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In situ investigation of supercritical CO_2 assisted impregnation of drug into polymer by high pressure FTIR micro-spectroscopy

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Abstract

An original experimental set-up combining a FTIR micro-spectrometer with a high pressure cell has been built in order to analyze *in-situ* the impregnation of solute into microscopic polymer samples, such as fibers or films, subjected to supercritical CO_2. Thanks to this experimental set-up, key factors governing the impregnation process can be simultaneously followed such as the swelling of the polymeric matrix, the CO_2 sorption, the kinetic of impregnation and the drug loading into the matrix. Moreover, the solute/polymer interactions and the speciation of the solute can be analyzed.

We have monitored *in situ* the impregnation of aspirin and ketoprofen into PEO (Polyethylene Oxide) platelets at T=40°C and P=5; 10 and 15 MPa. The kinetic of impregnation of aspirin was quicker than the one of ketoprofen and the final drug loading was also higher in case of aspirin. Whereas the CO_2 sorption and the PEO swelling remain constant when PEO is just subjected to CO_2 under isobaric conditions, we noticed that both parameters can increase while the drug impregnates PEO. Coupling these results with DSC measurements, we underlined the plasticizing effect of the drug that also leads to decrease the crystallinity of PEO *in situ* thus favoring the sorption of CO_2 molecules into the matrix and the swelling of the matrix. The plasticizing effect increases with the drug loading.

Finally, the speciation of drug was investigated considering the shift of the carboxyl bands of the drugs. Both drugs were found to be mainly homogeneously dispersed into PEO.

1 – INTRODUCTION

To add a desired property to a previously prepared polymer, one can impregnate the matrix with a molecule which possesses this property. This impregnation process requires the use of a solvent to carry the solute into the polymer. Replacing the traditional organic solvents with supercritical carbon dioxide ($scCO_2$) is currently an attractive field of research. Actually, $scCO_2$ possesses numerous advantages among these a low environmental impact, and both a high diffusivity and a high density. $ScCO_2$ is soluble in many amorphous and semi-crystalline polymers and can act as a plasticizer. Consequently, $scCO_2$ can increase the free volume of these polymers.[1-3] Moreover, it is a good solvent for nonpolar solutes with low molecular weight because of its high density. Therefore, it can solubilize such a solute and carry it into the matrix, thus resulting into the impregnation of the polymer.[4, 5] The high diffusivity of $scCO_2$ makes the process easy and fast. Finally, using $scCO_2$ enables to recover an impregnated material free of any solvent residues as $scCO_2$ is removed by depressurization

Some key parameters influence the impregnation process. First, the quantity of CO_2 sorbed into the polymer and the swelling of the matrix will facilitate and accelerate the transport of solute.[6] Then, the relative affinity of the solute between $scCO_2$ and the polymer will improve the efficiency of impregnation.[7, 8] Practically, the amount of solute loaded into the polymer can be tuned by adjusting the operational conditions such as the pressure, the temperature and the time of impregnation. Therefore, in order to optimize the process and to control the impregnation, it is necessary to understand the thermodynamic behavior of each system (polymer+$scCO_2$+solute).

Such $scCO_2$ assisted impregnation has already been applied in different fields, the kind of solute depending on the targeted application. Fluorescent dyes have been loaded into polymer optical fibers [9], dyes into textiles [10], monomers into polymeric matrix for polymer blending [11,

[12], and bioactive molecules into biocompatible polymeric device for the biomedical field [13, 14]. In the literature, a large part of the studied systems has implied thin polymeric films or fibers (50 - 1000 µm) [15-21]

Various techniques are implemented in the literature to study the thermodynamic behavior of such impregnation systems. Gravimetric technics [16, 17] are the simplest and the main technics used to determine the quantity of CO_2 sorbed and the amount of solute loaded into the polymer. However, these methods need to be coupled with an optical measurement or an equation of state (Sanchez-Lacombe) to estimate polymer swelling. Moreover, two separated experiments must be carried out: the first one without solute to determine the weight uptake due to the sorbed CO_2 and the second one with solute to measure the total weight uptake due to both the CO_2 and the solute. The amount of solute impregnated into the matrix is then estimated as the weight difference between the two experiments, making the approximation that the CO_2 sorption is not impacted by the solute. Otherwise, spectroscopic technics enable to determine the key parameters simultaneously: the quantity of CO_2 sorbed into the polymer, the swelling, the amount of impregnated solute and the kinetic of impregnation as well as the molecular interactions between the different components.

The two spectroscopic technics that are mostly implemented are the Near and mid-FTIR (Fourier Transformed InfraRed) and ATR-IR (Attenuated Total Reflectance InfraRed) spectroscopies. Nonetheless, both of them are not well appropriated to investigate thin film or fibers. Indeed, FTIR spectroscopy is more suited to analyze liquid and/or large samples (cm^3). [22, 23] Besides, ATR-IR spectroscopy requires a good contact between the polymer and the crystal that is why the polymeric samples are generally melted to create a film on the crystal.[19] Consequently, the melting step impacts the polymer structure and so its behavior under $scCO_2$.

In order to investigate *in situ* the thermodynamic behavior of microscopic polymer samples (~ 5-500µm) subjected to $scCO_2$ without melting them, we have developed a new and original method. The experimental setup consists in a FTIR microscope coupled with a high pressure cell that enables to operate up to 15MPa and under a controlled temperature. As the FTIR microscope technic leads to adjustable size and focus of the IR beam, this technic is adapted to analyze microscopic scale samples. Thanks to the newly developed experimental set-up, we have recently reported as a function of the CO_2 pressure (from 2 to 15 MPa) the CO_2 sorption and the polymer swelling at T=40°C of four polymer samples, among it PEO (Polyethylene Oxide)[24]. Then, the aim of the present study is to investigate the impregnation of the PEO sample by two anti-inflammatory drugs, namely aspirin and ketoprofen using $scCO_2$ and in particular to perform the simultaneous measurement of CO_2 sorption, polymer swelling and drug loading using *in situ* high pressure FTIR micro-spectroscopy.

Aspirin and ketoprofen were selected as two models molecules that both bear a carboxylic acid function, at least an aromatic ring and a carbonyl group, which makes them both soluble in $scCO_2$ [17, 25-27], but they possess different molecular weight and molecular structure (figure 1). The PEO sample was selected as a model polymer matrix since preliminary tests have shown that it can be impregnated with a significant amount of aspirin and ketoprofen. Moreover, this polymer does not possess characteristic bands in the range 1600-1800cm^{-1} that could overlap the characteristic bands of the two drugs, making possible the follow up of the impregnation.

Therefore, in this article, the impregnation process is followed for two different systems, namely {PEO+CO_2+Aspirin} and {PEO+CO_2+Ketoprofen} using *in situ* high pressure FTIR micro-spectroscopy. In particular, the influence of the pressure on the impregnation process has been studied in terms of drug loading as well as of kinetic of impregnation. The comparison of the results obtained with the systems {PEO+CO_2} and

{PEO+CO_2+drug} has enabled us to specifically study the influence of the loaded drug on the thermodynamic behaviors i.e. on the polymer swelling and on the amount of sorbed CO_2 as a function of time during the impregnation. Comparing the two systems implying the two drugs, we have also investigated the influence of the drug on the drug loading and on the kinetic of impregnation. Finally, the speciation of the molecules of drug into the polymer and their molecular interactions with the polymer have been studied.

2 – EXPERIMENTAL DETAILS

2.1. Materials

Carbon dioxide N45 (purity 99,95%) was supplied by Air Liquide. PEO (Mw=1 000 000 $g.mol^{-1}$), Ketoprofen (Keto) and Acetylsalicylic Acid (i.e. Aspirin) were purchased from Sigma-Aldrich. The PEO powder was pressed to form a platelet of about 60µm thickness. Then, the platelet was cut to form films of 3mm length and 1.5mm width.

