

# Waterborne hybrid polyurethane coatings containing Casein as sustainable green flame retardant through different synthesis approaches

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## ABSTRACT

Waterborne polyurethane (WPU) dispersions were prepared for flame retardant coatings. Specifically, alkoxy-silane-capped polycaprolactone-based WPUs were synthesized employing the acetone process, and Casein, as a green and sustainable flame retardant additive, was added by two different methods (*in situ* and *ex situ*). These two strategies made possible to evaluate the effect of the Polyurethane/Casein interaction in the final properties of the dispersions and films. FTIR and solid-state <sup>29</sup>Si NMR, confirmed the formation of the siloxane network during film generation process. The addition of Casein during the synthesis (*in situ*) resulted in a covalent bonding between the polyurethane and Casein, which significantly increased the particle size. However, the incorporation after phase inversion of the WPU (*ex situ*), did not change the particle size. Tensile tests revealed that the covalent bond promoted an increase in the brittleness of the material compared to *ex situ* approach due to a better dispersion of the Casein in the system. TGA results showed that Casein increased the thermal stability of all the coatings, especially of those obtained by the *ex situ* route. Moreover, and according to the microscale combustion calorimeter (MCC) and vertical burning test (UL-94) measurements Casein delayed the combustion of the material. Consequently, due to their characteristics, these Casein-WPU dispersions could potentially be used as combustion retardant coatings, where good physicochemical properties are essential for effective performance.

## 1. Introduction

Nowadays the use of waterborne polyurethanes (WPU) is experiencing a huge growing since they do not contain volatile organic compounds (VOC) and accordingly they can be considered safe for the human health and the environment [1].

The main process to obtain WPUs is called acetone process and involves the synthesis in solution of a polyurethane containing an anionic internal surfactant and the subsequent emulsification. The final VOC free dispersion is obtained after the solvent removal [2]. Due to the presence of the internal emulsifier (usually containing carboxylate groups), waterborne polyurethane dispersions present poor water resistance and accordingly their water absorption is high, limiting their applications. One of the most useful methods employed to overcome this drawback involves the crosslinking of the material during the film

formation process. For this purpose, several methods are utilized such as the introduction of high functionality monomers [3], and the functionalization of the polyurethane chains with acrylic double bonds or with alkoxy-silane groups [4,5]. In the latter, alkoxy-silane hydrolysis and condensation reactions take place during the drying process giving rise to an organic/inorganic hybrid polymer network with improved mechanical and thermal properties.

Cross-linkable waterborne Polyurethane dispersions can be successfully employed to coat, “*inter alia*”, leather and wood [6,7]. In this application, the fire behavior of the coating is important to protect substrates, and therefore great varieties of methods towards reducing the flammability of the coatings have been developed. Thus, different types of flame retardant additives can be added to polyurethanes *via* blending or covalent bonding [8,9].

Halogenated compounds are very effective flame-retardants but

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unfortunately the gases generated in their burning processes are extremely toxic [10]. Phosphorous containing compounds represent a safer alternative and they have proved to be effective as intumescent additives improving the fire behavior of waterborne polyurethanes [11,12]. Recently phosphorous containing biomolecules such as DNA and Casein have been employed as green sustainable flame retardant additives [13–15]. Considering that Casein is a byproduct in the cheese production and that it is a cost effective product, its use as sustainable flame retardant coating for cotton has been explored [16–20]. Casein has also been used as sustainable flame retardant for acrylic coatings [21,22], poly lactic acid [23] and poly propylene [24]. It has also been used to improve the fire resistance of polyurethane foams [25,26]. Moreover, Casein has been successfully employed as filler in waterborne polyurethanes [26–28] but its effect as flame retardant has not been established.

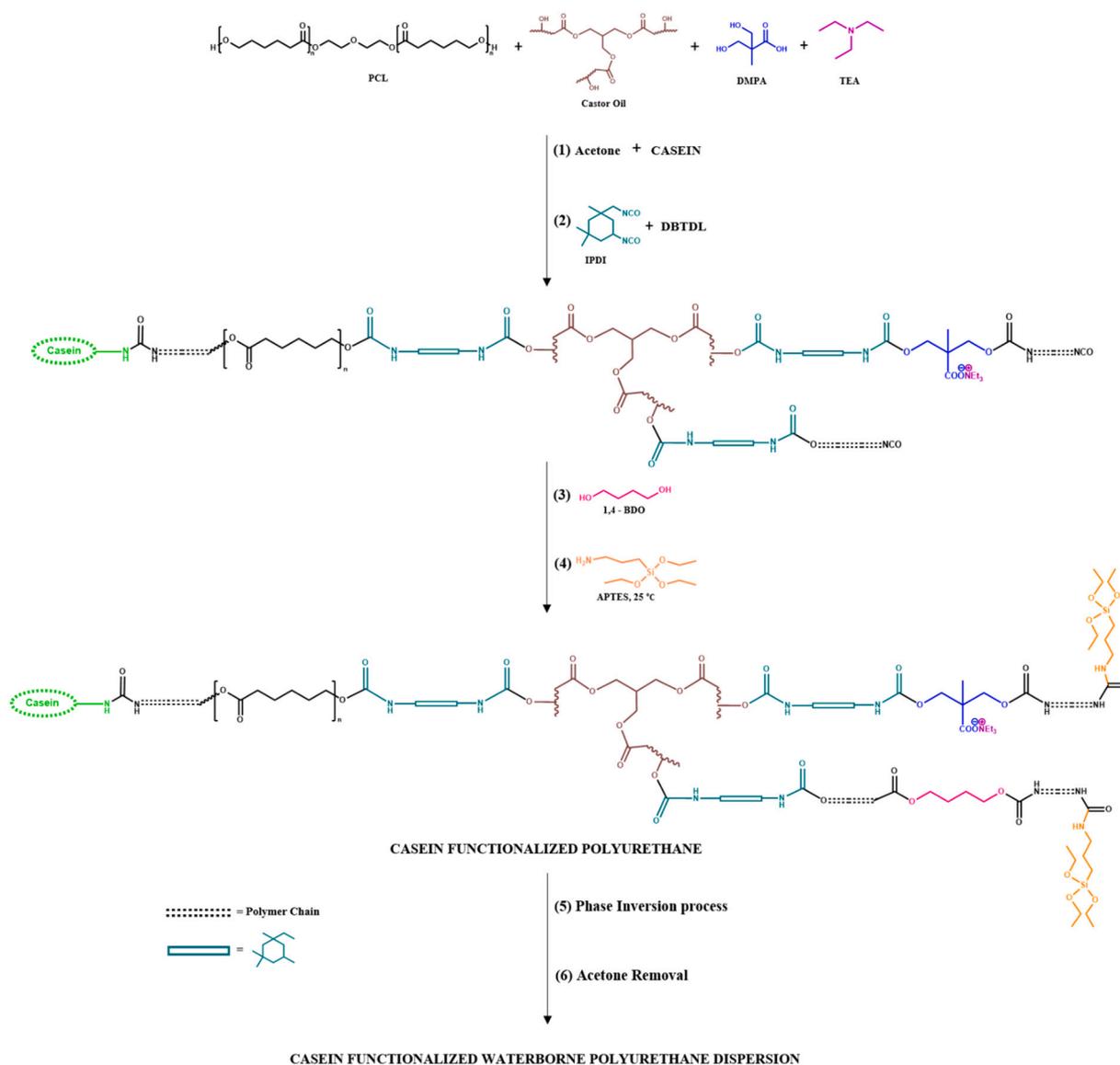
In light of the above, the main objective of the present study was to improve the fire resistance as well as other physical properties of waterborne polyurethanes by adding Casein and crosslinking the samples *via* hydrolysis and condensation of alkoxy silanes. Thus, alkoxy silane capped polycaprolactone based WPU were obtained by the acetone process and Casein was added through two different synthesis approaches (*in situ* and *ex situ*). These two synthesis methods made

possible to evaluate the effect of the polyurethane/Casein interaction, which in turn was closely related to the final properties of the films.

## 2. Experimental section

### 2.1. Materials

Isophorone diisocyanate (IPDI, purity 98 %), polycaprolactone diol (PCL) ( $M_n = 2000 \text{ g}\cdot\text{mol}^{-1}$ ), Casein (from bovine milk, technical grade), 2-bis(hydroxymethyl) propionic acid (DMPA, purity 98 %), 1,4-butanediol (BDO, purity 99 %), triethylamine (TEA, purity 99 %), dibutyltin diacetate (DBTDA, technical grade), (3-aminopropyl)triethoxysilane (APTES, purity 99 %), castor oil (CO), acetone (purity  $\geq 99.8$ ) and *o*-Phthalaldehyde (OPA, purity  $\geq 99$  %) were purchased from Sigma Aldrich. Polycaprolactone diol (PCL) with molecular weight of  $M_n = 1250 \text{ g}\cdot\text{mol}^{-1}$  was obtained from Polysciences. The deionized demineralized water (conductivity  $< 1 \mu\text{S}\cdot\text{cm}^{-1}$  and resistivity  $1,000,000 \text{ Ohm}\cdot\text{cm}$ ) used for the aqueous dispersions of the polyurethanes was acquired from Quimibacter S.L. All chemicals were used as received without further purification.



**Scheme 1.** The schematic illustration for the synthesis of Casein functionalized WPU.

## 2.2. Synthesis of Casein-WPU dispersions

A sequence of Casein functionalized WPU dispersions were prepared through the acetone method. The schematic illustration of the synthesis is shown in Scheme 1 and the amounts of the reagents in Table 1. For all the reactions, the molar ratios of the reagents were maintained, except for Casein, whose content varied from 2 to 7 wt% (in relation to total polymer mass). Firstly, the desired amounts of Casein, PCL, DMPA (internal anionic surfactant), TEA (to completely neutralize the acidic groups, at TEA:DMPA molar ratio 1.3:1), CO (for hydrophobicity of the system) and acetone (40 wt% of the total mass of PU) were added to a 250 mL jacketed reactor. This was equipped with a mechanical stirrer (IKA Eurostar 20), a reflux condenser and a nitrogen inlet. A water bath (Thermo Haake AC 200) was also employed to control the temperature of the system. As the reagents were dissolved in acetone by stirring at 200 rpm, the temperature was raised to 56 °C. Once the temperature was reached and the solution homogenized, the IPDI and DBTDA (0.08 wt% of the total polymer mass) were fed into the reactor. The reaction proceeded until the ratio between stretching band of the isocyanate group (around 2260 cm<sup>-1</sup>) and stretching band of the C—H groups (3000 cm<sup>-1</sup>) did not change, for which about 1 h was required. Thus, the prepolymer with terminal isocyanate groups was obtained. Then, the chain extender BDO was introduced and the reaction continued for 4 h at 56 °C. Afterwards, the temperature was reduced to 25 °C and APTES was added. The synthesis proceeded for another hour to obtain functionalized Casein-PU in acetone. Finally, a phase inversion process at room temperature was carried out to obtain the desired dispersions in water. For this purpose, the obtained formulations were set to 60 wt% polymer. Afterwards, under vigorous stirring (450 rpm), deionized water was added drop-wise to the reactor by addition pump (Lab Pump Jr. RHSY) at 3 mL·min<sup>-1</sup>. In this step, the hydrolysis of the alkoxy silane groups attached to the PU chains took place and silanol groups were formed. Lastly, acetone was removed at 25 °C using a rotary evaporator (IKA Labortechnik RV 06-ML connected to IKA LVS 105 T-ef vacuum pump) at 100 mbar for 1 h.

The resulting dispersions had a solid content of around 30 wt% and pH values around 9. Furthermore, one synthesis was performed without Casein and another with 2 wt% of Casein but employing a PCL of 1250 g·mol<sup>-1</sup>. Dispersions were named PU-X (PCLY), where X denoted the weight percentage of Casein and Y the molecular weight of PCL.

## 2.3. Ex situ synthesis of Casein-WPU dispersions

Casein-WPU dispersions were also performed *via ex situ* method. First, a 10 wt% Casein dispersion at basic pH was prepared by adding 26 g of Casein, 230 g of deionized water and 4 g of TEA in a 250 mL flask with a condenser. The mixture was heated at 60 °C for 2 h using an oil bath and magnetic stirring. Afterwards it was cooled at room temperature. Then, for each Casein content, 25 g of PU-0(PCL2000) dispersion were placed aside under constant stirring and the desired amounts of Casein solution were gently added, considering the solids content (30 wt %). The quantities of Casein added were 2, 3, 5 and 7 wt%. Finally, the mixtures were maintained at room temperature at 200 rpm for 3 days. The obtained dispersions were named *exPU-X*(PCL2000), where X

**Table 1**  
Compositions of Casein-WPU dispersions.

Samples	IPDI/g (mmol)	PCL/g (mmol)	CO/g (mmol)	DMPA/g (mmol)	TEA/g (mmol)	BDO/g (mmol)	APTES/g (mmol)	Casein/g
PU-0 (PCL2000)	7.52	25.50	2.28	0.98	0.96	0.45	2.30	0
PU-2 (PCL2000)	(33.9)	(12.8)	(2.4)	(7.3)	(9.5)	(5.0)	(10.4)	0.8
PU-3 (PCL2000)								1.2
PU-5 (PCL2000)								1.9
PU-7 (PCL2000)								2.7
PU-2 (PCL1250)	30.00 (135.0)	63.75 (51.0)	9.10 (9.7)	3.90 (29.1)	3.80 (37.8)	1.80 (20.0)	9.10 (41.1)	2.4

denoted the weight percentage of Casein.

## 2.4. Preparation of Casein-WPU films

The homogeneous Casein-WPU dispersions were casted onto silicon plates and dried at room temperature for 10 days. As the water evaporated, film formation took place through the condensation reaction of terminal silanol groups to form a stable siloxane network. The final film was removed from the mold, taking care not to deform it.

## 2.5. Characterization

Fourier transform infrared spectroscopy (FTIR) was used to analyze the structure of the polymers and to monitor the polymerization process. FTIR spectra of PU prepolymers diluted in acetone were obtained at room temperature and at different reaction times employing Nicolet 6700 equipment in reflection with the Golden Gate ATR accessory at a resolution of 8 cm<sup>-1</sup> for sixteen scans. The conversion of the NCO groups was calculated using Eq. (1):

$$\text{Conversion (\%)} = \left( 1 - \frac{\left( \frac{A_{2260}}{A_{3000}} \right)_t}{\left( \frac{A_{2260}}{A_{3000}} \right)_{t_0}} \right) \times 100 \quad (1)$$

where  $A_{2260}$  is the area of the stretching band of the isocyanate group at 2260 cm<sup>-1</sup> and  $A_{3000}$  is the area of the bands due to the stretching vibration of the methyl and methylene groups at 3000 cm<sup>-1</sup>.

