	0,20674	0,86594
PCL_Chi_40	2,6411	0,24759	0,88105
PCL_Chi_50	3,28954	0,23099	0,87317

Photograph of PCL-Chi, Figure 3, shows regions with different colors, indicating that the absorption can occur differently. The swelling values predicted using TPSIM allow analyzing, simultaneously, the effect of time and Chi amount in arnica absorption. The TPSIM surface (Figure 4) was similar to that of Silva et al. 2019, reinforcing the chitosan's ability to absorb arnica and improve the polymer's absorption properties. However, Sw presents positive and negative variation, due probaby to PCL chains compression promoted by Chi swelling.

Figure 3: Photograph of samples after the swelling process. (A) pure PCL; (B) PCL/Chitosan

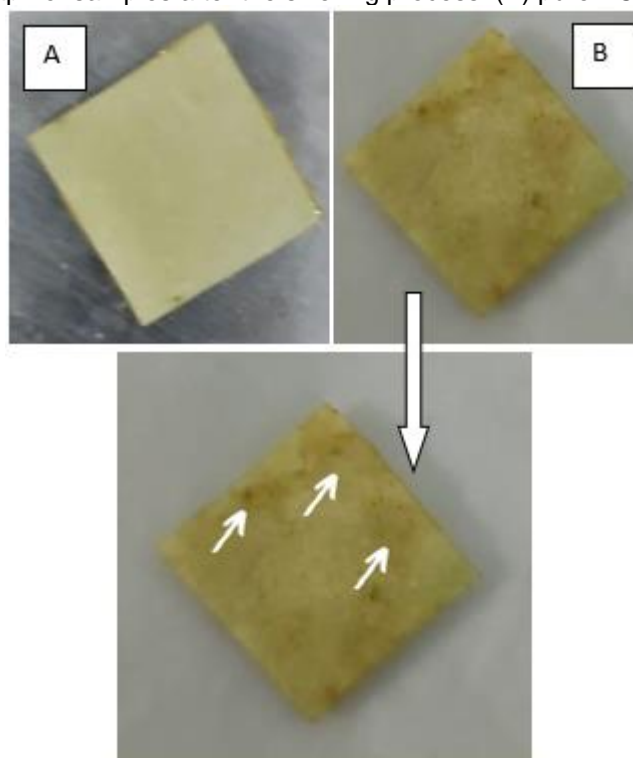
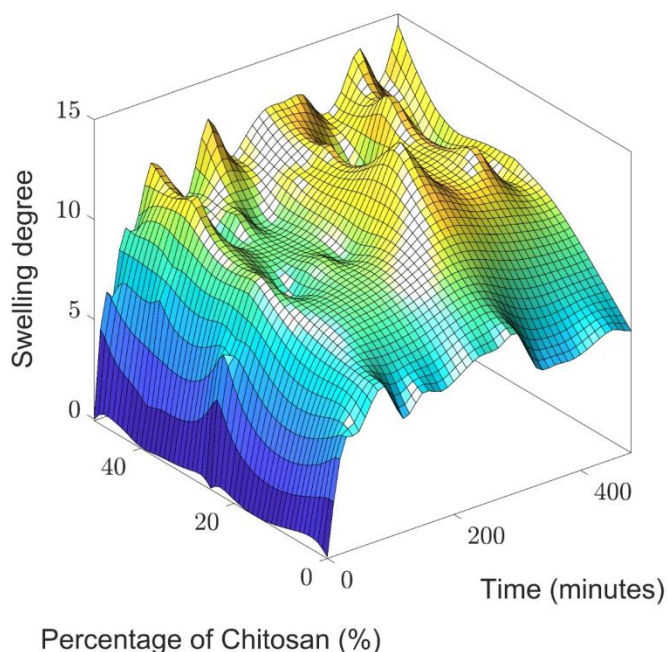