Ketoprofen was used as received since the particles were thin enough to ensure a good solubilization of ketoprofen in $scCO_2$, whereas acetylsalicylic acid was finely ground to form thinner particles powder.

2.2. Infrared micro-spectroscopy

2.2.a. Experimental set-up

A schematic representation of the experimental set-up is presented in figure 2. The set-up has been already described elsewhere [24]. It consists in a FTIR microscope working in transflection mode coupled with a high pressure cell. The infrared absorption measurements were performed using a ThermoOptek interferometer (type 6700) equipped with a globar source and KBr/Ge beamsplitters coupled to an Infrared microscope (NicPlan, Nicolet) equipped with an MCT (Mercury Cadmium Telluride) detector in order to investigate the spectral range (400-7500 cm^{-1}). Single beam spectra recorded with a 2 cm^{-1} resolution were

obtained after the Fourier transformation of 50 accumulated interferograms. The home-made stainless steel was equipped with a CaF_2 window and a mirror in between the polymer sample was maintained as shown in figure 2. A 100 µm Kapton® foil was placed between the window and the cell body to compensate for any imperfections between the two surfaces. Two thermocouples were used to verify the homogeneity of the temperature into the cell, the first one located close to a cartridge heater for the temperature regulation and the second one close to the sample area to measure the temperature of the sample with an accuracy of about 1°C. The cell was connected via a stainless steel capillary to a hydraulic pressurizing system, which permits the pressure to be raised up to 50 MPa with an absolute uncertainty of ± 0.1 MPa and a relative error of ± 0.3%. The size of the infrared beam was tuned thanks to a rectangular diaphragm in order to focus the beam at the center of the polymer sample (in x,y plane).

2.2.b. Experimental Procedure

First of all, the polymeric sample was fitted into the cell and held in contact between the window and the mirror thanks to a spring disposed between the mirror and the bottom of the cell (figure 2). Aspirin or ketoprofen powder was placed on the bottom of the cell and in excess to ensure the saturation of $scCO_2$ with drug. The mirror used was made of stainless steel polished to obtain a good reflection of the IR beam. In this set-up, the CO_2 diffused into the polymer from its lateral sides to its center. Once the cell was mounted, it was placed under the infrared beam and successive adjustments were performed in order to optimize infrared spectra (see the supporting information a).

Our two systems {PEO+CO_2+Aspirin} and {PEO+CO_2+Ketoprofen} were subjected to a specific experimental protocol. The systems were successively subjected to 5 MPa during 2h; 10 MPa during 5h; and 15 MPa during 3h or more. In order to follow the evolution with

time of the different parameters characterizing our systems, infrared spectra have been recorded every 2 minutes. Following this protocol, we were able to investigate the influence of pressure and the evolution of different variables as a function of time, i.e., the weight percentage of CO_2 sorbed into the PEO, the PEO swelling and the drug loading. Finally, two impregnation experiments have been carried out, following the same experimental protocol described above: one focusing the IR beam on the PEO sample in order to investigate the impregnation of the polymeric system and the other experiment, focusing the IR beam just next to the polymeric sample in order to measure the variation of the pathlength which is necessary to calculate the polymer swelling by using the method presented in part 3.2.a. (see supporting information b).

Concerning the system {PEO+CO_2}, the results for the CO_2 sorption (figure 7) and the polymer swelling (figure 8) correspond to the values obtained in our previous paper and are presented as straight lines for each pressure since the thermodynamic equilibrium are reached after 10minutes and no more changes have been observed after 2 hours at each pressure [24].

Prior to this work, the solubility of aspirin and ketoprofen in CO_2 has been measured at 40°C as a function of the pressure by FTIR analysis (see supporting information c). The results are summarized in table 1 and 2.

2.3 Differential Scanning Calorimetry (DSC)

The differential scanning calorimetry (DSC) analysis was used to confirm *ex-situ* the phenomena observed *in situ* such as the speciation of drug and the modification of the microstructure of PEO. More specifically, the DSC aims at observing a possible crystallization of drug into PEO and to determine the melting temperature of the raw PEO, of PEO only subjected to CO_2 and of PEO after impregnation, using the Instrument DSC Q100

from TA instrument. 5 to 10mg of the PEO samples were placed in an aluminum pan, which was sealed and then heated from 40°C to 200°C with a ramp of 10°C/min.

3 – INFRARED SPECTRA AND DATA PROCESSING

3.1. Infrared absorption spectra

The infrared spectra of a PEO film impregnated with aspirin using supercritical CO_2 were recorded *in situ* at 40°C at 0.1; 5; 10 and 15 MPa. Figure 3 illustrates the evolution of the IR spectra. A number of significant peaks associated with fundamental and combination modes of PEO, CO_2, and aspirin can be observed. One of the obvious changes is the increase of the CO_2 and the aspirin peaks with an increase of pressure.

3.1.a. Assignment of spectral bands of the polymer

The spectrum of raw PEO is shown in figure 4. A number of significant peaks are detected in the range 2900-3100 cm^{-1} associated to fundamental C-H stretch vibrations and that observed in the range 4000-4500 cm^{-1} are associated to C-H combination modes. The peaks observed at about 3000 cm^{-1} are saturated in our experimental conditions. Consequently, we have used the peak centered at 4333 cm^{-1} to determine the swelling of PEO.

3.1.b. Assignment of spectral bands of CO_2

Concerning CO_2, as shown in figure 3 and 4, one can detect four peaks at 2300, 3590, 3695 and 4950 cm^{-1} which are assigned to the antisymetric stretch ν_3 and the combination modes $2\nu_2+\nu_3$, $\nu_1+\nu_3$, and $\nu_1+2\nu_2+\nu_3$ of the CO_2 molecule respectively [28]. Note that the most intense peak observed in figure 3 (centered at 2300 cm^{-1}) is saturated in our experimental conditions and cannot be used for our purpose. The band positions confirm that these peaks

correspond to CO_2 sorbed into the polymer and not to surrounding CO_2, since the peaks of surrounding CO_2 are detected at higher wavenumbers (3610, 3710 and 4970 cm^{-1} for the combination modes). This kind of shift was also reported in the literature. For example, Brantley and al. observed that the peak centered at 4966 cm^{-1} was shifted to 4954 cm^{-1} when the CO_2 was sorbed into the PET [29] because of interactions between the polymer and CO_2.

The bands at 3590, 3695 cm^{-1} or at 4950 cm^{-1} were used to estimate the evolution of the weight percentage of CO_2 sorbed into the polymers, depending on the saturation or not of the peaks at 3590 cm^{-1} and 3695 cm^{-1}.

3.1.c. Assignment of spectral band of the drugs

The ATR-IR spectra of Aspirin and Ketoprofen have been reported in figure 4. Both drugs present characteristic bands in the range of 400 and 1800cm^{-1} as well as a broad peak centered at 3000cm^{-1}. The characteristic bands used to follow the drug loading have been chosen taking into account the spectral range limitations of the set-up and the presence of the characteristic peaks of CO_2 and PEO. Our criteria for the selection of the bands of the drugs were as follows: the selected band has to be isolated (not overlap CO_2 or PEO bands), to be independent on the speciation of the molecules of drug, and its molar extinction coefficient has to be known. In order to determine the molar extinction coefficient of specific modes of aspirin and ketoprofen, we have performed Infrared absorption measurements on both drugs diluted in supercritical carbon dioxide at T=40°C and various pressures from 5 up to 30 MPa (see supporting information c and d).