The structural characterization of the reagents and the functionalized PU in acetone was also carried out by liquid <sup>1</sup>H nuclear magnetic resonance (NMR) obtained in Bruker 300 MHz spectrometer. The solvent used was deuterated chloroform (CDCl<sub>3</sub>) (Sigma Aldrich, 99.9 %), except for Casein for which deuterated water with TEA was used. Solid-state <sup>29</sup>Si NMR and <sup>13</sup>C NMR spectra of WPU films were recorded using a Fourier Transform Bruker 300 MHz (model Advance 300 DSX) with reference to tetramethylsilane (TMS).

Dynamic light scattering (DLS) technique was used for particle size characterization. Brookhaven Instruments 90 Plus analyzer and 1.5 mL PMMA disposable cuvettes were employed. For each sample, three measurements were obtained at 25 °C with a refractive index of 1.52. In all the cases, one drop of PU dispersion was diluted in distilled water.

Z-Potential measurements were registered on a Malvern Zetasizer Ultra (Malvern Paralytical Instruments). Samples were diluted with distilled water until a final concentration of 0.5 mg·mL<sup>-1</sup> was obtained and sonicated for 1 h at room temperature. The measurements were performed on plastic zeta DTS1070 cuvettes and a backscatter angle of 175°.

The Casein/Polyurethane reaction was evaluated after derivatization with OPA in a spectrophotometric assay [29]. To separate the non-reacted Casein a multiple centrifugation and redispersion process was applied [30]. About 10 g of the dispersions were centrifuged at 20000 rpm for 4 h at 5 °C. The supernatant was separated and the solid was redispersed with 5 mL of a TEA/water solution (1.7 wt%) and it was stirred for 12 h to remove the non- reacted Casein. The procedure of

centrifugation/separation/redispersion was repeated twice. The UV absorption at 340 nm was analyzed in a Shimadzu UV-3600 spectrometer.

The non-soluble part of Casein-WPU films was determined from the measurement of the weight loss of the samples after being in water and water/TEA mixture (690 g of water and 12 g of TEA) using a microwave with pressurized cells (MARS 5, CEM) that allows working at high pressures. Extractions were carried out at 110 °C and 200 Psi using a power of 400 W for 15 min, after which the solid part was separated from the soluble part. The solvents (water and TEA/water) were allowed to evaporate first under infrared light and then, in order to obtain a constant weight of both the soluble and insoluble parts, the extractions were kept in the oven at 50 °C with vacuum for at least 6 days.

The molecular weight distribution of the samples before the phase inversion was studied by Gel permeation chromatography (GPC). Specifically, GPC Ultimate 3000 (Thermo Scientific) equipped with a RI RefractoMax 250 (ERC) and four Phenogel columns (Phenomenex) in series was employed. Tetrahydrofuran was used as eluent at flow rate of 1 mL·min<sup>-1</sup>. All the samples were filtered prior to injection into the GPC (filter pore size = 0.45 µm, Scharlab). The system was calibrated using different molecular weight polystyrene standards and at 25 °C.

The tensile properties of the Casein-WPU films were examined using an MTS Insight 10 testing machine. The gauge length was set at 10 mm and the crosshead speed of testing at 50 mm·min<sup>-1</sup> at room temperature with a 250 N load cell. Before testing, the films were cut into a dog-bone shape and dried at 50 °C under vacuum for 3 days to ensure equilibration of the moisture uptake in the films. The Mega-Check 5F-ST thickness gauge was used to obtain the average thickness of all samples (ten measurements per sample). The minimum thickness recorded was 0.93 ± 0.11 mm and the maximum was 1.13 ± 0.12 mm. Six identical specimens of each composite sheet were tested and the average values were reported.

Differential scanning calorimetry (DSC) analyses were performed on DSC Q2000, TA Instruments equipment at a heating rate of 10 °C·min<sup>-1</sup> under nitrogen atmosphere (flow rate of 50 mL·min<sup>-1</sup>), in order to evaluate the thermal transitions of the Casein-WPU films. The samples were heated from -80 °C to 150 °C, maintained at this temperature for 1 min to erase the thermal history, then cooled to -80 °C at a cooling rate of 10 °C·min<sup>-1</sup> and heated again to 150 °C. The cold crystallization temperature (T<sub>cc</sub>) and cold crystallization enthalpy (ΔH<sub>cc</sub>) were determined from the second heating scan, while the glass transition temperature (T<sub>g</sub>), melting temperature (T<sub>m</sub>) and enthalpy (ΔH<sub>m</sub>) were measured from the first and second heating scans. The degree of crystallinity (X<sub>c</sub>) for PCL samples was calculated from the second heating scan and using Eq. (2):

$$X_c(\%) = \left[ \frac{\Delta H_m}{\Delta H_m^0} \right] \times 100 \quad (2)$$

where ΔH<sub>m</sub> is the melting enthalpy of the film samples and ΔH<sub>m</sub><sup>0</sup> is the melting enthalpy of the 100 % crystalline PCL (135.44 J·g<sup>-1</sup>) [31].

Thermogravimetric analyses (TGA) were performed on a TA instruments TG-Q-500 at a heating rate of 10 °C·min<sup>-1</sup> under nitrogen and air atmosphere (flow rate of 90 mL·min<sup>-1</sup>), within the temperature range of 40–800 °C. In the experiment in nitrogen, once the maximum temperature was reached, it was maintained for 5 min and then air atmosphere (O<sub>2</sub>) was incorporated for 15 min.

Microscale combustion calorimeter (MCC) was used to evaluate the flammability of Casein-WPU films according to ASTM D730913. For MCC tests, FAA Micro Calorimeter was employed and 8–10 mg of samples were heated to 700 °C at a heating rate of 1 K·s<sup>-1</sup> in a stream of nitrogen flowing at 80 mL·min<sup>-1</sup> and then mixed with oxygen at 20 mL·min<sup>-1</sup> before incoming a 900 °C combustion furnace.

The vertical burning tests (UL-94) of WPU films were carried out with the dried samples according to ASTM D3801-1996 standard. The thickness of the samples was around 1 mm.

Morphological features of the different Casein-WPU films were analyzed by scanning electron microscopy (SEM). A Hitachi TM3030Plus Tabletop Microscope SEM device with an accelerating voltage of 15 kV took images of the fractured surfaces. For all the cases, the specimens were fractured after being in liquid nitrogen for 1 h and then covered with gold under an Argon atmosphere at SC 500 sputter coater.

The adhesion properties between Casein-WPU films and a metal surface was determined by PosiTest pull-off adhesion tester and using a resin adhesive (EP11HTGray). The diameter of the dolly used was 20 mm. The greatest tensile pull-off force for which the coating could adhere to the substrate was evaluated in terms of mega Pascal. In order to prepare the test specimens, the Casein-WPU film was formed on a metal surface. Three measurements were carried out for each sample.

## 2.6. Data analysis - statistical analysis

Data from mechanical properties were evaluated by Shapiro-Wilk to corroborate the normal distribution. Once it was verified that the data were parametric, an analysis of variance (ANOVA) could be used to determine if there were differences between the samples. Tukey's *post hoc* test was used when there was homogeneity of variances between the groups and the Games-Howell test if the variances were different. The confidence interval selected was 95 %, so that with a *p*-value < 0.05 the null hypothesis that the samples were equal could be rejected. Calculations were performed with SPSS 26 statistical software (IBM SPSS Statistic).

## 3. Results and discussion

### 3.1. Structural characterization

The synthesis process of Casein-WPU was monitored by FTIR at different reaction times (Fig. 1.A). Briefly, the polymerization could be described in three main steps. At the first hour, the reaction between the less reactive alcohols with the excess of diisocyanate took place. During this period, the stretching band of the isocyanate group (2260 cm<sup>-1</sup>) decreased in intensity, but it was not after the second step, the addition of BDO as a chain extender, when it markedly decreased. In the last step, APTES was added to react with the remaining free isocyanate groups in order to obtain an alkoxy silane capped PU and thus, the complete disappearance of the band of isocyanate groups occurred. Likewise, as the reaction progressed, the characteristic peaks of the urethane groups increased in intensity: -NH stretching vibration at 3325 cm<sup>-1</sup>, -C=O stretching vibration at 1710 cm<sup>-1</sup>, and -NH bending vibration at 1533 cm<sup>-1</sup> [32].

Additionally, the extent of the reaction was calculated using the isocyanate NCO stretching vibration band decrease, assuming that there were no side reactions. Fig. 1.B shows the conversion of the isocyanate functional groups during the PU-3 (PCL2000) reaction that was calculated with the normalized area of the NCO band, measured at different reaction times, and the area of this band at the initial reaction time, using Eq. (1).

As observed, conversion increased with time. A huge increase was noticed at 60 and 300 min due to the addition of BDO and APTES. At the end of the reaction total conversion was reached. The same behavior was observed for all the syntheses performed in this work where the conversion of the isocyanate groups exceeded 98 %.

Considering the conversion of the isocyanate groups (P), the Casein-WPU kinetics analysis could be performed as follows (Eq. (3)):

$$P(t) = \int_0^t dP \quad (3)$$

In literature, different kinetic models describing the reaction conversion based on empirical rate laws are reported [33–37]. However, in this study, the kinetic model of order *n* proposed by Li et al. [36] was

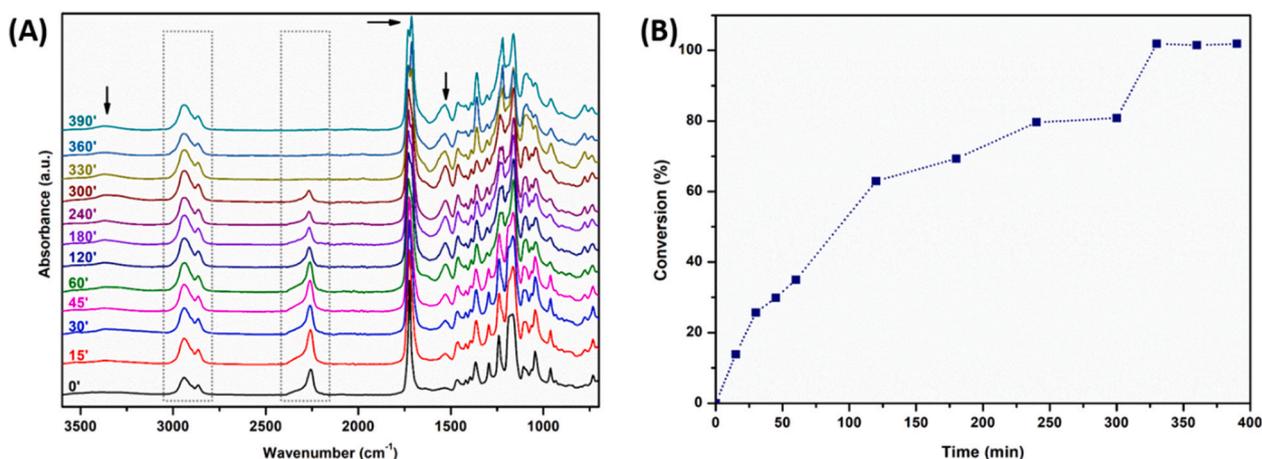


Fig. 1. (A) FTIR spectra at different reaction times and (B) isocyanate conversion as a function of time for PU-3 (PCL2000).

applied (Eq. (4)).

$$\frac{dP}{dt} = K_0(1 - P)^n \quad (4)$$

where parameter  $n$  is related to the reaction order and  $K_0$  to the value of rate constant. According to this equation, the data obtained were fitted to three exponentials.

If  $n = 1$

$$\ln(1 - P) = -K_0t + C \quad (5)$$

If  $n = 2$

$$\frac{1}{(1 - P)} = K_0t + C \quad (6)$$

If  $n = 3$

$$\frac{1}{(1 - P)^2} = K_0t + C \quad (7)$$

Fig. 2 shows (solid line) the results for the first 30 min of reaction using the equation for reaction order one (Eq. (5)). The dashed lines correspond to the linear regression analysis. The fit to the other two equations can be found in the supplementary information (Fig. S1). For all cases, the value of the correlation coefficient ( $R^2$ ) was good, but the best fit was found for a first order reaction. Therefore, considering the highest value of the regressions, it could be concluded that the Casein-

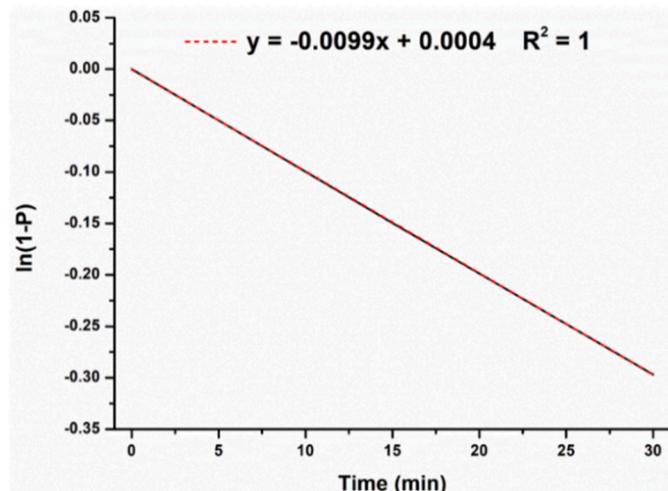


Fig. 2. First order rate law representation for PU-3 (PCL2000).

WPU synthesis studied followed one-order kinetics. At this point, it should be emphasized that the Li et al. model used could only be applied during the first reaction times, because during the PU synthesis process, the mixture became more viscous, and, consequently, the reaction mechanisms changed, leading to a deviation in the kinetic order. According to the values of the apparent constant ( $K_0$ ) shown in Table 2, which were determined from the slope of each straight line, it could be asserted that the addition of 2 % Casein led to a decrease in the value of the constant, but as the content increased, the value remained constant. Furthermore, it could be noted that the change of the molecular weight of PCL did not provide differences in the values of the constant.