Figura 4 - Thin plate spline interpolation - Swelling degree prediction



According to da Silva et al., 2021 and Oliveira et al., 2021, PCL presented an endothermic transition around  $63.23^{\circ}\text{C}$ . It means that, around this temperature, PCL molecular mobility trends occur. Thus, in the present work, samples of PCL-Chi were immersed in arnica glycolic extract at  $50^{\circ}\text{C}$ . The temperature increment promotes an increase in arnica absorption. In one hour, the amount absorbed was more than the observed for 8 hours at room temperature. It is essential to say that although the swelling occurs fast, a disadvantage is that sample fragilization may occur.

Figure 5 - Swelling degree at room temperature and 50°C

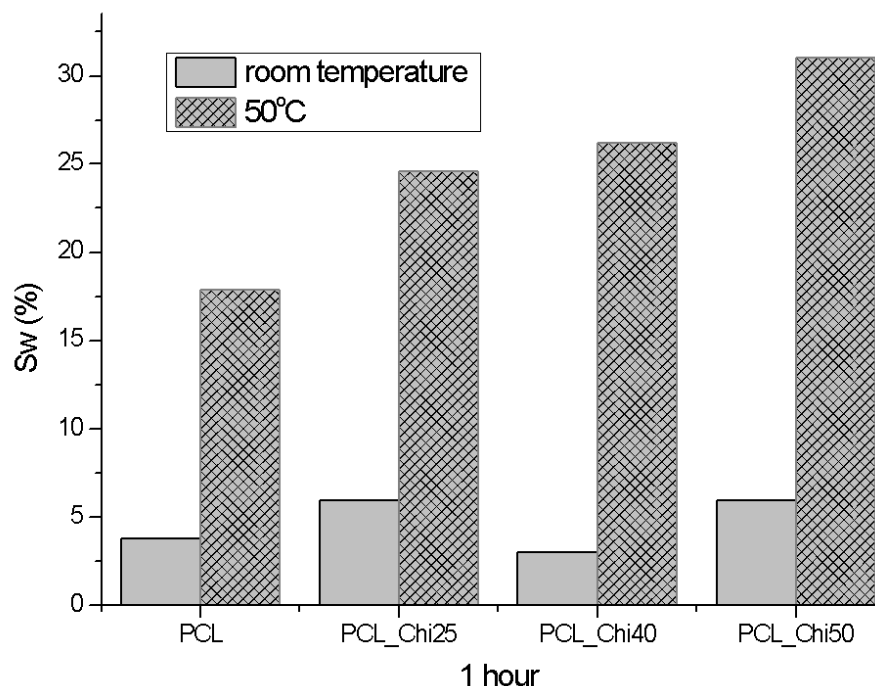


Figure 6 shows matrices FTIR spectra before the swelling process. It is possible to observe bands related to Chi and PCL. The resulting spectra were similar to the ones observed in the literature (da SILVA et al., 2021; Ghosal et al., 2018; Zanetti, et al., 2019). Silva et al. (2019)) studied matrices of PHB and chitosan and observed that the polymer could encapsulate chitosan.

Figure 6: FTIR Spectra of PCL, PCL\_Chi25, PCL\_Chi40, and PCL\_Chi50 separate (A) and together (B).