Aspirin

The assignment of the characteristic peaks of aspirin and the determination of their respective molar extinction coefficient is given in detail in supporting information c. In the range of 1600 and 1800cm^{-1}, we have assigned the peak at 1608cm^{-1} to $\nu_{C=C}$ stretching mode

of the phenyl group; the peaks centered at 1706 cm^{-1} and 1750cm^{-1} corresponds to $\nu_{C=O}$ stretching vibrations of the aspirin carboxyl group in its dimer and monomer form respectively. The peak centered at 1780cm^{-1} corresponds to $\nu_{C=O}$ of the aspirin ester group. The broad peak observed in the range of 2200 and 3400cm^{-1} is assigned to ν_{O-H} stretching vibrations. Since the bands at 1706 and 1750cm^{-1} depend on the speciation of aspirin, the band centered at 1608cm^{-1}($\nu_{C=C}$) was used for measuring the impregnation because it is isolated, independent of the speciation of aspirin, it does not overlap PEO characteristic bands and its molar extinction coefficient has been determined previously (ε_{1608}=147 $L.mol^{-1}.cm^{-1}$). Once this peak saturated, the shoulder at 3200cm^{-1} of the broad peak centered at 3000cm^{-1} was used after calculation of its molar extinction coefficient (see supporting information d). The molar extinction coefficients of the peaks were considered to be independent on the environment of the molecules of drug (i.e. in $scCO_2$ or impregnated into the polymer).

Ketoprofen

The assignment of the characteristic peaks of ketoprofen is given in detail in supporting information c. Similarly to the choice of the characteristic bands of aspirin, the peak at 1660cm^{-1}($\nu_{C=O\ ketone}$ stretching vibrations) was used for measuring the concentration of ketoprofen impregnated in the polymer and its molar extinction coefficient has been determined (ε_{1660}=380.5 $L.mol^{-1}.cm^{-1}$). The shoulder at 3200cm^{-1} of the broad peak centered at 3000cm^{-1} was also used and the calculation of the corresponding molar extinction coefficient is explained in supporting information d.

3.2 Determination of polymer swelling, CO_2 sorption and drug loading: data processing

In the following calculations, the absorbance has been measured considering the peak heights instead of integrated area because the baseline choice produces larger errors when the integrated area method is used.

3.2.a. Polymer swelling

As initially proposed by Guadagno and al.[23] and given in detail elsewhere [24], the polymer swelling S is given by:

$$S = \frac{A_0}{A} \cdot \frac{l}{l_0} - 1 \qquad (1)$$

where A_0 and A are the absorbances of the polymer bands before and after exposure to CO_2, respectively; l_0 and l are the pathlengths before and after exposure to CO_2, respectively (cm).

In FTIR transmission measurements, the pathlength corresponds to the length of the cell hence it is fixed along the experiment [22, 29]. On the contrary, the pathlength can change during the impregnation process in the developed set-up thanks to the use of the spring (figure 2). Indeed, the spring is compressed during polymer swelling and thus, the pathlength can vary depending on the working conditions. Thus, the variation of pathlength has been evaluated during the impregnation process as explained in supporting information b [30].

Once the swelling of PEO is estimated, the density of PEO ρ can be calculated using the following equation:

$$\rho = \frac{\rho_0}{1+S} \quad (2)$$

where ρ is the density of PEO subjected to CO_2 or {CO_2+drug} mixture (g.cm^{-3}); ρ_0 is the initial PEO density and equal to 1.21 g.cm^{-3}; S is the swelling.

3.2.b. CO_2 sorption

In order to determine the concentration of CO_2 (C_{CO_2}) sorbed into the polymer, the Beer-Lambert law has been applied to the CO_2 peaks. In order to directly use the concentration of CO_2 for further calculation, it has been calculated in g.cm^{-3} using the equation 3:

$$C_{CO_2} = \frac{A}{\varepsilon.l} \times M_{CO_2} \times 10^{-3} \quad (3)$$

where A is the absorbance of the CO_2 band; ε is the molar extinction coefficient; l is the pathlength; M_{CO2} is the molar mass of CO_2 and equal to 44 g.mol^{-1}. The molar extinction coefficient considering the peaks height were estimated to be about $\varepsilon_{3695cm\text{-}1}$= 8.28 L.mol^{-1}.cm^{-1}; $\varepsilon_{3590cm\text{-}1}$= 3.17 L.mol^{-1}.cm^{-1} and $\varepsilon_{4950cm\text{-}1}$= 0.25 L.mol^{-1}.cm^{-1} (see supporting information e) [31]. Then, the weight percentage of CO_2 $(\%mass_{CO_2})$ sorbed into the polymer was calculated using the following equation:

$$\%mass_{CO_2} = \frac{C_{CO_2}}{C_{CO_2}+\rho} \quad (4)$$

where C_{CO2} is the concentration of CO_2 (g_{CO2}.cm^{-3}) and ρ is the polymer density calculated with equation 2 (g.cm^{-3}).

3.2.c. Drug loading

The concentration of drug C_{drug} (mol.L^{-1}) was obtained by applying the Beer-Lambert law using the previously selected characteristic peak for each drug. Then it was converted in the units of g_{drug}.cm^{-3} in order to calculate the drug loading DL which corresponds to the weight fraction of drug in the polymer by using the equation (5):

$$DL = \frac{C_{drug}}{C_{drug} + \rho} \quad (5)$$

where C_{drug} is the concentration of drug (g_{drug}.cm^{-3}) and ρ is the polymer density (g.cm^{-3}).

As explained in section 3.1.c, the bands centered at 1608 and 1660cm^{-1} were selected for the calculation of drug loading DL for aspirin and ketoprofen respectively. When these peaks saturated, the peaks at 3200cm^{-1} were used for both drug.

Finally, using such a methodology and taking into account all the source of errors associated with our methodology (baseline correction, constant molar extinction coefficient, spectrometer stability), we have estimated a relative error of about ± 10% on our concentration values. We emphasize that the reliability of in situ IR spectroscopic measurements has already been demonstrated in previous investigations on the mutual solubility of epoxide with CO_2 [32, 33] and water with CO_2 [34] where a satisfactory agreement with literature data was shown.

4 – RESULTS AND DISCUSSION

4.1 Thermodynamic behavior of the {PEO+CO_2+Aspirin} system during the impregnation process

In order to show the main results obtained via our set-up, we have selected the system {PEO+CO_2+Aspirin} as a model system. The same kind of investigation has been carried out with {PEO+CO_2+Ketoprofen}.

The developed set-up enables to follow simultaneously the density of PEO (i.e. concentration of PEO ρ), the concentration of CO_2 (C_{CO2}), and also the concentration of drug (C_{drug}), as a function of time.

Figure 5 shows the evolution of these three concentrations in the {PEO+CO_2+Aspirin} system throughout the experimental protocol. These results enable to point out the changes that occur at each increase of pressure and also to correlate the different phenomena during the impregnation process.

First of all, when the pressure is increased to 5MPa, the CO_2 sorbs immediately into PEO and it entails a sudden decrease of the PEO density (in less than 2 minutes). Both the concentration of sorbed CO_2 and PEO density remain constant during 2 hours at 5 MPa. Despite of CO_2 sorption, the impregnation of aspirin into PEO does not occurred.

When the pressure is increased to 10 MPa, the concentration of CO_2 increases quasi-instantaneously and the PEO density decreases. After about 15min, the impregnation begins and the concentration of aspirin increases slightly throughout the experiment until the end. At 10 and 15MPa, the concentration of CO_2 increases and the density of PEO decreases continuously. The thermodynamic equilibrium has been reached neither at 10 MPa after 5h, nor at 15 MPa after 7h since the concentrations of PEO, CO_2 and aspirin do not reach any plateau-like values.

From these data, some parameters can be deeper investigated such as the influence of the pressure on the drug loading and the impact of the drug on the CO_2 sorption and PEO swelling. Moreover, the thermodynamic behaviors of the two impregnation systems of {PEO+CO_2+Aspirin} and {PEO+CO_2+Ketoprofen} can be compared to analyze the influence of the drug on the impregnation process.