In order to determine the chemical structure of the PUs synthesized with the *in situ* method,  $^1\text{H}$  NMR spectroscopy analysis of the reaction products, before the phase inversion process, was performed. As it can be distinguished in Fig. 3, in PU-0(PCL2000) the presence of APTES was confirmed by the signals at 0.6 and 3.8 ppm (assigned with the letters **b** and **c**) due to the protons of the methylene groups attached to the silicon and the methylene protons of the ethoxysilane groups, respectively. Likewise, according to H. Sardon et al. [38] the chemical linkage between polyurethane and alkoxy silane was confirmed by the presence of the signal at 3.2 ppm (**a**) which was assigned to the methylene groups attached to the urea groups. The  $^1\text{H}$ -NMRs spectra of the rest of the dispersions presented the same signals and are shown in the supplementary information (Fig. S2).

Casein-PU films were dried at room temperature for 10 days. The films were not soluble in any solvent evidencing the high crosslinking degree of the samples and their chemical resistance. Solid-state  $^{29}\text{Si}$  NMR and  $^{13}\text{C}$  NMR analysis were conducted to evaluate the Si-O-Si and to assess the chemical structure of the final coating. The acquired results for sample PU-3 (PCL2000) are plotted in Fig. 4 and Fig. 5. In the  $^{29}\text{Si}$  NMR spectrum (Fig. 4) two peaks could be distinguished, approximately at  $-60$  and  $-69$  ppm, which were assigned to silicon atoms with two ( $T_2 = 12\%$ ) and three ( $T_3 = 88\%$ ) siloxane linkages, respectively. The absence of peaks relative to  $T_1$  or  $T_0$  and the high  $T_3$  intensity suggested that the condensation reaction was practically complete, thus demonstrating the formation of highly cross-linked Casein-WPU films [11].

Table 2

The rate apparent constant values of *in situ* Casein-WPU.

Samples	$K_0 \cdot 10^2$ (min $^{-1}$ )
PU-0 (PCL2000)	3.0
PU-2 (PCL2000)	2.0
PU-3 (PCL2000)	1.0
PU-5 (PCL2000)	1.0
PU-7 (PCL2000)	1.0
PU-2 (PCL1250)	2.0

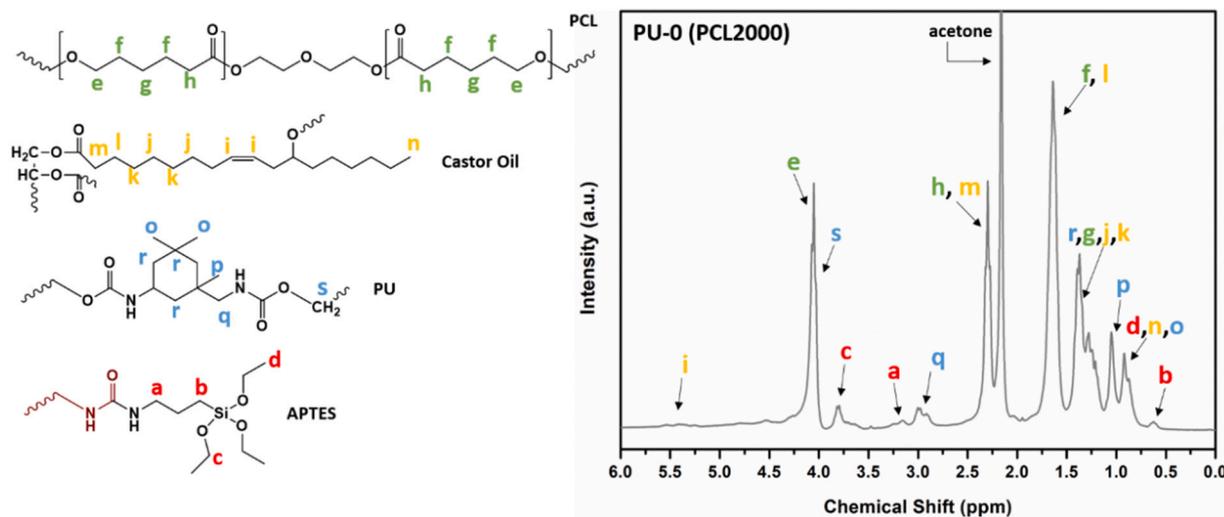


Fig. 3. Schematic illustration of the components used for WPU synthesis and the  $^1\text{H}$  NMR spectrum of PU-0 (PCL2000) in  $\text{CHCl}_3$ .

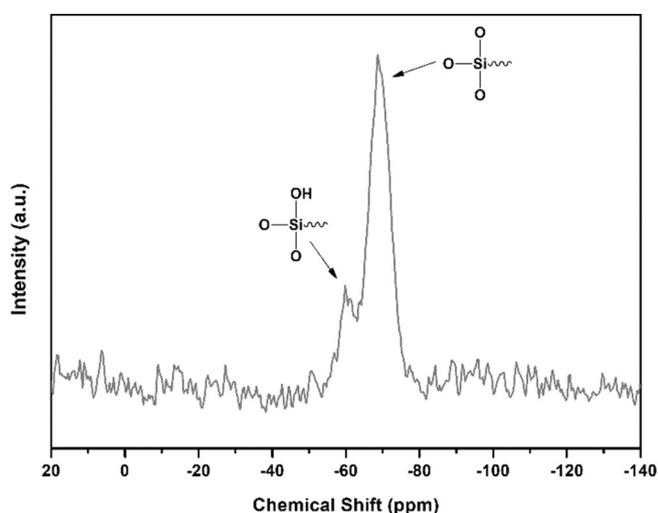


Fig. 4. Solid-state  $^{29}\text{Si}$  NMR of PU-3 (PCL2000).

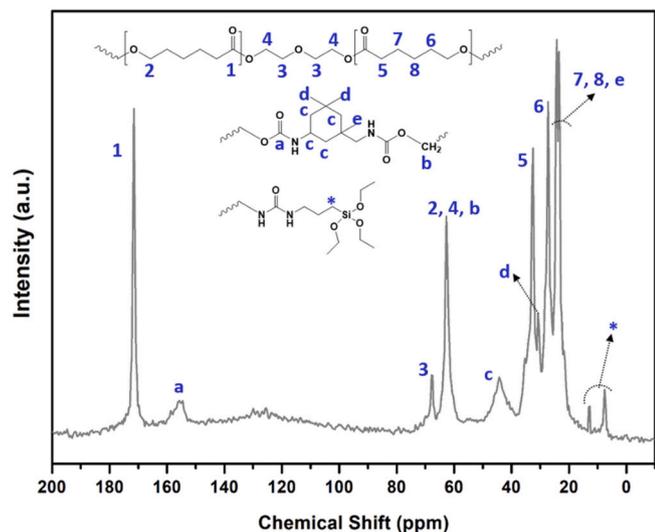


Fig. 5. Solid-state  $^{13}\text{C}$  NMR of PU-3 (PCL2000).

In the solid-state  $^{13}\text{C}$  NMR (Fig. 5), many of the signals associated to castor oil and Casein were overlapped with the PCL signals. Briefly, the signals at 171.5 and 155.8 ppm belonged to the carbonyls of the polyester and PU, respectively. The following signals at 67.7 ppm and 62.7 ppm were attributed to PCL. Of these two, the most intense was assigned to the carbon whose position is next to the oxygen of ester group (2), to the carbon (4) in the center of the PCL, and to the carbon (b) next to the oxygen of the urethane. The IPDI ring carbons (c) were observed at 44.3 ppm. The strong signals between 33 and 24 ppm were mainly due to the methylenes of PCL along with those of castor oil and Casein (solid-state  $^{13}\text{C}$  NMR of Casein powder is shown in the supplementary information Fig. S3). The methyl groups (d) on the IPDI ring could be seen at 30.7 ppm, while the methyl attached to the asymmetric carbon (e) would be masked by the signal from the methylenes. The two signals at the lower chemical shift were ascribed to APTES (\*).

### 3.2. Dispersions and films properties

After water addition and acetone removal the final dispersions were obtained. It is worth mentioning that the synthesized dispersions were complex and contained free polyurethane, free Casein, and Casein-PU copolymer. In addition, these species could be distributed in both phases, particles and water.

The average particle size and GPC data of the Casein-WPU dispersions are reported in Table 3. The addition of different Casein contents in PU-0 (PCL2000) *via ex situ* method has no effect on particle size. Jinazhong Ma et al. [39] in their research, employing caprolactam-modified with Casein and then added to WPU, report linkages between Casein and PU, through the condensation reaction between  $-\text{NH}_2$

Table 3  
Particle size and GPC results for Casein-WPU dispersions.

Samples	Particle Size (nm)	PDI	GPC			
			Mn	Mw	PDI	
<i>Ex situ</i>	PU-0 (PCL2000)	111.8 ± 0.4	0.22	11,243	29,511	2.62
	PU-2 (PCL2000)	110.1 ± 4.9	0.20			
	PU-3 (PCL2000)	115.1 ± 4.8	0.19			
	PU-5 (PCL2000)	112.0 ± 5.0	0.20			
	PU-7 (PCL2000)	114.7 ± 5.4	0.18			
<i>In situ</i>	PU-2 (PCL2000)	387.7 ± 8.8	0.16	8638	16,938	1.95
	PU-3 (PCL2000)	278.5 ± 9.4	0.25	7030	12,547	1.79
	PU-5 (PCL2000)	442.8 ± 1.6	0.24	7358	13,557	1.84
	PU-7 (PCL2000)	318.3 ± 8.8	0.26	6644	11,943	1.80
	PU-2 (PCL1250)	118.4 ± 1.4	0.21			

and  $\text{-COOH}$  groups. This interaction led to a reduction in the self-aggregation of modified-Casein molecules. For this purpose, they kept the mixtures stirred at  $80\text{ }^\circ\text{C}$  for 2 h. However, in our case this linkage can hardly occur because the WPU and the Casein dispersion were not heated to high temperatures. These differences in the process seem to be the reason for no change in particle size. Therefore, this behavior suggested that in *ex situ* route Casein remained dispersed in water (at basic pH) without being part of the WPU structure. The WPU particles were generated in the phase inversion so that a subsequent addition of Casein did not affect the structure of the polyurethane at room temperature.

In contrast, the addition of Casein through *in situ* approach led to an increase in particle size from 112 nm for PU-0 (PCL2000) to at least 278 nm. With the addition of 5 wt% the highest particle size value was achieved, 443 nm. However, there was not a clear relation between the particle size and the Casein content. According to the results obtained by Niangui Wang et al. [27], this effect can be attributed to the reaction between the  $\text{-NCO}$  groups of the prepolymer dispersion and the  $\text{-NH}_2$  of the Casein resulting in  $\text{-NHCONH-}$  bonds, as is shown in Scheme 2. In our case, this bonding was supported by the FTIR results obtained before the addition of APTES (Fig. S4). It was possible to distinguish a decrease in the absorbance band of the isocyanate group ( $2260\text{ cm}^{-1}$ ) with respect to the stretching band of the methyl and methylene groups ( $3000\text{ cm}^{-1}$ ). The chemical bonding decreased the number of NCO-terminal groups. Likewise, the increase in Casein content made the decrease in isocyanate groups more pronounced. These data demonstrated the Casein/PU linkage. Additionally, to corroborate this assertion, the Casein fraction that remained soluble in water/TEA after centrifugation and subsequent cleanings for the dispersions PU-7 (PCL2000) obtained by the *in situ* and *ex situ* methodology was measured by UV assays after reaction with OPA (UV absorption spectra are shown in the supplementary information Fig. S5). From these data, after calibration with pure Casein, the percentage of protein reacted with the PU during the synthesis was calculated and values of 1 and 89 wt% were obtained for the *ex situ* and *in situ* samples, respectively.

Additionally, the effect of DMPA content in the different dispersions was analyzed. For all *in situ* syntheses, the DMPA emulsifier content was the same, 2.5 wt% based on the total polymer mass, but without considering the Casein weight. Hence, as the Casein content increased, the real DMPA content decreased to 2.3 % for PU-7 (PCL2000), which mean less stabilizer per gram of polymer and in turn, the larger particle sizes could be expected [40,41]. Anyway, for *in situ* syntheses, the reduction of emulsifier was not remarkable and it was considered that the increase at the particle size with the Casein concentration was related to the urethane-Casein linkage, that increased the system viscosity before the phase inversion. However, in line with the reported findings of H. Sardon et al. [41] the results for PU-2 dispersion (PCL1250) showed that DMPA content was a significant factor. For this reaction, the molar ratio of the reagents was maintained the same as for the dispersions with PCL2000, which resulted in a higher amount of PCL and thus, a DMPA content of 3.3 wt%. As a result, the higher the amount of stabilizer, the smaller the particle size. This increase in emulsifier led to a particle size very similar to that of the dispersion without Casein.

The stability of the dispersions was compared according to the values

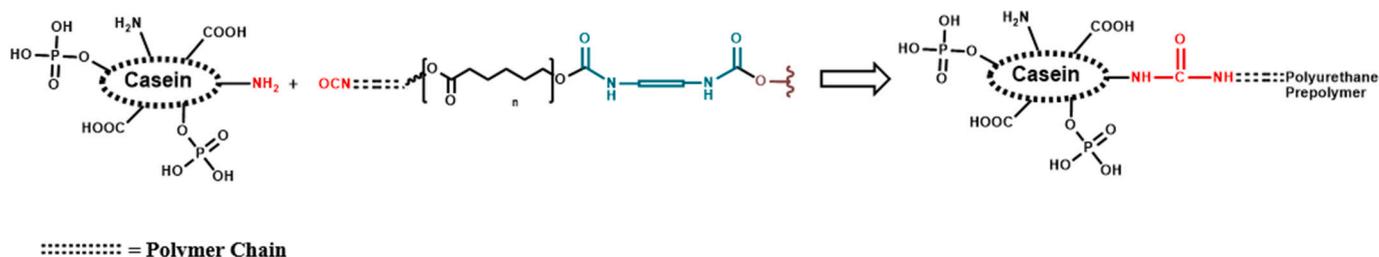
of the Z potential at pH 7.0 (Table S1). As observed, the Z potential value of pure PU was  $-72\text{ mV}$ . However, the addition of 7 wt% of Casein increased Z potential values to  $-49.0$  and  $-44.0\text{ mV}$  for the *ex situ* and *in situ* routes respectively. Therefore, the introduction of Casein changed the surface chemistry of the particles reducing the dispersion stability. However, the stability of all the dispersions was acceptable [42]. In the *in situ* samples the reduction of the stability could be related to a reaction between the carboxylic acid groups of the polyurethane with the amine groups of the Casein. In the *ex situ* route, as stated before, no reaction happened between the Casein and the Polyurethane. The change in the Z potential of this dispersion could be related to the absorption of Casein in the surface of the PU particle. The increase of the diameter of the particles provoked by the possible absorption was theoretically calculated assuming that all the Casein was in the surface of the particles. A value of 3 nm was obtained for the sample containing 7 wt% of Casein obtained *ex situ*. Therefore, the possible Casein absorption did not result in a change of the diameter or the particles as reported in Table 3.