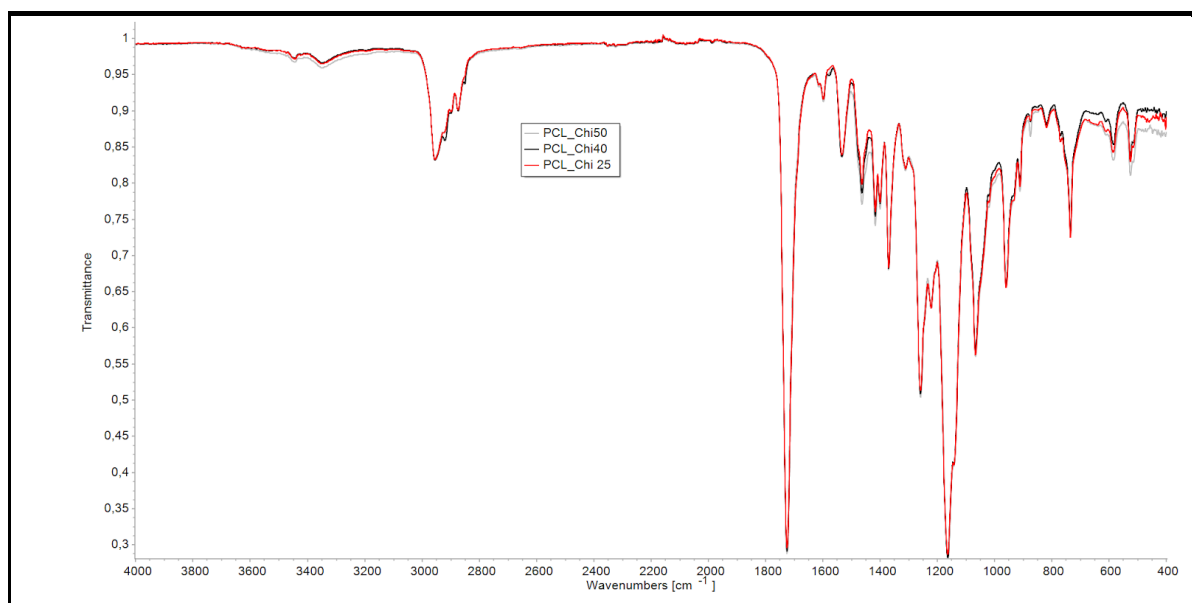
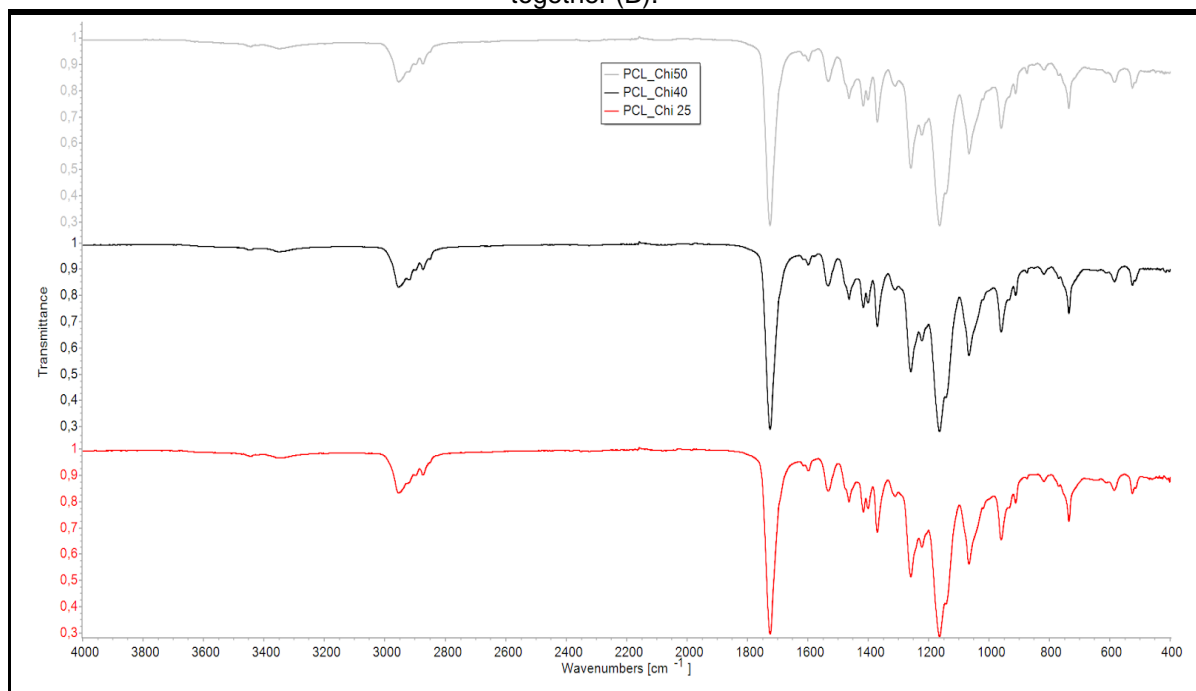
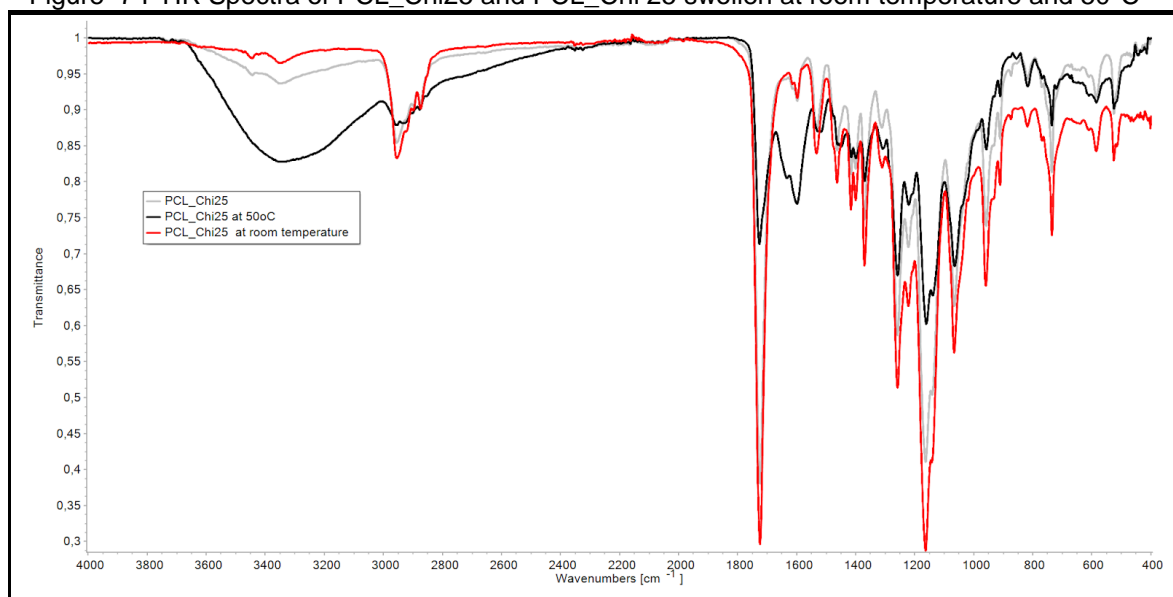