4.1.a Influence of pressure on the drug loading of aspirin into PEO

The drug loading of aspirin into PEO has been calculated using equation 5 and the kinetic evolution is presented in figure 6.

In figures 6, we observe that no impregnation occurs at 5 MPa. According to table 1, aspirin is not soluble in CO_2 at 5 MPa because CO_2 is gaseous and have a poor solvating power. Consequently, the mass transfer of aspirin into PEO cannot be achieved in such conditions.

Once the pressure is raised to 10 MPa, the impregnation begins (figure 6). The mass transfer is possible since aspirin is solubilized in CO_2 which is in its supercritical state (table 1). The impregnation is only observed after a 20 minutes period of time. This delay can be accounted by two factors. The first one is the kinetic of solubilization of aspirin in $scCO_2$ which is estimated to be about 10 minutes in absence of any stirring. The second one is the

diffusivity of aspirin into the PEO sample. Indeed, the analyses were performed in the center of the sample and the aspirin could only diffuse from the lateral sides to the center of the sample. The surface corresponding to the lateral sides is small, which makes the diffusivity quite low.

According to the data reported in table 1, the solubility of aspirin into $scCO_2$ increases with pressure. However, the kinetic of drug loading does not show any significant changes when the pressure is increased to 15 MPa. The drug loading continues to rise slowly with time, because of the progressive transport of aspirin into PEO.

As presented in figure 5, the evolutions of the concentrations of CO_2 and of aspirin follow a similar trend with time. As the concentration of CO_2 increases, the total amount of aspirin carried by CO_2 and transferred into the matrix become higher. Moreover, the diffusion of aspirin into the matrix is facilitated by the higher swelling of the PEO matrix.

4.1.b Influence of the loaded aspirin on the thermodynamic behaviors of the system: CO_2 sorption and PEO swelling

In order to investigate the influence of aspirin on the CO_2 sorption and on PEO swelling, we have compared the results obtained for the two systems {PEO+CO_2+Aspirin} and {PEO+CO_2}.

The weight percentage of CO_2 and the PEO swelling were calculated with equations 4 and 1 respectively and the results are reported in figures 7 and 8.

The curves corresponding to {PEO+CO_2+Aspirin} and {PEO+ CO_2} superimposed at 5 MPa for both the weigh percentage of sorbed CO_2 and PEO swelling. This is concordant with the fact that PEO is only subjected to CO_2 in the system {PEO+CO_2+Aspirin} since aspirin is not soluble in CO_2 at 5 MPa.

In figure 7 and 8, some differences appear between the two systems at 10MPa for the weight percentage of sorbed CO_2 and PEO swelling, especially after 160minutes (i.e. 40 min carried out at 10 MPa) and 300 minutes (i.e.180min carried out at 10 MPa) respectively. Indeed, the weight percentage of CO_2 and the PEO swelling both increase with time in the system {PEO+CO_2+Aspirin} whereas both remain constant in {PEO+ CO_2}. These differences are observed once the impregnation starts i.e. after 140minutes (i.e. 20 minutes carried out at 10 MPa) according to figure 6. After 5h at 10MPa the weight percentage of CO_2 is 25% higher in the system {PEO+CO_2+Aspirin} than in {PEO+CO_2} whereas it is 55% higher at the end of the protocol.

The impact of aspirin on the sorption and swelling of PEO can be attributed to a plasticizing effect of aspirin on PEO. CO_2 alone acts as a plasticizer by interacting with the ether group of PEO which allows a high CO_2 sorption (17% at 10MPa and 20% at 15MPa), but it appears that the plasticizing power of the mixture {CO_2+Aspirin} is even higher considering the higher CO_2 sorption. The molecule of aspirin may enhance the mobility of the polymeric chains that can rearrange to sorb more CO_2. Besides, the crystallinity of PEO is probably decreased at 10MPa under $scCO_2$ as reported previously [24], and the presence of drug could emphasize this phenomenon by decreasing the melting temperature of PEO *in situ* even more than CO_2 does. A decrease of the melting temperature is generally due to the presence of smaller crystals. Therefore, the large scale movements of the chains would be facilitated which would allow a larger swelling of PEO as it is observed in figure 8. The plasticizing effect of aspirin and the T_m depletion seems to be enhanced while the drug loading rises.

Üzer et al. and López-Periago et al. have observed a similar impact of solutes on the swelling of PMMA when impregnated in $scCO_2$ at 35-40°C and in a range of pressure between 8 and 20 MPa [35, 36]. They observed that the naphthalene impregnation into PMMA entailed an increase in volume expansion up to 50% higher than when PMMA was only

swollen with $scCO_2$ in the same conditions. The use of EtOH or MetOH as a cosolvent also increases the plasticizing effect of $scCO_2$ by enhancing the swelling of P(D,L)LA or cross-linked PDMS [7, 37, 38]. However, to the best of our knowledge, there is no previous study that investigated the impact of a solute on the CO_2 sorption and on the thermodynamic behavior of the impregnated polymer.

In order to confirm the hypothesis that aspirin enhance the melting of the crystals in PEO, DSC thermograms were performed on samples impregnated in the batch reactor (40°C; 10 MPa; 5h). In figure 9, we compare the DSC thermograms of raw PEO, PEO only subjected to CO_2 and PEO impregnated with aspirin at 40°C and 10 MPa. The melting temperature of PEO is not significantly impacted by the CO_2 treatment. On the contrary, the melting temperature of the impregnated PEOs is decreased by the presence of aspirin up to 13°C which shows that the microstructure is impacted *in situ*. It is worth noting that these thermograms do not reflect the crystallinity of the sample *in situ* and the observed crystallinity could have been formed during the depressurization step. Nevertheless, it can be supposed that the original crystals in PEO melted partially (and maybe disappeared) *in situ* and smaller ones have been created in presence of drug. These modifications can explain the increase mobility of the chains during impregnation and the resulting higher swelling of PEO.

The increase of swelling and CO_2 sorption favor the diffusion of the {CO_2+drug} mixture which leads to increase of the drug loading. Consequently, the higher drug loading in the matrix enhances the chains mobility and so on. This self-sustained phenomenon probably happens until the impregnated PEO totally melt.

4.1.c Influence of the drug on the impregnation and on the thermodynamic behaviors: comparison of aspirin and ketoprofen loadings

The experimental protocol presented in part 2.2.b was also applied to the system {PEO+CO_2+Keto} in order to compare the impregnation processes of ketoprofen and aspirin into PEO.

In figure 10 are reported the drug loadings of aspirin and ketoprofen throughout the experimental protocol. The kinetic of impregnation of aspirin in PEO is faster than the one of ketoprofen during the whole experiment. At the end of the experiment, the drug loading DL of aspirin reached 12.8% whereas it reached only 4.7% for ketoprofen.

The drug loading is influenced by different parameters such as the solubility of the drug in scCO_2, the molecular weight of the drug, the CO_2 sorption, the polymer swelling and the interactions between the polymer and the drug.

The solubility of ketoprofen into CO_2 is reported in table 2. The solubility of aspirin and ketoprofen into scCO_2 are of the same order for each pressure. However, the molecular weight of aspirin (180g.mol^{-1}) being lower than the one of ketoprofen (254g.mol^{-1}), its diffusivity in PEO may be facilitated that can explain a faster uptake and a higher drug loading.