The molecular weight of the samples was measured in THF, before the phase inversion. Samples were filtered before the injection and as Casein (and the Casein grafted to the polyurethane) are not soluble in THF, the measurements corresponded to the neat polyurethane. The results (Table 3) revealed that there were no major differences, but a slight decrease was considered with respect to PU-0 (PCL2000) that became more prominent with increasing Casein content for the *in situ* sample series. This behavior supported the hypothesis of chemical bonding between the  $\text{-NCO}$  groups of the prepolymer and  $\text{-NH}_2$  of the Casein, since in this way there were fewer free  $\text{-NCO}$  ends in the chain to grow with the addition of the chain extender. At the same time, polydispersity was reduced and remained practically constant with Casein content, indicating more homogeneous molecular weight chains. In the supplementary information, an example of the obtained results graph is presented (Fig. S6). The *ex situ* dispersion series was not evaluated by GPC, as all samples were obtained from the same PU-0 (PCL2000) to which the Casein was added once the waterborne polyurethane was formed.

Samples were casted and films were obtained. Fig. 6 shows the infrared spectra of all the coatings. The signals of  $\text{-NH}$  stretching ( $3300\text{ cm}^{-1}$ ),  $\text{-CH}$  stretching ( $<3000\text{ cm}^{-1}$ ),  $\text{-C=O}$  stretching of urethane and PCL ( $1720\text{ cm}^{-1}$ ),  $\text{-NH-}$  bending ( $1550\text{ cm}^{-1}$ ) as well as the  $\text{C-O-C}$  asymmetric stretching of PCL and urethane ( $1150\text{ cm}^{-1}$ ) confirmed the polyurethane structure. A shoulder at  $1650\text{ cm}^{-1}$  was assigned to the  $\text{-C=O}$  stretching of the amide group present in the Casein structure that was overlapped with the urea  $\text{C=O}$  stretching generated because of the bonding of the APTES to the prepolymer chain.

In order to evaluate the chemical linkage between the polyurethane and the Casein, the soluble and non-soluble part of the films were studied and analyzed, both in water and in water/TEA. The percentages of the sample that did not dissolve are shown in Table 4. The non-soluble part of the final films was high in all cases, between 88 and 99 %. These results confirmed the ability of alkoxy silane to form a three-dimensional network, a fact that agreed with the  $^{29}\text{Si}$  NMR in the solid state (Fig. 4) discussed above.

According to the data shown in Table 4, PU-0 (PCL2000) presented



Scheme 2. Schematic illustration of the bond between the Casein and the isocyanate group of the polyurethane prepolymer.

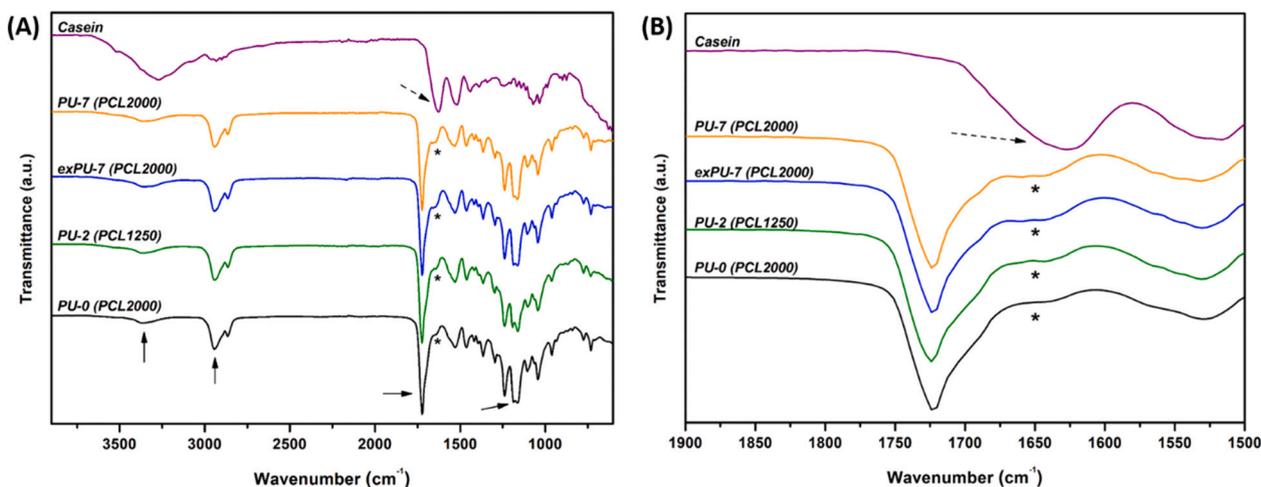


Fig. 6. (A) FTIR spectra of the different Casein-WPU films and (B) scale expanded spectra in the carbonyl region.

Table 4

Data of the non-soluble fraction of the Casein-WPU films *in situ* and *ex situ*.

Samples	Non-soluble part of the film (%)			
	<i>In situ</i>		<i>Ex situ</i>	
	Water	Water/TEA	Water	Water/TEA
PU-0 (PCL2000)	98.6 ± 0.2	99.0 ± 0.2	98.6 ± 0.2	98.0 ± 0.3
PU-2 (PCL2000)	97.8 ± 0.1	98.6 ± 0.2	98.6 ± 0.2	97.9 ± 0.1
PU-3 (PCL2000)	98.0 ± 0.1	96.7 ± 0.4	98.2 ± 0.2	97.7 ± 0.2
PU-5 (PCL2000)	96.6 ± 0.2	90.3 ± 0.2	98.1 ± 0.1	96.6 ± 0.3
PU-7 (PCL2000)	96.9 ± 0.3	88.3 ± 0.1	98.8 ± 1.0	94.1 ± 0.1
PU-2 (PCL1250)	96.9 ± 0.6	90.9 ± 0.5		

1–2 % soluble content in both extractions. The FTIR spectra of the soluble part of PU-0 (PCL2000) in water and in water/TEA shown in Fig. 7 were very similar to those showed in Fig. 6. This result demonstrated that in both cases the short PU chains not taking part in the cross-linked network were extracted. In addition, regardless the formulation, the water solubility was lower than 4 wt% which means that the final film (after curing) presented an appropriate water resistance to be used as coating.

According to the water and water/TEA extraction of Casein-WPU

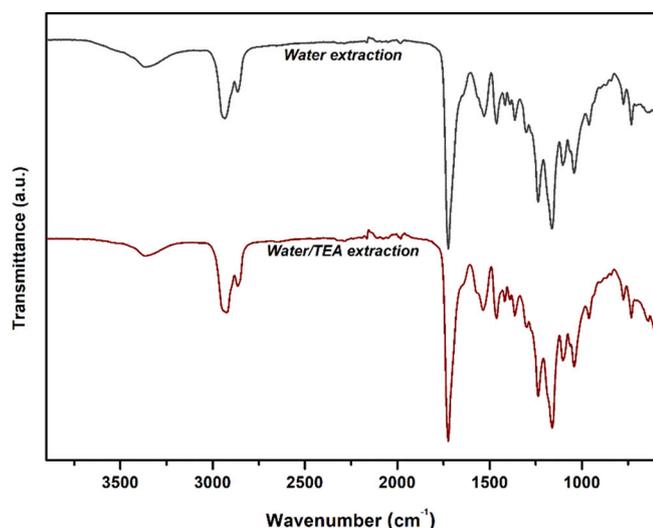


Fig. 7. FTIR spectra of soluble part of the extraction in water and water/TEA of PU-0 (PCL2000) film.

films, the non-soluble fraction was higher when the samples were extracted with water. The reason for this different behavior can be found in the Casein solubility. As Casein is hardly soluble in pure water only short polyurethane chains were observed in this medium, regardless the synthesis method.

The spectra in Fig. 8.A, showed that the water-soluble fractions presented the same signals as the PU-0 (PCL2000), which confirmed the extraction of PU and the lack of Casein. Moreover, and according to the values in Table 4, the water non-soluble fraction of the *ex situ* films remained almost constant with the Casein concentration whereas for the *in situ* films it decreased slightly. This was explained by the formation of a higher number of short PU chains in the presence of Casein during the synthesis.

The FTIR spectra of the water/TEA soluble fraction are shown in Fig. 8.B. As observed, the film containing 7 wt% of Casein obtained through *ex situ* route displayed a spectrum pattern quite similar to that of Casein. Hence, the spectra presented an intense signal at 1642 cm<sup>-1</sup> attributed to the amide group of Casein but no signals from the urethane bond. However, the spectrum of the *in situ* counterpart showed, in addition to the bands attributed to Casein, absorptions related to PU chains. This result confirmed that the *in situ* route provoked the formation of small PU chains. <sup>1</sup>H NMR spectra corroborated that Casein was extracted exclusively in the water/TEA medium (content presented in the supplementary information Fig. S7).

Furthermore, based on the data in Table 4, the non-soluble fraction of the samples extracted with water/TEA decreased with Casein amount, but this trend was more prominent in the samples obtained by *in situ* approach. Thus, for the latter, the soluble fraction increased from 1 % to 12 % for the samples with 0 and 7 wt% of Casein. Therefore, when Casein was part of the reagents used in the WPU synthesis the soluble fraction of the film increased significantly. This behavior was related to the chemical bonding between Casein and PU demonstrated before. Accordingly, the Casein-WPU linkage decreased the –NCO end groups of the prepolymer available to react with APTES and, as a result, the formation of the three-dimensional network was hindered increasing the fraction of short PU chains. In view of these results, it was concluded that Casein was covalently linked to PU. However, not all the Casein was part of the polymer crosslinked structure (as demonstrated in the UV assays) since the water/TEA extraction yielded, in addition to short PU chains, a small amount of the protein.

### 3.3. Mechanical properties

The typical stress-strain curves of *ex situ* and *in situ* Casein-WPU films are showed in Fig. 9. The specimens displayed elastomeric behavior with

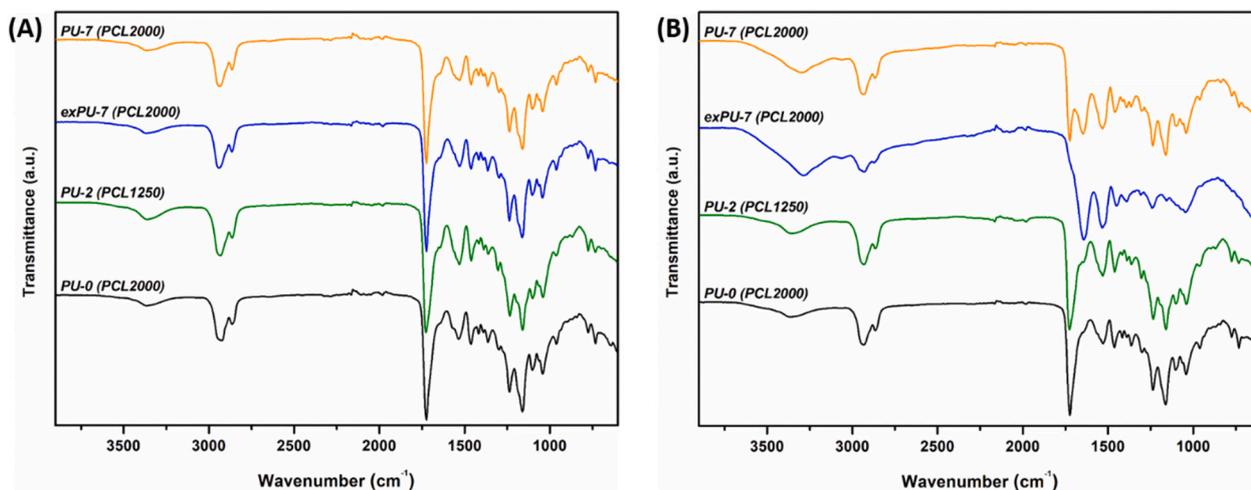


Fig. 8. FTIR spectra of the soluble part of PU-0 (PCL2000), PU-2 (PCL1250), exPU-7 (PCL2000) and PU-7 (PCL2000) in (A) water and (B) water/TEA.

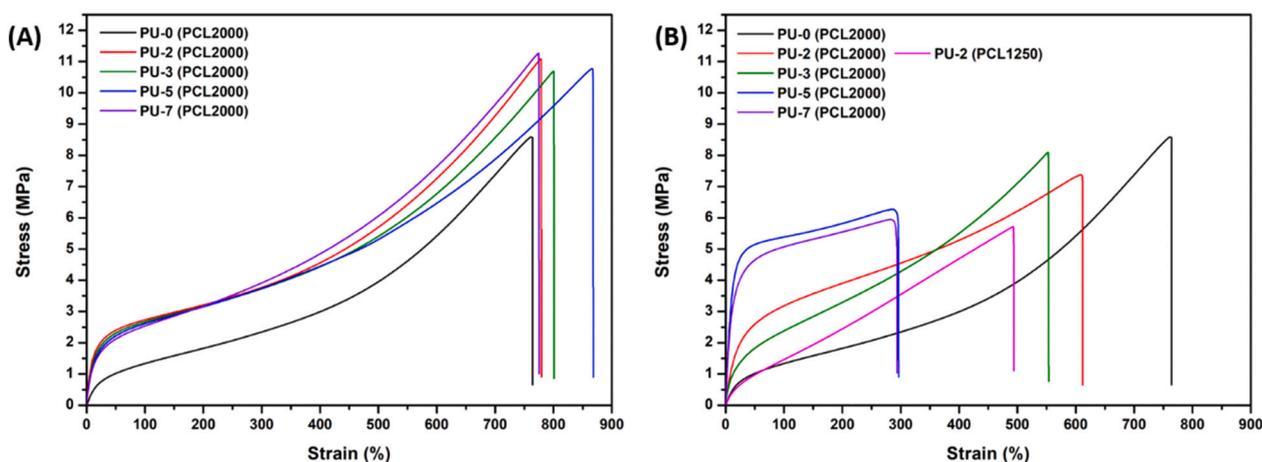


Fig. 9. Stress-strain plots of (A) *ex situ* and (B) *in situ* Casein-WPU films.

high deformation values. The data collected from the test are recorded in Table 5 and Fig. S8. The *ex situ* films exhibited higher deformations (Fig. 9.A) and somewhat higher maximum stresses than the *in situ* counterparts, while the maximum values of elastic modulus were achieved for *in situ* films (Fig. 9.B).