Figure 7 FTIR Spectra of PCL\_Chi25 and PCL\_Chi 25 swollen at room temperature and 50°C



FTIR analyses was applied to investigate the effect of heating on the chemical interactions.

Analyzing the carbonyl band at  $1750\text{ cm}^{-1}$ , we notice an intensity reduction. The relative intensity of PCL\_Chi bands compared to this one for pure PCL was 0.95. However, we detected that the temperature affects the swelling process. Although they absorb more arnica at  $50\text{ }^{\circ}\text{C}$ , the band intensity is lesser (Figura 10). PCL also absorbs arnica at room temperature. However, probably due to the chain mobility, the amount absorbed was more significant. All composition studied in this work presents similar behavior at  $50\text{ }^{\circ}\text{C}$ . But, in this case, the relative intensity decreases when the amount of Chi increase. This fact reinforces the hypothesis of the positive effect of polymeric chain mobility in arnica absorption. Moreover, the spectra seem to present more characteristics of PCL. Contrarily, at room temperature, we observe that the relative intensity increases with the increase of Chi in the sample. Although, the relative band intensity is lesser than the state at  $50\text{ }^{\circ}\text{C}$ . At room temperature, the bands related to OH groups are more strongly; after the swelling process, Chi characteristics bands are more distinguished. PCL and Chi are involved in the absorption process, but the temperature and sample composition influence the swelling behavior.