The evolution of the weight percentage of sorbed CO_2 and the PEO swelling are compared for the systems {PEO+CO_2+Ketoprofen}; {PEO+CO_2+Aspirin} and {PEO+CO_2} in figure 11. As no drug is loaded at 5 MPa, the three systems behave similarly. Increasing the pressure to 10 MPa, the CO_2 sorption ($\%mass_{CO_2}$) increases rapidly within 10 minutes to about 17 % and is found to be similar for all the systems within the experimental errors. During the following 140 minutes, an increase of CO_2 sorption is observed during the impregnation of aspirin whereas no obvious changes appear during impregnation of ketoprofen compared to the system {PEO+CO_2}. When the pressure is further increased up to 15 MPa, the weight percentage of CO_2 increases as a function of time for both drugs and in a much higher extent during the impregnation of aspirin.

Therefore, ketoprofen impacts the thermodynamics of the {PEO+CO_2} in a slighter extent than aspirin. This is confirmed by the DSC thermogram of PEO impregnated with ketoprofen (figure 9) which shows a slighter decrease of the melting temperature of PEO when impregnated with ketoprofen (6°C) than with aspirin (13°C). This difference may be due to the lower drug loading of ketoprofen into PEO, thus reducing the possible plasticizing effect on PEO. Nonetheless, it might be explained by different interactions between PEO and the two drugs.

4.2. Speciation of drug into PEO

The interaction between the polymer and the two drugs can be investigated by analyzing the characteristic bands of the drugs in the $\nu_{C=O}$ region between 1600 and 1800cm^{-1} since both drugs are carboxylic acids. Besides, the infrared spectra can provide information about the molecular state of the drug into the polymer, which is a major parameter that determines the ability of the final system to release the drug and the bioavailability of the drug in case of drug release application.

Aspirin

In figure 12 are shown (a) the IR spectrum of aspirin loaded into PEO recorded *in situ*, (b) the transmission IR spectrum of aspirin solubilized into $scCO_2$, and (c) the ATR-IR spectrum of aspirin powder which is crystallized.

Compared with the IR spectrum of aspirin solubilized into $scCO_2$ (b), the spectrum of aspirin loaded into PEO (a) shows noticeable changes regarding the appearance and disappearance of some peaks and the shifts between 1625 and 1800 cm^{-1}.

FTIR spectrum of aspirin powder shows two characteristic peaks centered at 1752 and 1687cm^{-1}, which are assigned to the $\nu_{C=O}$ stretching vibration of the ester group and of the carboxyl group stretching vibrations, respectively, for aspirin molecules organized in a crystalline structure [39]. The peak observed at 1752cm^{-1} of the ester group is shifted to 1780 and 1770cm^{-1} in $scCO_2$ and into PEO respectively owing to modification of the molecular environment. Regarding the spectrum of aspirin solubilized in $scCO_2$, the peak centered at 1706 cm^{-1} is assigned to the stretching vibrations of the carboxyl group of aspirin under its dimeric form solubilized in $scCO_2$. On the other hand, a new peak appears at 1749cm^{-1} which is assigned to $\nu_{C=O}$ of the stretching vibration of the carboxyl group of aspirin in its monomeric form. As reported before for carboxylic acids, both cyclic dimers and monomers are present in $scCO_2$, the equilibrium between both forms depending on the temperature and pressure conditions [39].

The spectrum of aspirin loaded into PEO presents two peaks at 1725 and 1662cm^{-1}. The peak at 1725cm^{-1} corresponds to the $\nu_{C=O}$ stretching vibration of the monomeric carboxyl band which is shifted to lower wavenumber compared to that observed for aspirin in $scCO_2$ at 1749cm^{-1}. To explain this shift, the carboxyl group of aspirin could interact with the OH terminal group of PEO through hydrogen bonds as it has been proposed by Chan et al. for the ibuprofen/PEO system [40]. However, in our case, the molecular weight of the PEO sample is 10^6 g/mol and therefore the ratio between the number of aspirin molecules and the terminal OH groups is expected to be much lower than in the study by Chan et al. [40] as they have investigated a PEO sample with a molecular weight of about 400 g/mol. In our study, the molecular weight of aspirin being180.2 g/mol, the aspirin/PEO ratio is about $180.2*2/10^6$, i.e. 0.036%. Thus, above a drug loading of 0.036% of aspirin, all the hydrogen bonding sites, i.e. the terminal OH groups of PEO, should be occupied by an aspirin molecule. However, the spectrum reported in figure 12 (a) corresponds to a drug loading of about 3%. We emphasize

that even at lower or higher drug loading, the position of the $\nu_{C=O}$ monomeric carboxyl band is always about the same at 1725 cm^{-1}. Therefore, the shift to lower wavenumber of the $\nu_{C=O}$ monomeric carboxyl band is not due to hydrogen bond interaction but mainly related to van der Waals interactions between aspirin molecules and the backbone of the polymer chains. These interactions are strong enough to prevent dimerization of aspirin molecules in PEO.

Finally, a weak contribution is detected at 1662cm^{-1} that we assign to the $\nu_{C=O}$ stretching vibration of the carboxyl group of aspirin molecules organized in a crystalline structure as observed in the spectrum of aspirin powder reported in figure 12 (c). However, the presence of large crystallite can be excluded by DSC analysis performed on the final sample where no melting peak corresponding to crystallized aspirin has been observed (figure 9). Consequently we attribute this IR band of low intensity to trace of crystallized aspirin in PEO, i.e. small crystallites created by few molecules which are not large enough to be visible through DSC analysis.

Ketoprofen

Similar conclusions have been observed for ketoprofen loaded into PEO. Two peaks are present in the spectrum of crystallized ketoprofen at 1695 and 1655cm^{-1} which correspond respectively to the $\nu_{C=O}$ stretching vibration of the carboxyl group and the ketone group of ketoprofen molecules organized in a crystalline structure (see figure 13).

In the spectrum of ketoprofen in $scCO_2$, the peak centered at 1763cm^{-1} is assigned to $\nu_{C=O}$ stretching vibration of the carboxyl group of ketoprofen in its monomeric form. The bands at 1716 and 1672cm^{-1} correspond respectively to the $\nu_{C=O}$ stretching vibration of the carboxyl group of ketoprofen in its dimeric form and to the $\nu_{C=O}$ stretching vibration of the ketone group. For ketoprofen loaded into PEO, the peak centered at 1736cm^{-1} corresponds to

the $\nu_{C=O}$ stretching vibration of the carboxyl group of monomers which is shifted from 1763cm^{-1} in scCO_2 probably due to Van der Waals interactions that might occur between ketoprofen molecules and the surrounding polymer chains. The one at 1664cm^{-1} is assigned to the $\nu_{C=O}$ stretching vibration of the ketone group. Contrary to aspirin loaded into PEO, no clear band is observed that could correspond to crystallized ketoprofen (which was confirmed by DSC analysis), showing that ketoprofen is molecularly dispersed into the polymeric matrix. The interactions between the polymer and the two drugs are of the same nature and the drugs were molecularly dispersed into the PEO matrix. The analyses of the $\nu_{C=O}$ region cannot account for the different drug loading and the different impact of the two drugs on the thermodynamic behavior of PEO.

Since the drugs are molecularly dispersed, the final impregnated PEO should be adequate for biomedical applications. Indeed, the decrease of the crystallinity of drugs by dispersing it into a water-soluble matrix is suitable to improve its solubility in water and so its bioavailability, both aspirin and ketoprofen being poorly soluble in water[41, 42].

5- CONCLUSION

A new set-up consisting in coupling a FTIR microscope with a high pressure cell has been developed, in order to analyze *in situ* microscopic polymer samples (~ 5-500µm) either only subjected to CO_2 or during their supercritical CO_2 assisted impregnation.

The developed system enables to measure simultaneously various key parameters implied in impregnation process such as the swelling of the polymeric matrix, the CO_2 sorption, the kinetic of impregnation and the quantity of solute loaded into the matrix during the impregnation. Moreover, the molecular state of the drug into the polymer as well as the molecular interactions between the drug and the polymer can be assessed.