The tensile properties of PU-0 (PCL2000) changed considerably with the addition of Casein for both synthesis methods (Table 5). Thus, the elastic modulus of all Casein-WPU films was higher than that of PU-0 (PCL2000), being this change more remarkable for *in situ* films. Likewise, the Casein content affected the stiffness of the films, increasing its value up to 341 % when *in situ* method was employed and 7 wt% of Casein was added. However, the increase of this magnitude in the *ex situ*

route did not show statistically significant differences ( $p > 0.05$ ) with the Casein percentage. This behavior was also observed in the elongation at break, since the values for this synthesis method did not show statistically significant differences ( $p > 0.05$ ) with the contents. For the *in situ* route, the elongation at break gradually decreased, which was in line with the increase in stiffness. Therefore, a significant increase in the elastic modulus, as occurred in *in situ* films with high Casein content, led to a decrease in the ductility of the material. As for the stress at break, the effect of Casein was different depending on the synthesis method. This property increased slightly with the content for *ex situ* films compared to PU-0 (PCL2000), but it was not until the addition of 5 wt% that the results showed statistically significant differences ( $p < 0.05$ ). In

Table 5  
Mechanical properties of *ex situ* and *in situ* Casein-WPU films.

	Samples	Young's Modulus (MPa)	Elongation at break (%)	Stress at break (MPa)
<i>Ex situ</i>	PU-0 (PCL2000)	8.2 ± 1.3	805.9 ± 59.8	8.1 ± 1.2
	PU-2 (PCL2000)	15.9 ± 2.5	741.3 ± 55.0	9.5 ± 1.9
	PU-3 (PCL2000)	14.9 ± 1.0	845.1 ± 68.0	10.7 ± 1.8
	PU-5 (PCL2000)	14.8 ± 1.7	844.2 ± 56.0	11.5 ± 1.8
	PU-7 (PCL2000)	14.8 ± 1.9	807.2 ± 32.4	11.3 ± 1.3
<i>In situ</i>	PU-2 (PCL2000)	14.4 ± 0.6	534.9 ± 91.2	6.7 ± 1.1
	PU-3 (PCL2000)	12.1 ± 1.5	533.8 ± 84.2	7.8 ± 1.4
	PU-5 (PCL2000)	35.7 ± 7.3	320.6 ± 50.0	6.4 ± 0.9
	PU-7 (PCL2000)	36.2 ± 3.9	355.0 ± 40.3	6.2 ± 0.2
	PU-2 (PCL1250)	4.2 ± 0.1	466.5 ± 33.7	5.5 ± 0.2

contrast, for the *in situ* route, the presence of Casein yielded a small decrease in the maximum stress at break values for all contents. However, it should be noted that this variation was practically within the experimental error, so it could be considered constant for the different amounts with the exception of 7 wt% of Casein which showed statistically significant differences ( $p < 0.05$ ). In this case, the stress at break decreased because of the lower ductility of the material, a behavior that was not observed in the *ex situ* specimens since their deformation did not change. The mechanical outcomes indicated that Casein caused an increase in the stiffness and brittleness of the material when added during WPU synthesis since the modulus increased and the elongation decreased. This phenomenon could be explained by a better dispersion of the Casein as a reinforcement in the system, which resulted in a better interface, and therefore greater interactions between the macromolecules [43–45]. This led to efficient charge transfer between the Casein and the WPU. Additionally, the variation in results between films with the same amount of Casein but prepared by different synthesis method supported the hypothesis of Casein attachment to the WPU structure.

It was worth mentioning that the decrease in molecular weight distribution recorded for *in situ* films with PCL 2000 (Table 3) did not result in a decrease in elastic modulus as might be expected; the reinforcement provided by Casein linked to WPU had a greater effect. In the *ex situ* route, Casein acted as a reinforcement, but without remarkable differences in terms of the added amount, suggesting a poor dispersion. For this series of films, the molecular weight was the same, leading only to the effect that Casein may provide, which in this case was low due to poor dispersion and to the absence of covalent bond to the polyurethane structure.

With respect to PU-2 (PCL1250) the tensile properties recorded were worse in comparison to its PCL 2000 counterpart from both synthesis routes, as well as for the WPU without Casein. Therefore, the molecular weight of PCL had higher effect on mechanical properties than the addition of 2 wt% of Casein. These results showed that Casein-WPU films with different mechanical properties could be obtained by adjusting the Casein content, together with the molecular weight of the PCL and, of course, depending on the synthesis method which turned out to be of great importance.

### 3.4. Thermal properties and flame-retardant behavior

The influence of different Casein contents and synthesis method on the thermal transitions of WPU was evaluated by differential scanning calorimetry (DSC). The DSC runs of the Casein powder and the PCLs are presented in the supplementary information Fig. S9. The PCLs showed two fusion peaks, both in the first and second heating, being more prominent in the PCL of higher molecular weight. Casein is a type of biomacromolecule containing several different proteins. The arrangement of the chains is disordered, which leads to the non-crystallization behavior of Casein itself. Close to the values found in the literature [22], this phosphoprotein presented in the second heating scan a glass transition temperature at 176 °C, whereas in the first one it exhibited broad melting peak, attributed to the release of water [46], since the capsule for this reagent was holed.

For the Casein-functionalized WPU films, the first and second heating scan are shown in Fig. 10, while the cooling scans are exposed in the supplementary information (Fig. S10). The DSC data for all the samples

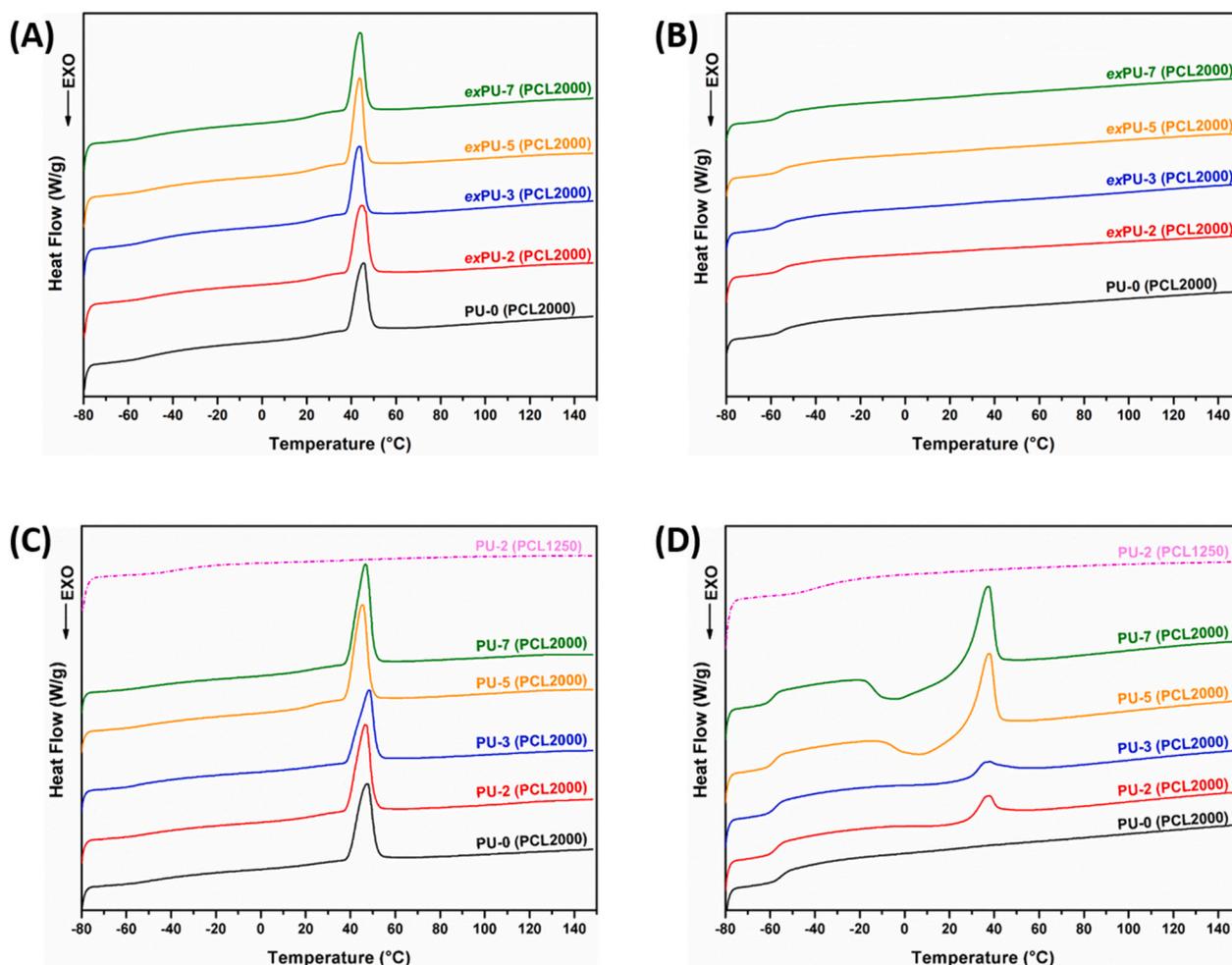


Fig. 10. DSC scans. (A,C) First heating and (B,D) second heating for *ex situ* (A,B) and *in situ* (C,D) Casein- WPU films.

are summarized in Table 6. In the first heating scan, the addition of Casein, slightly reduced the glass transition temperature ( $T_g$ ) of WPU based on PCL 2000 in both synthesis methods. However, in the second heating scan the  $T_g$  was only altered for the *in situ* samples compared to PU-0 (PCL 2000), namely, it decreased by approximately 2–4 °C as the Casein content increased. For the *ex situ* films, the  $T_g$  was practically the same as for the sample free of Casein. According to Fig. 10.A and Fig. 10. C, both *ex situ* and *in situ* samples showed an endothermic melting peak at the first heating run, whose enthalpy did not vary notably with Casein content; but, the global values were higher for the *in situ* samples and  $T_m$  was somewhat lower for *ex situ* samples. Therefore, the incorporation of Casein during WPU synthesis led to materials with higher crystallization capacity.

For the sample with the lowest molecular weight of PCL, no melting peak was observed in any of the heating scans (Fig. 10.C,D). This behavior proved that during Casein-WPU film formation, PCL 2000 was capable of crystallizing, but largely when Casein was added during synthesis, whereas the PCL 1250, which as standard has a much lower degree of crystallinity, was unable to crystallize during film formation. Moreover, in the cooling run for none of the samples exothermic peaks were detected (Fig. S10), which implied that the speed of the cooling test (10 °C·min<sup>-1</sup>) did not allow the organization of the chains.

According to the graphs in Fig. 10, in the second heating noticeable differences were observed in the thermal transitions of the WPU due to the Casein addition method. Analyzing the results of the *ex situ* samples (Fig. 10.B), it could be inferred that the addition of the protein after WPU synthesis did not cause variations in the transitions detected for the sample without Casein. In all cases, no endothermic melting peaks were observed, only the  $T_g$  which did not change with content. This behavior confirmed a low interaction between Casein and WPU, a fact that agreed with the results described for the mechanical properties. In the absence of interaction between the two components, the PU chains behave independently, giving the same DSC results as for the Casein-free sample.

On the contrary, when Casein was added at the beginning of WPU synthesis, the DSC scans were completely different compared to the *ex situ* counterparts (Fig. 10.D). At the second heating, an exothermic peak, known as cold crystallization, followed by an endothermic melting peak could be distinguished for all Casein contents. Moreover, the cold crystallization enthalpy and the melting enthalpy increased with the content. This finding suggested that the Casein-WPU chains were unable to organize at the selected cooling rate, as they vitrified before crystallizing, but during the second heating, they acquired sufficient energy and movement to form crystals, which melted as the temperature increased. This result was the effect of a good dispersion of the Casein

along the WPU, as a consequence of the chemical bonding that occurred between the –NCO prepolymer groups and the –NH<sub>2</sub> present in the Casein when it was added during the reaction. In addition, the well-dispersed protein seemed to have a certain nucleating character, since with increasing content, cold crystallization was promoted,  $\Delta H_{cc}$  increased and  $T_{cc}$  occurred earlier, suggesting easier crystal formation.

The thermal stability of all Casein-functionalized WPU films was evaluated by TGA. Thermogravimetric (TG) and derivative thermogravimetric (DTG) curves of Casein powder, PU-0 (PCL2000), *ex situ* and *in situ* films in inert atmosphere are displayed in Figs. 11,12, and Table 7 summarizes the data collected from these curves.

Casein, from the phosphoproteins group, was degraded under nitrogen atmosphere in a four-step process, as can be distinguished in Fig. 11.A. The first stage, with a peak in the weight loss derivative at about 100 °C and with a weight loss of about 6 % was attributed to evaporation of the physically adsorbed water. The second stage, took place between 183 °C and 248 °C, with a weight loss of 4 % and a temperature of the maximum loss rate ( $T_{max}$ ) at 235 °C. According to the research conducted by Mocanu, A. M et al. on the thermal behavior of Casein by TG-FTIR technique [15], this range of loss could ascribed to the weight loss of ammonia and carbon dioxide due to the breaking of the weaker end bonds of the molecular structure. Then, it was during the third stage, from 248 to 412 °C when the greatest weight loss occurred (>51 %), which corresponded to the cleavage of amino acid chains, followed by the cross-linking and dehydration of the cleaved amino acid chains due to the isocyanic acid (HNCO). At the same time, carbon monoxide was released. Moreover, following the information on flame retardant composites found in the literature [47], the thermal decomposition of these types of phosphorus-containing composites also generated phosphoric acid, which rapidly condensed producing pyrophosphates and released water. This water diluted the gas phase, and phosphoric and pyrophosphoric acids could catalyze the dehydration reaction of terminal alcohols, promoting the formation of carbocations and carbon-carbon double bonds. In the last stage, which could be set from 412 to 590 °C, the carbonaceous residues formed during the previous stages were further decomposed, resulting in the release of methane, water, ammonia and final carbon residues. Finally, a nitrogen carbonaceous residue from the remaining degraded structures of about 24 % was found at 600 °C and was stable at least up to 800 °C. However, keeping this high temperature, the carbonaceous residue was reduced to 4 % when air atmosphere was added because of thermo-oxidative degradation.