Figure 8 -Spectra of PCL\_Chi40 and PCL\_Chi 40 swelled at room temperature, and 50Oc.

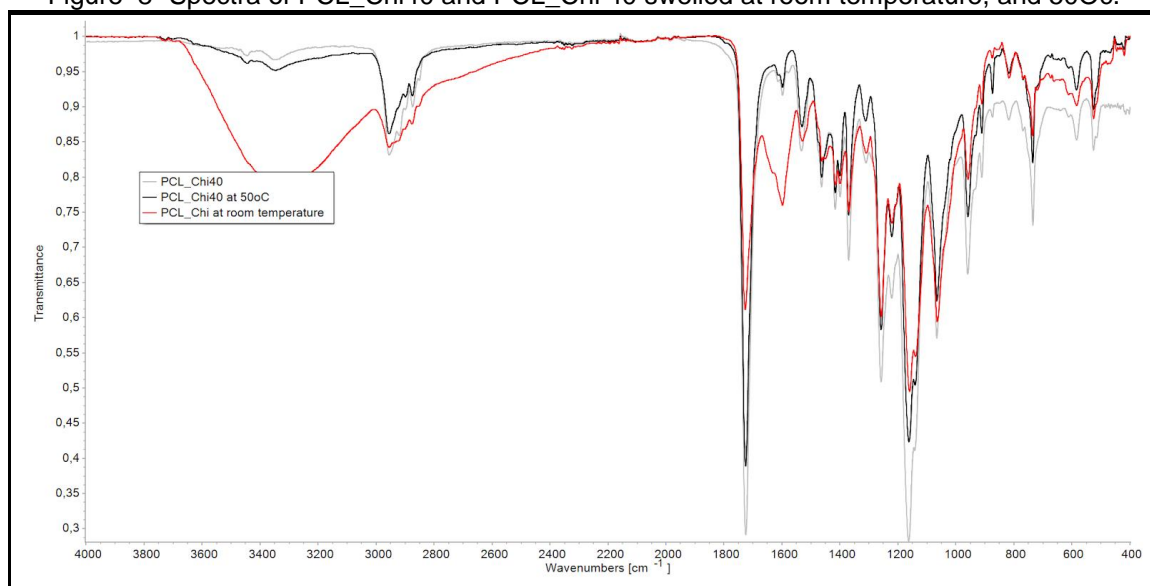


Figure 9 - Spectra of PCL\_Chi50 and PCL\_Chi 50 swelled at room temperature, and 50oC