The impregnation of PEO thin platelets (60µm) with aspirin and ketoprofen has been carried out and followed in situ at 40°C and 5; 10 and 15MPa successively.

Comparing the CO_2 sorption and the swelling of PEO during impregnation and of PEO only subjected to CO_2, we observed that both quantities are increased when the drug is loaded into the polymer. By its plasticizing effect, the drug enabled to increase the chains mobility and to decrease the crystallinity *in situ*. Consequently, it favored the impregnation process (the drug loading increases) even under isobaric conditions. This increase of drug loading tends to enhance the phenomena, so both CO_2 sorption, swelling of PEO and drug loading increase continuously. Therefore, it appears clearly that the CO_2 sorption, the PEO swelling and the drug loading are firmly correlated.

The kinetic of impregnation with ketoprofen was found to be slower than the one of aspirin which may be accounted by its higher molecular weight and by a sligther impact on the PEO behavior (CO_2 sorption and PEO swelling). Finally, the speciation of drug was investigated considering the shift of the carboxyl bands of the drugs. Both drugs were found to be mainly homogeneously dispersed into PEO.

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Table 1: Solubility of aspirin in CO_2 as a function of pressure at T=40°C

Pressure (MPa)	$y_{Aspirin}(molar\ fraction)$
5	Non detectable ($<10^{-7}$)
10	$4.79*10^{-5}$
15	$11.31*10^{-5}$

Table 2: Solubility of ketoprofen into CO_2 as a function of pressure at T=40°C

Pressure (MPa)	$y_{Keto}(molar\ fraction)$
5	Non detectable ($<10^{-7}$)
10	$1.68*10^{-5}$
15	$5.69*10^{-5}$

Figure captions

Figure 1: Chemical structure of a) aspirin and b) ketoprofen

Figure 2: In-situ FTIR microscope coupled with a transflection optical cell

Figure 3: IR spectra of a PEO film (thickness =60 μm) subjected to CO_2 and aspirin at T=40°C and 0.1, 5, 10 and 15 MPa

Figure 4: ATR-IR spectra of aspirin and ketoprofen and IR transmission spectra of a PEO film and CO_2 (P=5 MPa)

Figure 5: Simultaneous kinetic evolution of PEO density, of the concentration of CO_2 C_{CO2}, and of the concentration of aspirin $C_{Aspirin}$ during impregnation of aspirin into PEO

Figure 6: Kinetic evolution of the drug loading $DL_{Aspirin}$ during impregnation into PEO at 40°C as a function of the pressure of CO_2.

Figure 7: Comparison of the evolution of the weight percentage of CO_2 into PEO subjected only to CO_2 {PEO+CO_2} and during impregnation of aspirin {PEO+CO_2+Aspirin} throughout the experimental protocol

Figure 8: Comparison of the evolution of the PEO swelling when subjected only to CO_2 {PEO+CO_2} and during impregnation of aspirin {PEO+CO_2+Aspirin}

Figure 9: DSC thermograms for (a) raw PEO (b) PEO subjected to CO_2, (c) PEO impregnated with aspirin, (d) PEO impregnated with ketoprofen, (e) aspirin powder, (f) ketoprofen powder. (b),(c) and (d) have been carried out during 5h at 10 MPa

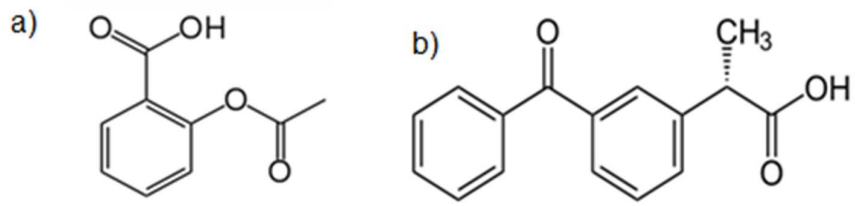
Figure 10: Comparison of the evolution of drug loadings of ketoprofen and aspirin into PEO

Figure 11: Comparison of the evolution of the weight percentage of CO_2 into PEO during impregnation of ketoprofen (Keto), of aspirin, or only subjected to CO_2.

Figure 12: IR spectra of aspirin loaded into PEO (DL=3%) (a) compared to Aspirin solubilized in scCO_2 (b) and Aspirin powder (c).

Figure 13: IR spectra of Ketoprofen loaded into PEO (a) compared to Ketoprofen solubilized in $scCO_2$ (b) and Ketoprofen powder (c).

Figure 1:



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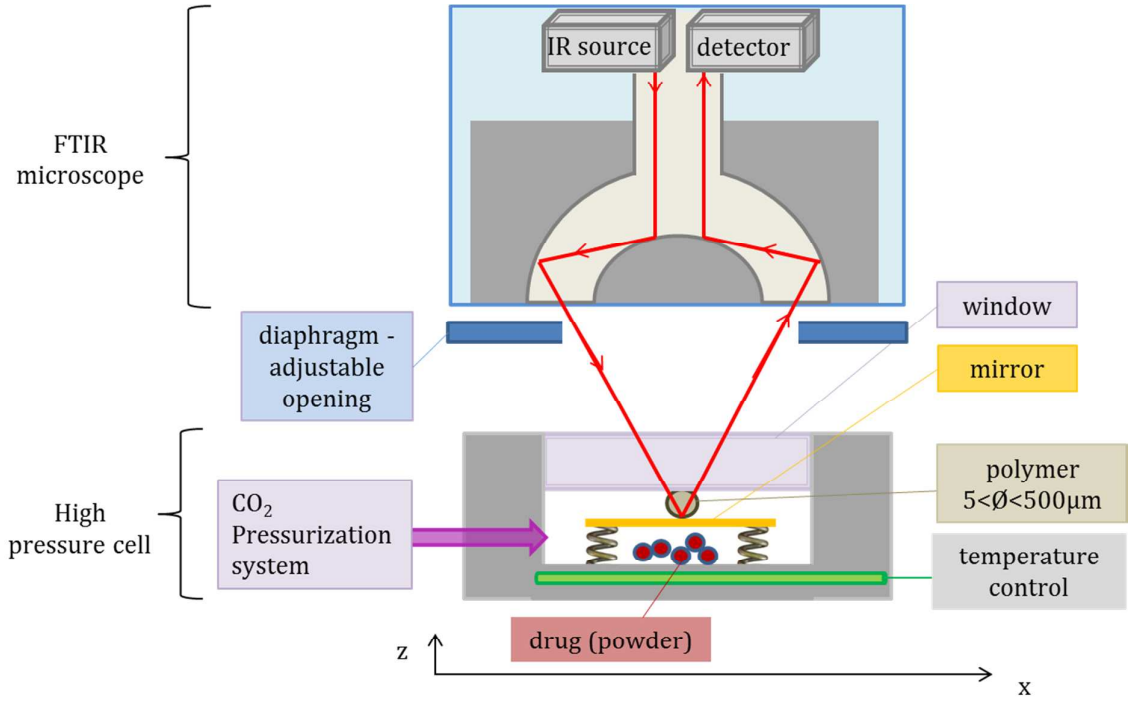