For PU-0 (PCL2000) film, the thermal degradation occurred in two main stages according to the thermogram shown in Fig. 11.B. The first stage with a mass loss of 16 % was found between 227 and 305 °C and

**Table 6**  
DSC data for Casein powder, PCLs, *ex situ* and *in situ* Casein-WPU films.

Samples	1st Heating Scan			2nd Heating Scan					$X_c$ (%)
	$T_g$	$\Delta H_m$	$T_m$	$T_g$	$\Delta H_{cc}$	$T_{cc}$	$\Delta H_m$	$T_m$	
	(°C)	(J/g)	(°C)	(°C)	(J/g)	(°C)	(J/g)	(°C)	
PCL 1250	-63.4	4.7	31.7	-70.2			17.7	35.5	13.1
PCL 2000	-59.6	16.1	49.7	-62.7			72.8	42.8	36.9 42.6
		65.1	49.4						
Casein		263.7	105.8	175.8					
PU-0 (PCL2000)	-50.8	23.6	47.3	-55.0					
<i>ex</i> PU-2 (PCL2000)	-53.0	17.1	44.7	-55.0					
<i>ex</i> PU-3 (PCL2000)	-53.1	14.9	43.6	-55.4					
<i>ex</i> PU-5 (PCL2000)	-53.3	16.4	43.7	-55.7					
<i>ex</i> PU-7 (PCL2000)	-52.6	16.7	43.8	-55.6					
PU-2 (PCL2000)	-51.0	25.1	46.7	-57.1	1.4	28.1	1.5	37.9	
PU-3 (PCL2000)	-55.2	22.3	48.2	-58.1	0.7	28.6	0.7	36.0	
PU-5 (PCL2000)	-52.4	26.0	45.3	-58.0	12.3	8.9	12.8	37.6	
PU-7 (PCL2000)	-51.5	28.3	46.5	-58.8	15.1	-2.5	15.3	37.1	
PU-2 (PCL1250)	-41.6			-42.0					

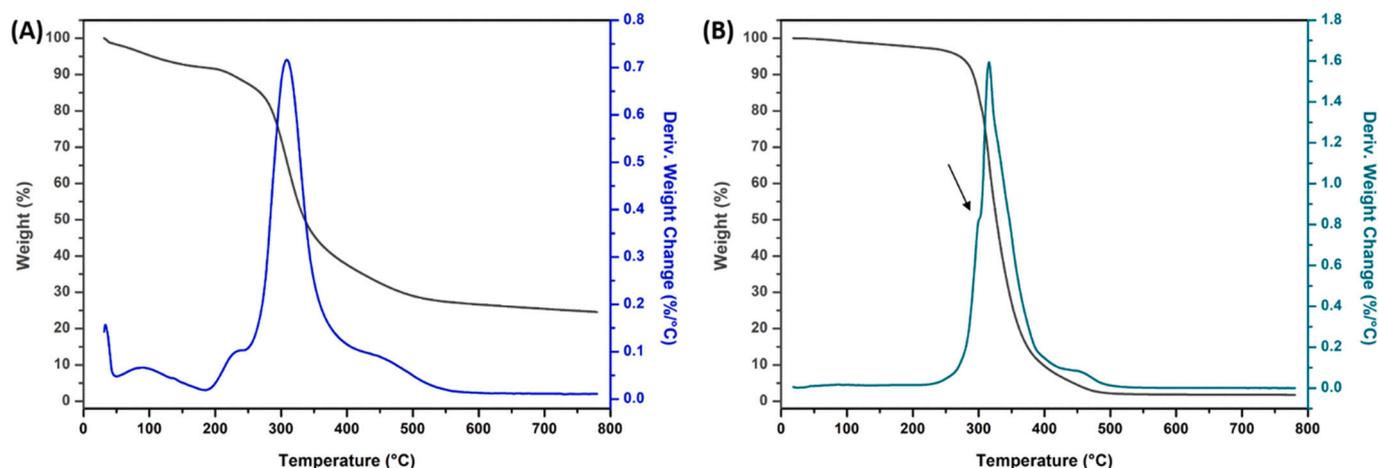


Fig. 11. TG and DTG curves of: (A) Casein powder and (B) PU-0 (PCL2000) in nitrogen.

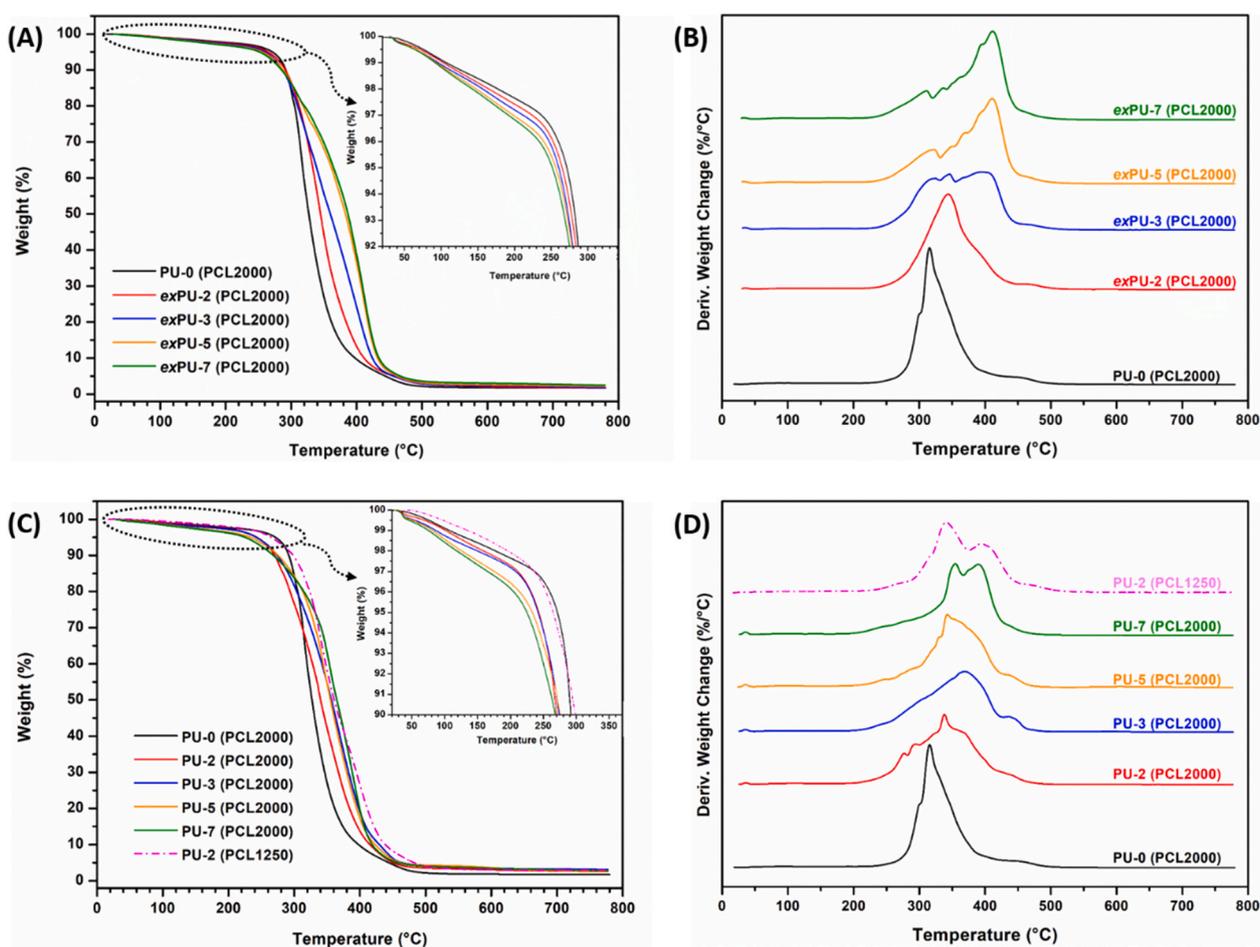


Fig. 12. TG (A,C) and DTG (B,D) curves of *ex situ* (A,B) and *in situ* (C,D) Casein-WPU films in nitrogen.

was attributed to the decomposition of urethane and urea bonds forming products such as alcohols and isocyanates, amines, primary and secondary olefins and carbon dioxide among others [48]. The second stage of degradation was at 305–500 °C with a weight loss of 79 % due to the decomposition of soft segments such as castor oil [49,50]. The amount of carbon residue for Casein-free WPU was 1.8 % with nitrogen, while with air atmosphere it was slightly reduced to 1.1 %. This residue was due to the silicon atoms of the APTES [51].

The presence of Casein significantly altered the degradation of PU-

0 (PCL2000), as the degradation profile of Casein-WPU films in the DTG curves changed in shape compared to pure WPU (Fig. 12.B,D). The first stage of polyurethane decomposition presented a single peak with a shoulder in the DTG curve, whereas after the addition of Casein the complete degradation curve became broad and different stages of degradation could be distinguished with peaks at higher temperature, especially in the *ex situ* samples with Casein contents higher than 3 wt%. According to Table 7,  $T_{5\%}$  (temperatures corresponding to 5 % weight loss) decreased in both synthesis methods with the Casein addition and

**Table 7**TGA data for Casein powder, *ex situ* and *in situ* Casein-WPU films in nitrogen.

Samples	T <sub>5%</sub>	T <sub>50%</sub>	1st stage		2nd stage		Residue	Residue
	(°C)	(°C)	Loss %	T <sub>max</sub> (°C)	Loss %	T <sub>max</sub> (°C)	(%) in N <sub>2</sub>	(%) in O <sub>2</sub>
Casein	104.6	336.0	53.1	309.0			24.1	4.0
<i>Ex situ</i>								
PU-0 (PCL2000)	271.4	327.0	95.1	315.8			1.8	1.1
PU-2 (PCL2000)	264.9	344.9	94.0	344.0			1.7	1.3
PU-3 (PCL2000)	260.3	360.0	43.9	262.3	50.2	401.6	1.8	1.2
PU-5 (PCL2000)	256.3	383.1	21.7	316.2	72.0	411.5	2.1	1.3
PU-7 (PCL2000)	251.6	386.8	16.3	309.9	76.4	411.8	2.4	1.3
<i>In situ</i>								
PU-2 (PCL2000)	243.0	340.0	93.9	338.2			2.5	2.0
PU-3 (PCL2000)	242.0	356.7	92.9	370.4			2.5	2.1
PU-5 (PCL2000)	233.2	355.2	92.4	342.1			2.5	2.3
PU-7 (PCL2000)	224.9	364.8	48.3	353.2	44.0	392.5	2.8	1.5
PU-2 (PCL1250)	264.8	360.8	56.9	339.8	37.5	392.6	2.9	2.8

content increase, reaching a reduction of 20 °C and 46.5 °C, respectively for PU-7 (PCL2000) film compared to Casein-free WPU. This onset of weight loss was driven by the decomposition of Casein, which started at much lower temperatures. In terms of the methods used to obtain the films, *in situ* samples showed a T<sub>5%</sub> lower than their *ex situ* counterparts (Fig. 12.A,C). This fact was related to a better dispersion of Casein (the component that first begins its degradation) throughout the film, due to the chemical bonding between Casein and polyurethane that occurred during *in situ* synthesis and prevented Casein agglomeration. With this result, it should be noted that well dispersed Casein promoted the sample decomposition.

Nevertheless, the T<sub>50%</sub> (temperatures corresponding to 50 % weight loss) increased for all cases, suggesting a delay in WPU complete degradation. Moreover, T<sub>max</sub> of the last degradation stage increased with increasing Casein content. The chemical components that make up the Casein structure, such as isocyanic acid, inorganic phosphate, and carboxyl/sialic acid groups reacted during degradation, causing the formation of non-combustible volatile gases, such as ammonia and carbon dioxide, but also a condensed phase [15]. This phase promoted the processes of dehydration and cross-linking of amino acids, which resulted in the formation of a protective layer of carbonaceous residues that hindered heat transmission and the emission of volatile gases. Hence, Casein provided thermal stability to the samples, which was evidenced by the shift to higher values of T<sub>50%</sub> and T<sub>max</sub>. However, this improvement was more prominent for films obtained by the *ex situ* approach (Fig. S11).

As concluded in previous sections the Casein dispersion in the matrix was enhanced in the *in situ* route because of the chemical linkage between the Casein and the Polyurethane. However, in the *in situ* route, Casein was introduced in the reactor from the beginning of the reaction that proceeded at 56 °C for 5 h while in the *ex situ* route Casein (dissolved for two hours at 60 °C) was added after the reaction, at room temperature. Accordingly, it could be argued that the Casein was more prone to thermal degradation processes in the *in situ* method and the change in the Casein structure could be in the origin of the poorer thermal behavior of the samples obtained by this methodology.

The higher thermal stability of sample PU-2 (PCL1250) could be explained upon the basis of a higher interaction between soft and hard segment that conform the polyurethane. As the molecular weight of PCL decreases, it becomes more difficult for the segments of different nature to behave as block copolymers, *i.e.* each segment interacts only with its part. In this way, being the PCL smaller, the soft and hard segment interact easily with each other, which seems to provide certain thermal stability, since key links were less exposed.

As for the final residue, the *in situ* Casein-WPU samples presented a higher amount of nitrogen residue at 800 °C than that of PU-0 (PCL2000), and in the case of the *ex situ* ones this result was observed from Casein contents higher than 5 wt%. When the oxidative atmosphere was applied, this residue decreased slightly, giving values between 2.3 and 1.3 % due to the thermo-oxidative degradation that

took place in the carbonaceous part, leaving only silicon oxide. As expected from the Casein-powder degradation results, the presence of this protein led to an increase in film residue, which seemed to increase with content. However, this trend was more noticeable for *ex situ* films.