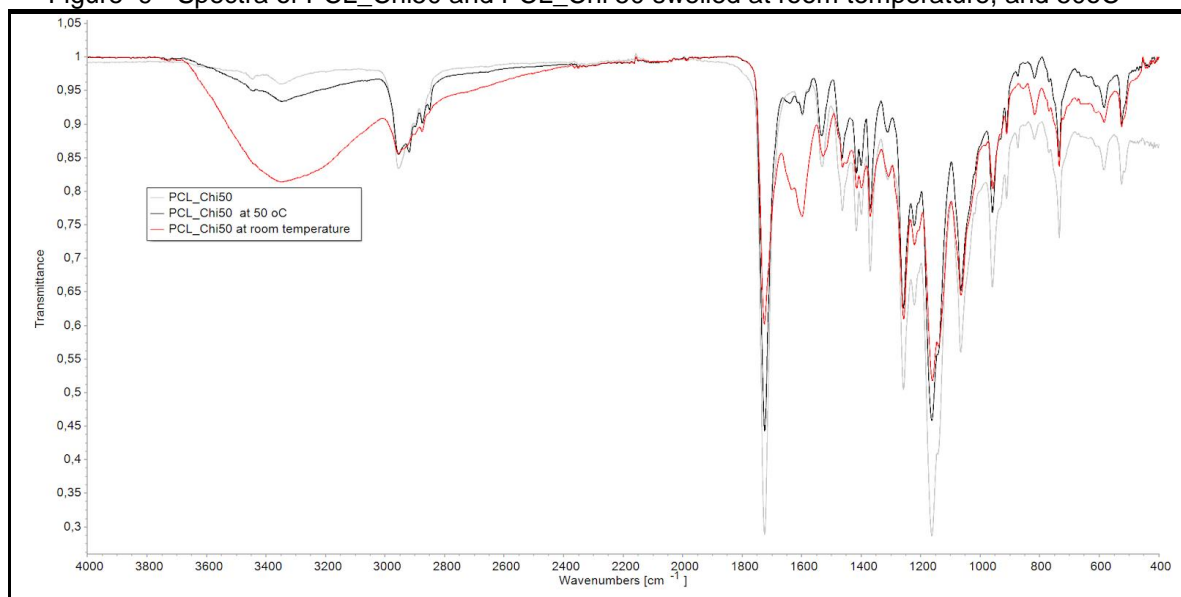
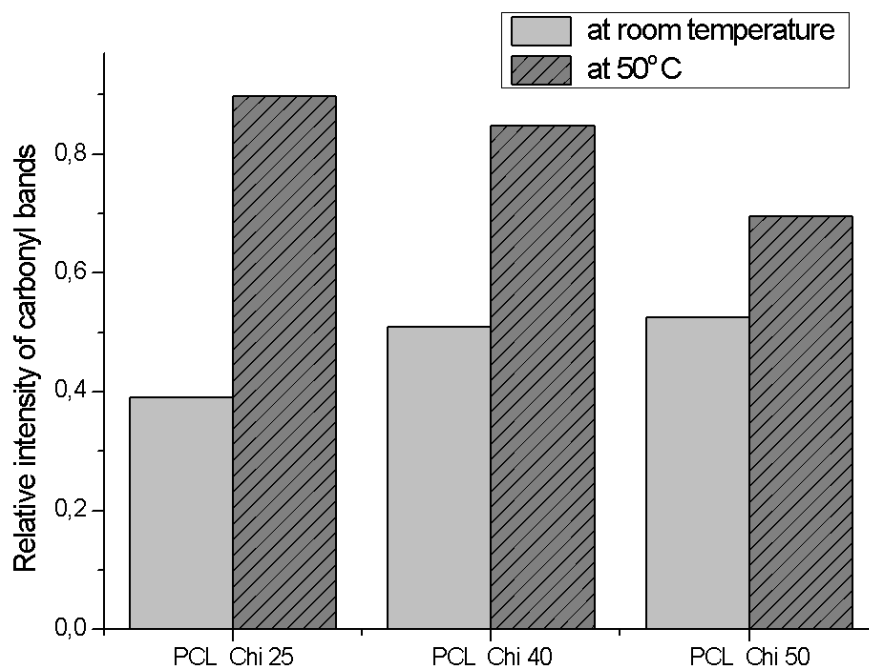


Figure 10 - Relative intensity of carbonyl bands in comparison to samples spectra before swelling



#### 4 CONCLUSION

Matrices of polycaprolactone and chitosan were prepared using the microwave. The effect of temperature on swelling degree was studied. The matrices were added to arnica alcoholic extract at room temperature and at 50o C. It was stated that swelling occurs fastly at 50oC. Moreover, the amount of arnica absorbed is more considerable at 50oC for all samples studied. FTIR analysis showed that interaction between PCL, Chi, and arnica is affected by temperature.





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