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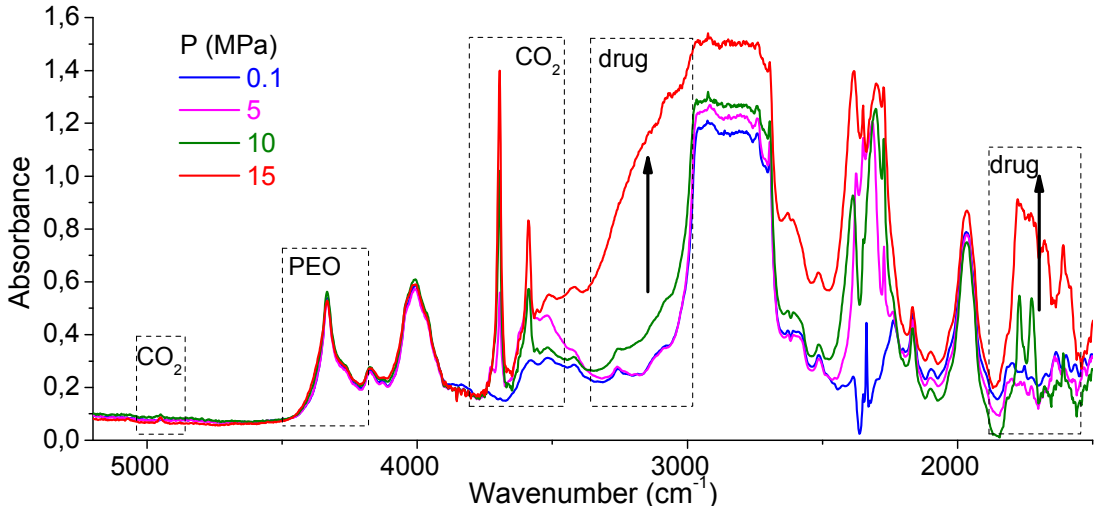

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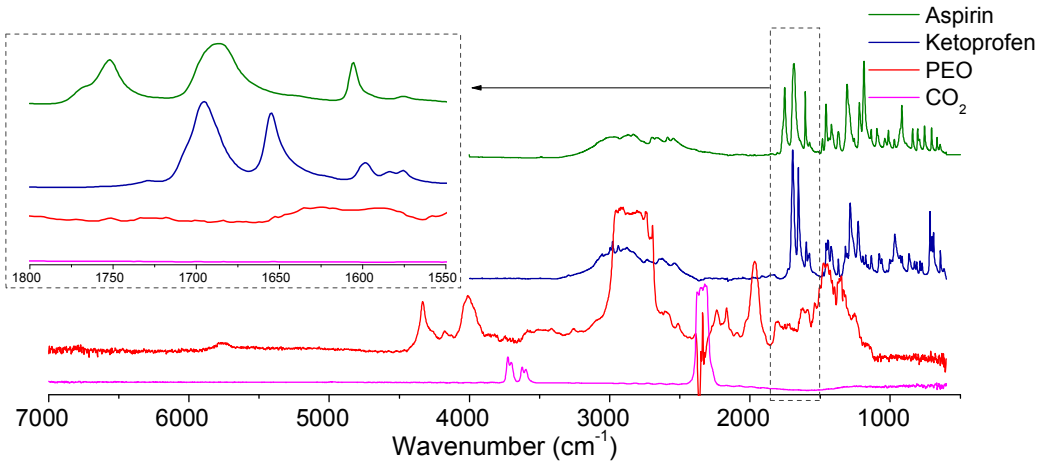

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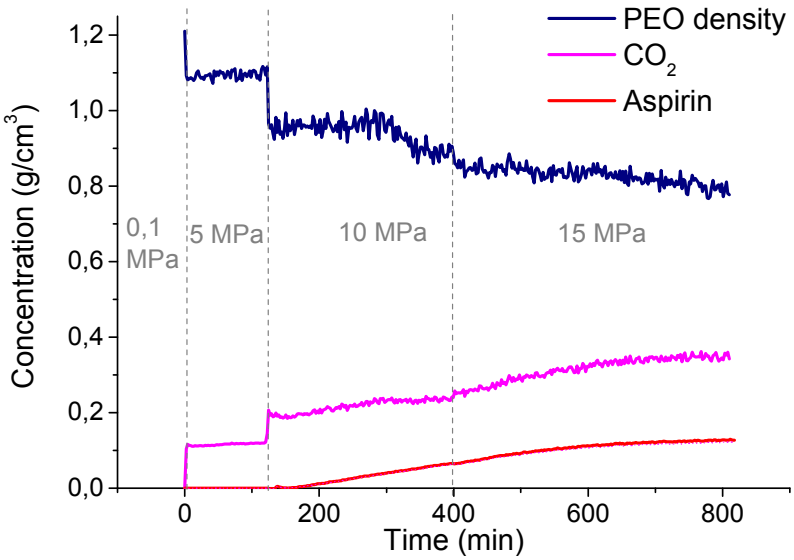

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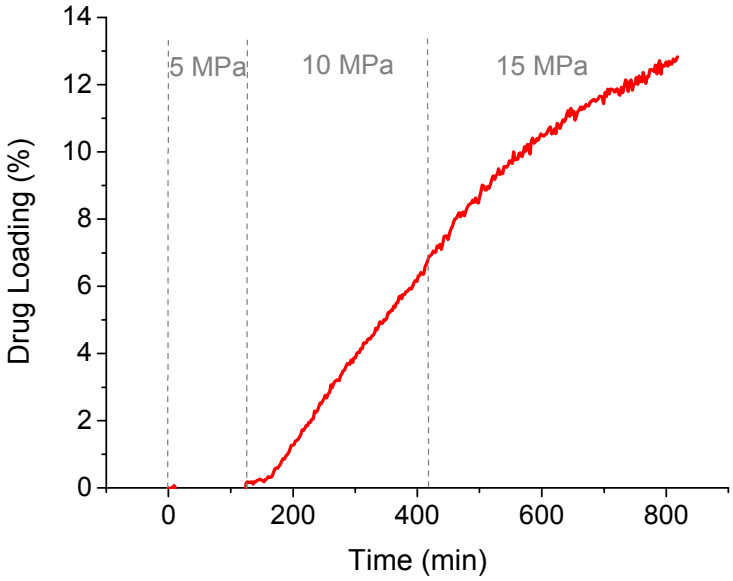

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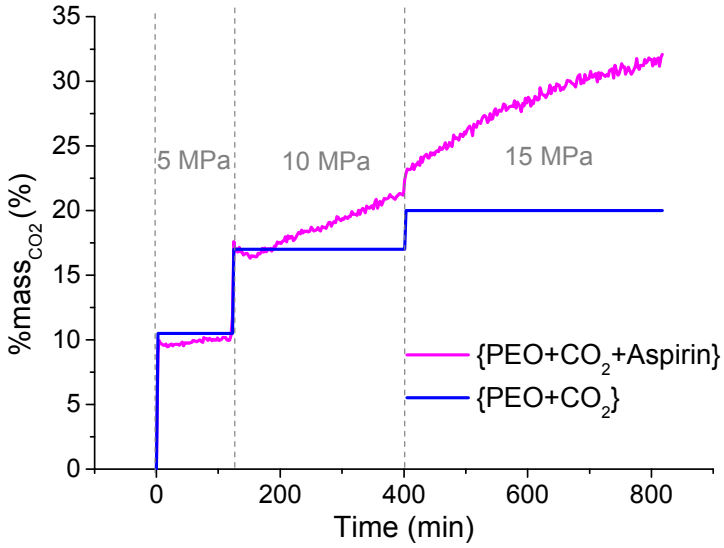

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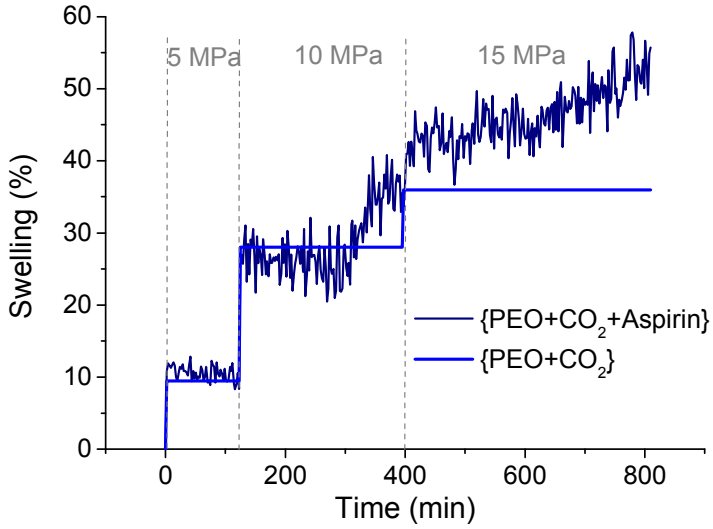