Thermal stability of the samples was also analyzed in air atmosphere by TGA. The thermogravimetric (TG) and derived thermogravimetric (DTG) curves of the samples under air are displayed in Figs. 13,14, and Table 8 summarizes the data reported from these curves.

In oxidative atmosphere, thermal degradation of Casein took place in five-stages, one more than in inert atmosphere. However, the first and second stages occurred at similar temperature ranges in air and nitrogen. In both cases, the respective weight loss was almost identical and the first stage was assigned to the evaporation of physically adsorbed water. Therefore, the chemical degradation of Casein began with the second stage of degradation. At this stage the weight loss of ammonia and carbon dioxide was designated due to the breaking of the weaker end bonds of the molecular structure. This result suggested the same degradation mechanism in both atmospheres up to temperature of 230 °C. It was during the third stage where more noticeable differences were observed between both degradation atmospheres. Under air, this stage extended up to 425 °C and was ascribed to the elimination of carbon monoxide and the isocyanic acid. Next, increasing the temperature, a well-defined stage between 450 and 650 °C was revealed in oxidative atmosphere. In line with the previously mentioned research [15], at this fourth stage carbon dioxide, water, carbon monoxide, isocyanic acid and ammonia were released. Finally, in the last stage between 642 and 778 °C, only detected under air atmosphere, the carbonaceous residues formed during the previous stages decomposed further, causing the release of more carbon dioxide and water.

As shown in Fig. 13.B, oxidative thermal degradation for PU-0 (PCL2000) film occurred in three phases. Similar to nitrogen, the first one occurred between 207 and 355 °C and was attributed to the decomposition of urethane and urea bonds. The second stage arose between 355 and 400 °C due to the decomposition of soft segments such as castor oil. In addition, under air atmosphere a very intense narrow peak was observed in the derivative weight change that did not occur under nitrogen, also related to the decomposition of soft segments.

Regarding the Casein-functionalized WPU films, according to the results presented in Fig. 14, the addition of Casein *via in situ* and *ex situ* method significantly altered the degradation of PU-0 (PCL2000) in air. The degradation profile of the Casein-WPU films changed shape compared to pure WPU and the T<sub>50%</sub> increased prominently for all cases, suggesting a delay in the complete thermo-oxidative degradation of WPU. However, this improvement was not significantly altered by the synthesis method employed. Moreover, in contrast to what occurs under inert atmosphere, in the weight loss derivative two peaks could be distinguished in the degradation stage relative to the soft segments. The T<sub>max</sub> of the narrow and intense peak changed slightly with the Casein content, but not proportionally.

The microscale combustion calorimeter (MCC) test made possible to

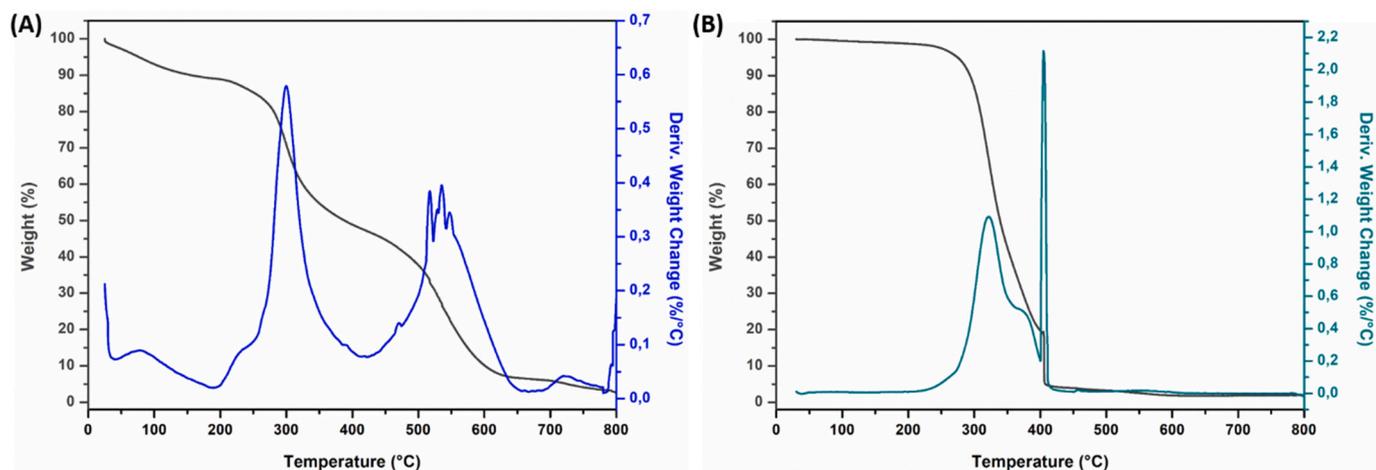


Fig. 13. TG and DTG curves of: (A) Casein powder and (B) PU-0 (PCL2000) in air.

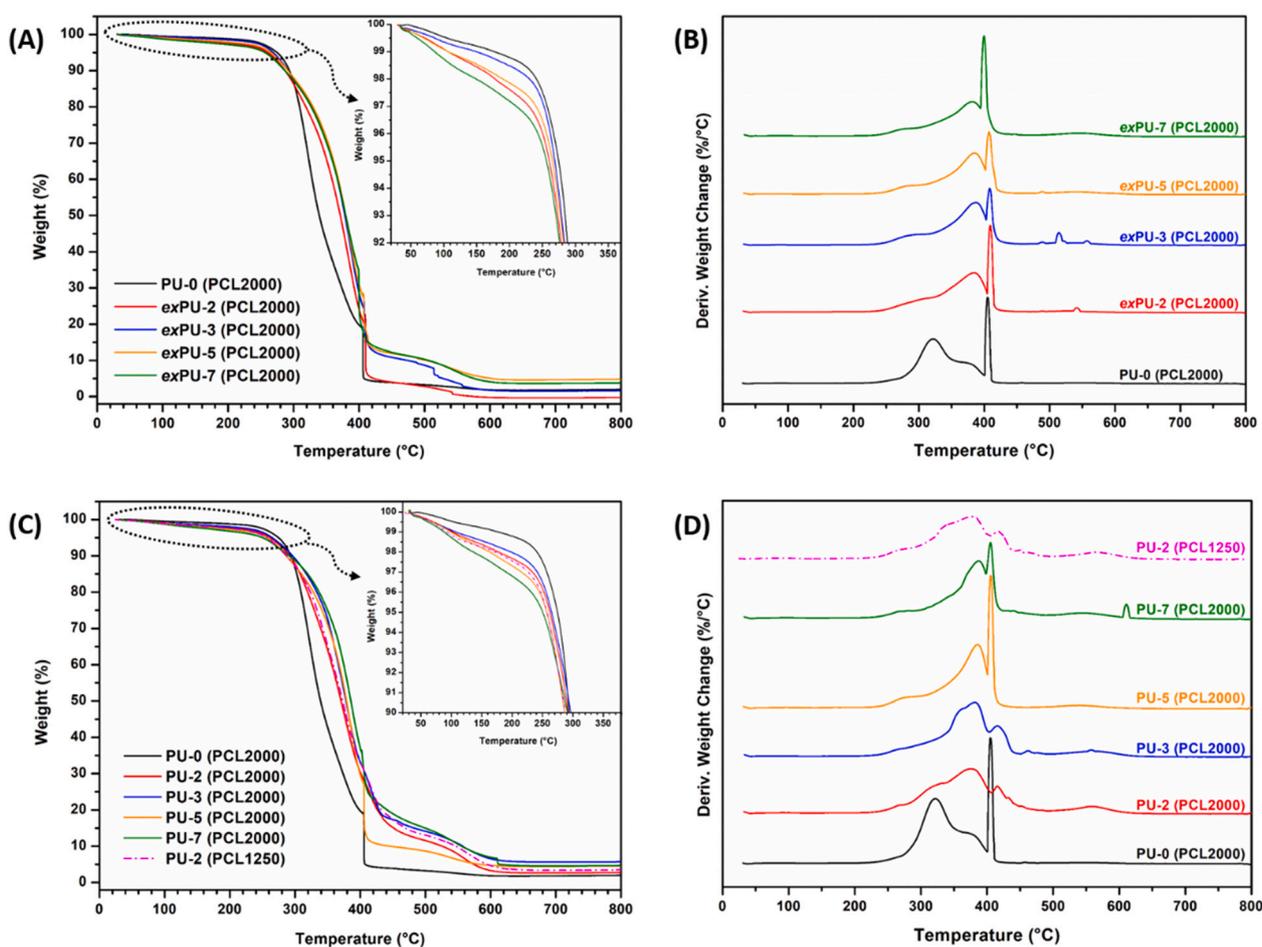


Fig. 14. TG (A,C) and DTG (B,D) curves of *ex situ* (A,B) and *in situ* (C,D) Casein-WPU films in air.

analyze at small scale the behavior of samples under thermal radiation. It is important to know the flame characteristics of the films, such as the heat release rate (HRR) as it contributes to the fire growth rate, fire size and heat of combustion. Table 9 summarizes this parameter and other calorimetric data, while Fig. 15 shows the heat release rate (HRR) curves.

Similar to that described for the DTG curves, the combustion of polyurethane without Casein presented a single peak with a shoulder in the HRR curve, whereas after the addition of Casein different shoulders

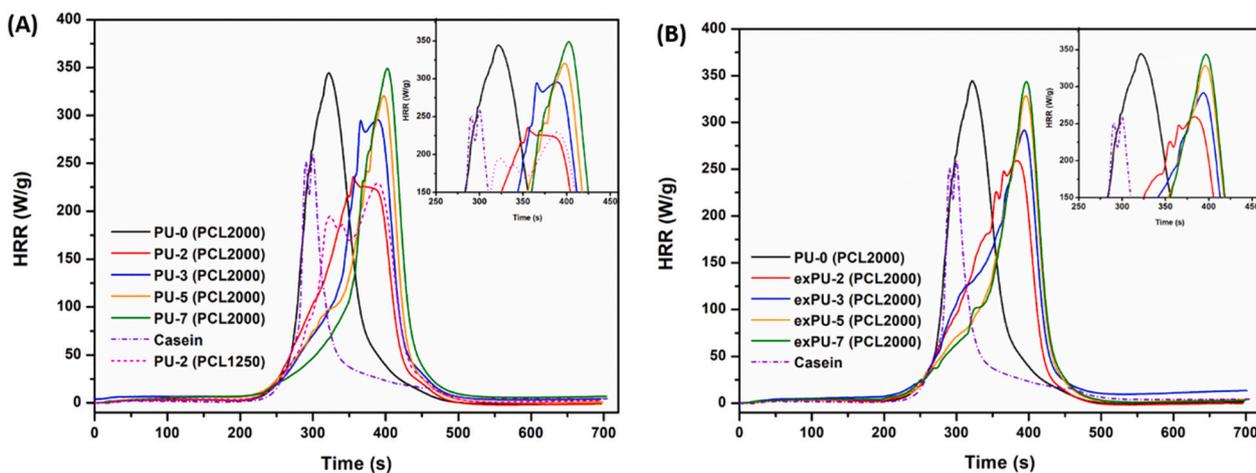
could be distinguished at the beginning, mainly in the *ex situ* samples. Concretely, for the PU-0 (PCL2000) film, the HRR curve was narrow, with a high maximum value (pHRR) compared to the rest of the samples, indicating that heat release occurred fast, which was a sign of a very high burning rate. In contrast, as seen in Fig. 15.A, for PU-2 (PCL1250) film two stages of degradation were clearly distinguished. The first stage (243–282 °C) with the lowest HRR region could be related to the degradation of Casein and the hard segments. Meanwhile, the second stage (383–570 °C) with the higher HRR region was ascribed to the

**Table 8**TGA data for Casein powder, *ex situ* and *in situ* Casein-WPU films in air.

Samples	$T_{5\%}$	$T_{50\%}$	1st stage		2nd stage		3th stage		Residue (%) in O <sub>2</sub>	
	(°C)	(°C)	Loss %	$T_{max}$ (°C)	Loss %	$T_{max}$ (°C)	Loss %	$T_{max}$ (°C)		
Casein	76.7	388.4	8.6	82.4	41.3	299.8	40.4	535.6	2.9	
<i>Ex situ</i>	PU-0 (PCL2000)	274.7	339.2	78.9	321.1	15.7	405.7		2.1	
	PU-2 (PCL2000)	260.6	371.3	75.4	384.5	17.8	409.7	4.2	541.9	0.0
	PU-3 (PCL2000)	268.8	378.3	71.7	386.5	16.1	408.7	9.0	514.6	1.6
	PU-5 (PCL2000)	265.3	379.5	67.8	385.5	18.0	407.7	6.6	541.9	4.7
	PU-7 (PCL2000)	256.2	379.6	59.0	381.5	24.9	399.0	7.5	543.9	3.8
<i>In situ</i>	PU-2 (PCL2000)	262.1	370.6	70.0	375.9	15.7	415.7	9.3	555.7	2.8
	PU-3 (PCL2000)	265.0	378.1	66.2	382.5	14.5	416.8	11.6	557.0	5.7
	PU-5 (PCL2000)	257.0	379.0	67.6	386.5	19.4	405.7	4.9	537.8	4.6
	PU-7 (PCL2000)	250.3	385.6	57.0	387.5	17.5	405.7	10.2	544.9	4.7
	PU-2 (PCL1250)	257.3	373.1	65.2	378.4	18.7	417.8	9.9	566.1	3.5

**Table 9**Cone calorimeter test parameters for *in situ* and *ex situ* Casein-WPU films.

Samples	HRC	pHRR	THR	$T_{HRR}$	tPHRR	Residue (wt%)	
	(J/g-K)	(W/g)	(kJ/g)	(°C)	(s)		
Casein	237	238	12.3	315/324	289/300	24.26	
<i>Ex situ</i>	PU-0 (PCL2000)	354	344	26.1	354	321	2.32
	PU-2 (PCL2000)	248	257	25.2	417	382	2.46
	PU-3 (PCL2000)	268	290	25.4	425	394	3.15
	PU-5 (PCL2000)	324	329	25.4	425	395	2.82
	PU-7 (PCL2000)	348	342	25.3	426	397	3.45
<i>In situ</i>	PU-2 (PCL2000)	241	224	25.1	413	378	3.82
	PU-3 (PCL2000)	301	295	26.0	423	389	4.47
	PU-5 (PCL2000)	312	320	25.4	429	398	2.87
	PU-7 (PCL2000)	345	349	25.3	431	400	3.85
	PU-2 (PCL1250)	227	226	25.6	353/418	323/390	2.07

**Fig. 15.** Heat release rate by MCC for (A) *in situ* and (B) *ex situ* Casein-WPU films.

decomposition of the soft segments. In the rest of the samples with PCL 2000, these stages were not clearly distinguishable.

As indicated in Table 9, the addition of 2 wt% Casein to the WPU using the *ex situ* and/or *in situ* approach reduced the pHRR value by 25 % and 35 %, respectively, and the time took for the material to reach that peak increased significantly, suggesting slower heat release and flame propagation. It could also be observed that the total heat released (THR) decreased slightly with the presence of Casein. Hence, these results evidenced a significant decrease in the flammability of PU due to the incorporation of Casein. The high phosphorus and nitrogen content present in this phosphoprotein eases its action in condensed and gas phases of the combustion processes, producing a protective layer, which hinders the release of heat. Therefore, this Casein content could hamper WPU combustion, evidencing its action as a flame retardant (FR).

Nevertheless, increasing Casein content the pHRR values did not decrease, the heat release rate tended to increase and the HRR curve (Fig. 15) narrowed gradually, but without exceeding the value of PU-0 (PCL2000) film, with the exception of the sample obtained by the *in situ* method with a content of 7 wt%. This behavior could be explained by a fracture in the carbonaceous layer. This fact allowed the release of part of the heat generated during combustion, producing the above-mentioned peaks at the beginning of the HRR curve (Fig. 15). Simultaneously, the times for the pHRR values shifted towards longer times as the content increases, and the onset of combustion occurred later, indicating a delay in burning. Consequently, these findings advocated that the combustion of the material was higher with Casein contents above 2 wt%, however, it could be pointed out that the combustion was delayed.

Comparing the synthesis methods employed, the calorimetric results showed no differences to be highlighted. The observed behavior between *in situ* and *ex situ* counterparts was practically the same, so the Casein addition route in WPU did not seem to have any effect on the film combustion. The residue found at the end of the flammability tests was higher in the presence of Casein, which agreed with the TGA results. However, the values obtained in the two techniques differ slightly, since the method of analysis was not the same. TGA uses a heating rate of  $10\text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$  under nitrogen atmosphere at  $90\text{ mL}\cdot\text{min}^{-1}$ , whereas the calorimeter uses a heating rate of  $1\text{ K}\cdot\text{s}^{-1}$  and a nitrogen stream flowing at  $80\text{ mL}\cdot\text{min}^{-1}$  and then mixed with oxygen at  $20\text{ mL}\cdot\text{min}^{-1}$ .

Several studies reported in the literature describe the efficacy of Casein as a flame retardant in cotton, polyester fabrics, poly(lactic acid) and polyethylene [19,20,23,24]. While these researches have shown that the presence of higher content of modified-Casein in the polymer matrix leads to a considerable reduction of pHRR, in this work, the results suggest that the best FR effect occurs at low Casein contents. Similarly, Czlonka, S. et al. [26] studied polyurethane composites reinforced with different contents of apricot filler modified with Casein and the best results were obtained with a content of 2 wt%.

The burning characteristics of the dried films were analyzed by UL-94 test and the results are summarized in Table 10. The outcomes showed that most of the samples presented poor fire response and accordingly were classified as V-2. However, regardless the synthetic route, the samples containing Casein presented lower persistence of the flaming than the pure polyurethane film. Specifically, in the majority of the samples, as Casein amount increased the after-flame time was reduced. These results showed that the samples containing Casein were able to produce a protective layer of carbonaceous residues that hindered heat transmission and the emission of volatile inflammable gases reducing the length of time which the sample continued to flame.

It is also noticeable that for the same Casein percent, the samples obtained by the *ex situ* procedure presented lower after flame time than their *in situ* counterpart. The same behavior was observed in the TGA experiments where a lower thermal stability of the *in situ* samples was registered. This behavior was explained considering the possible Casein structure change in the *in situ* route due to a prolonged exposure at high temperature.

The melting characteristics of the films were also registered during the burning test. The results showed clear differences between samples obtained by the different strategies since in the *ex situ* samples flaming material dropped from the specimen while *in situ* samples (excluding sample with the lowest Casein content) did not show this undesired

**Table 10**  
UL-94 test results.

	Sample	After-flame time (s)	Flaming particles	Classification
<i>Ex situ</i>	PU-0 (PCL2000)	$41.3 \pm 13.5$	Yes	V-2
	PU-2 (PCL2000)	$19.7 \pm 1.9$	Yes	V-2
	PU-3 (PCL2000)	$22.4 \pm 6.6$	Yes	V-2
	PU-4 (PCL2000)	$20.2 \pm 4.7$	Yes	V-2
	PU-7 (PCL2000)	$16.5 \pm 3.6$	Yes	V-2
<i>In situ</i>	PU-2 (PCL2000)	$39.6 \pm 14.4$	Yes	V-2
	PU-3 (PCL2000)	$38.3 \pm 9.6$	No	V-2
	PU-5 (PCL2000)	$28.7 \pm 2.7$	No	V-1
	PU-7 (PCL2000)	$22.1 \pm 2.8$	No	V-1
	PU-2 (PCL1250)	$32.4 \pm 11.8$	No	V-2

behavior. Moreover, the *ex situ* samples experienced a volume contraction process when heating before burning, that did not happen in the specimens obtained from the *in situ* route. This way of acting was related to the Casein/Polyurethane covalent linkage that reduced the ductility of the material as demonstrated in previous sections. Consequently, in the *in situ* route, the Casein acted on the one hand as a modifier of the mechanical properties and on the other hand promoting the formation of a carbonaceous layer that reduced the flame persistence. Both effects improved the global fire response of the material and thus, the samples obtained *in situ* with 5 and 7 wt% of Casein were classified as V-1.

Finally, the sample obtained by the *in situ* route but using lower molecular weight PCL (PU-2 (PCL1250)) presented, as expected, after-flame time values similar to those of the sample with the same Casein content but obtained from higher molecular weight PCL. However, this sample did not drop any flaming material when burning. This effect was related to the higher crosslinking density of the sample originated by the lower molecular weight of the PCL.

### 3.5. Morphology and adhesion properties

Fig. 16 displays SEM images of cryofractured cross-section of PU-0 PCL(2000), *ex*PU-7 (PCL2000) and PU-7 PCL(2000) films at different magnifications. SEM micrographs of the Casein-free WPU exhibited two distinct areas (Fig. 16.A). On the one hand, a clean and smooth surface and, on the other hand, an area with a wave-like morphology could be distinguished. The roughness shown in the images was attributed to interfacial adhesion between the polymer chains. In presence of Casein, the surface roughness increased. However, in the *ex situ* sample smooth areas could still be detected (Fig. 16.C) at low magnifications, suggesting a lack of good interfacial interaction and adhesion between Casein and WPU because of poor dispersion [44]. At the same content, a higher roughness was observed in the *in situ* sample (Fig. 16.E,F), which means a higher interaction and a better dispersion. Therefore, an improvement in the compatibility between WPU with the Casein was obtained when the Casein was added during the synthesis.

Regarding the surface of the films, a pull-off adhesion test was performed to evaluate the adhesion of the film to a metal substrate. This was considered a preliminary test for the films with the highest Casein content. The values given in Table 11 show that the highest tensile strength was achieved for the Casein-free sample. Moreover, comparing the addition methods, the lowest values were obtained in the *in situ* route. This pattern of results could be explained by the chemical bonding between Casein and WPU, as well as good dispersion. According to the results discussed in the mechanical properties, Casein provided a higher stiffness to the material and a decrease in plastic deformation when was bonded. This decrease led to a lower adhesion capacity with the substrate. Therefore, the higher the stiffness, the lower the adhesion.

## 4. Conclusions

In the present work, by means of two different synthesis strategies, a series of self-curable Casein-WPU waterborne dispersions based on PCL and containing APTES as crosslinking agent were prepared. The interaction between the protein and WPU was investigated. FTIR spectra registered at different reaction times revealed that the isocyanate/Alcohol reaction followed an order one kinetics. In addition, the presence of Casein as well as the increase of the content decreased the values of the apparent constant. Solid-state  $^{29}\text{Si}$  NMR results evidenced the formation of a siloxane network, while solid-state  $^{13}\text{C}$  NMR and FTIR confirmed that the synthesis of WPU was successful. The particle size was not altered when Casein was added after WPU synthesis, but with the *in situ* method, an increase was detected suggesting the attachment of the Casein to the prepolymer during the synthesis. This bonding was supported by FTIR and UV results. Mechanical properties data revealed an increase in Young's modulus as well as in the brittleness of the material when the Casein was part of the WPU. The behavior of the *in situ*

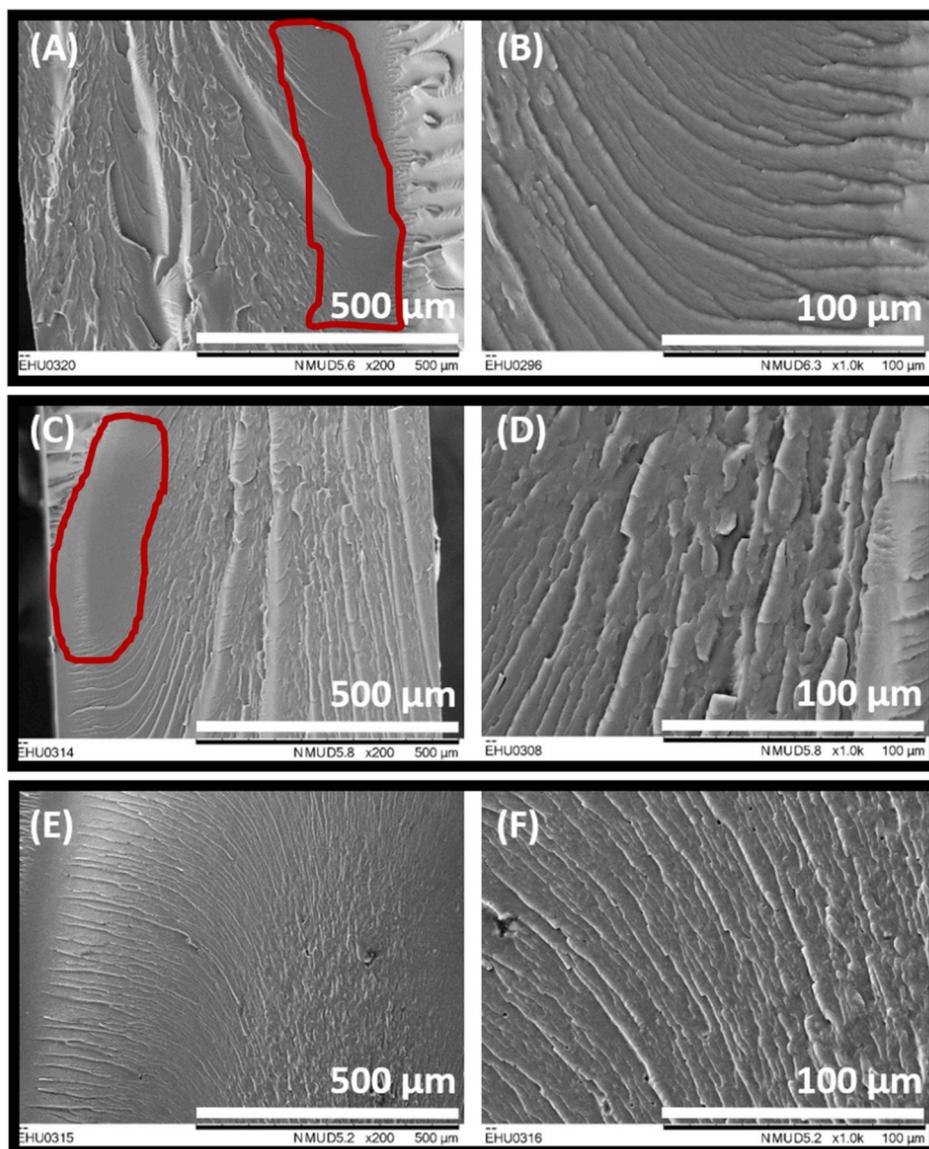


Fig. 16. SEM images of cryofracture surfaces of PU-0 (PCL2000) (A,B), exPU-7 (PCL2000) (C,D) and *in situ* PU-7 PCL(2000) (E,F) films at different magnifications.

**Table 11**

Adhesion results for Casein-free WPU, *in situ* and *ex situ* PU-7 (PCL2000) films.

Samples	Pressure (mPa)
PU-0 (PCL2000)	11.00 ± 2.15
exPU-7 (PCL2000)	7.39 ± 0.88
PU-7 (PCL2000)	4.02 ± 1.80

samples in the tensile test pointed out a better dispersion of the Casein in the system. Furthermore, the synthesis method provoked a strong effect on the thermal transitions of WPU, since only for the *in situ* samples cold crystallization was observed. Neither the *ex situ* samples nor the Casein-free film showed exothermic peaks at the second heating. According to TGA studies, all samples showed higher thermal stability compared to PU-0 (PCL 2000) film. The MCC results exposed that an increase in Casein content delayed the combustion of the material. UL-94 results showed the effectivity of Casein as flame retardant since it reduced the after-flame time and it improved the thermal properties of the sample when it was covalently linked to the Polyurethane. *Ex situ* samples

showed reduced fire persistence but they were classified as V-2 because of the drop of flammable material when burning. Thus, in order the global fire resistance of the *ex situ* samples to be improved the cross-linking points of the polyurethane should be increased.

Morphological characterization confirmed the good dispersion of Casein in the WPU when added by the *in situ* route. Considering all the results presented, it could be concluded that the incorporation of Casein at the beginning of the WPU synthesis led to the -NHCONH- bond formation, which resulted in a good dispersion that improved the properties of the material. Consequently, due to their characteristics, these Casein-WPU dispersions could potentially be used as combustion retardant coatings, where good physicochemical properties are essential for effective performance.

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.porgcoat.2022.107278>.

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