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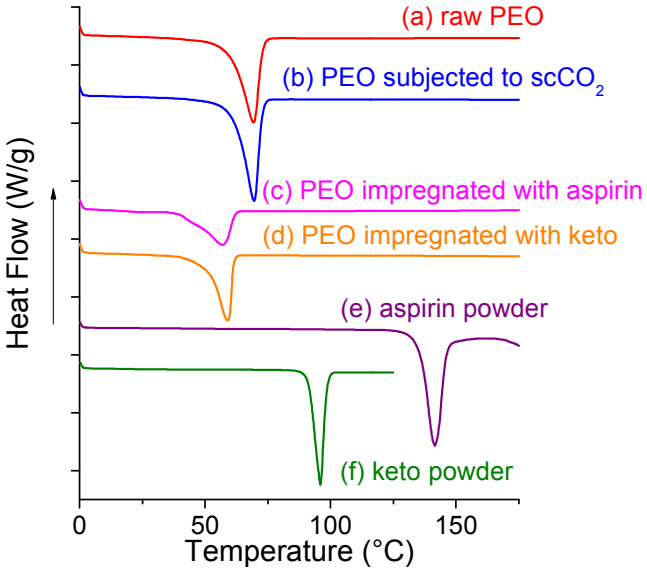
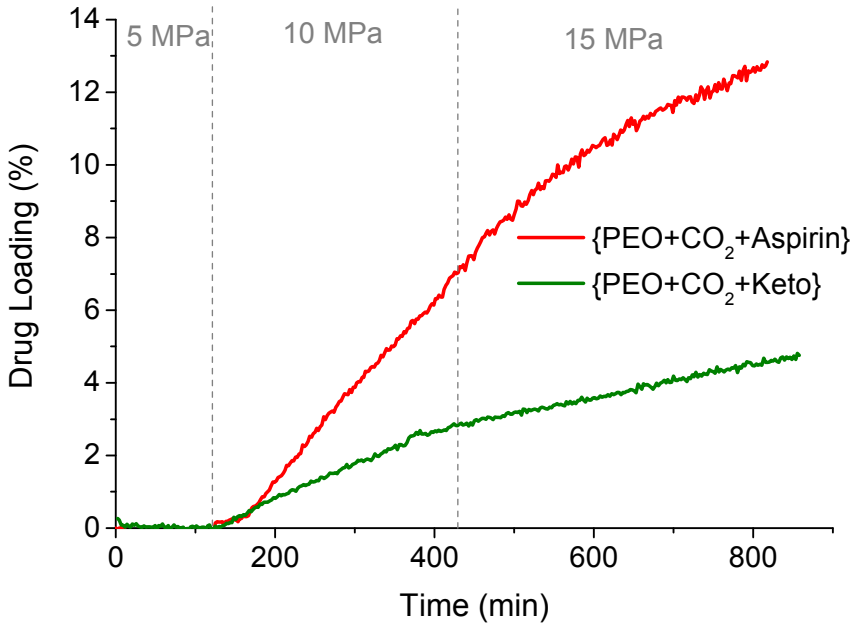


Figure 10



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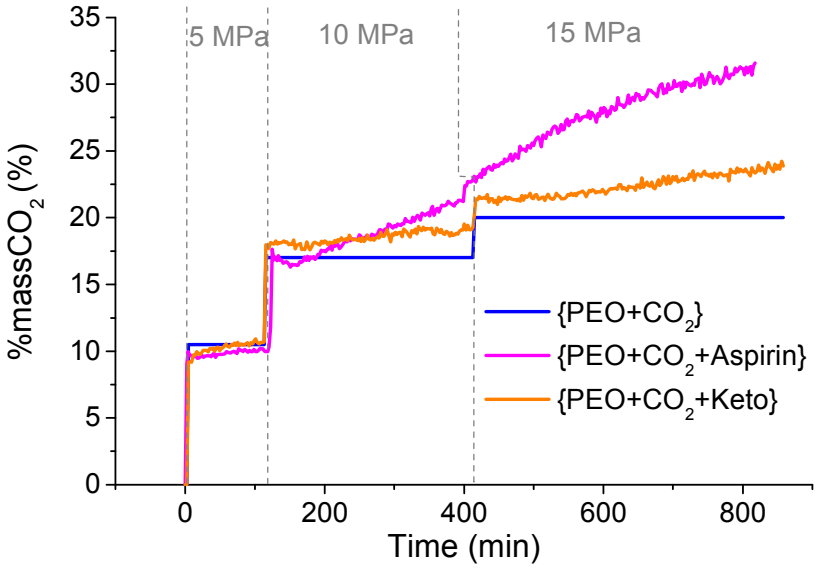
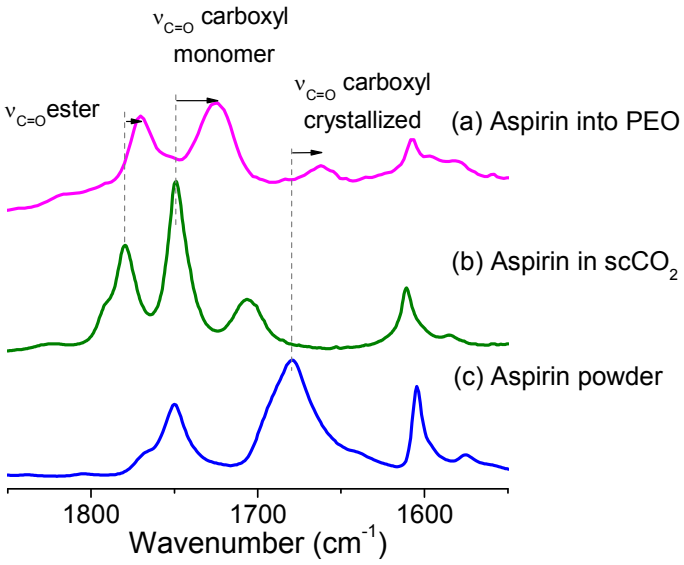


Figure 12



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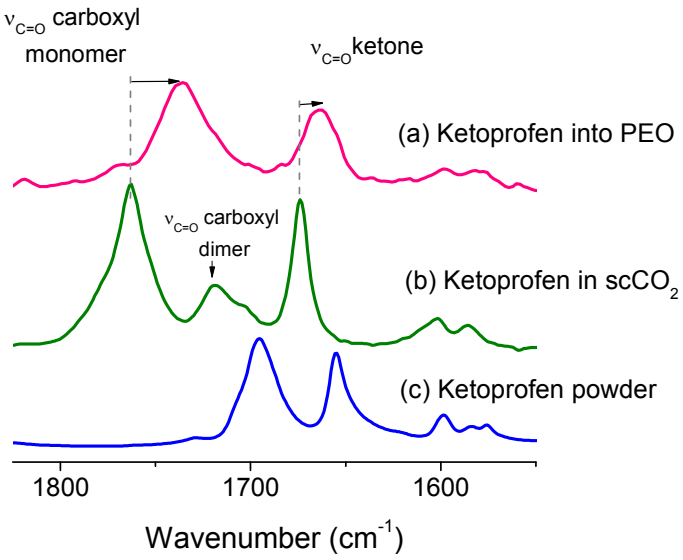

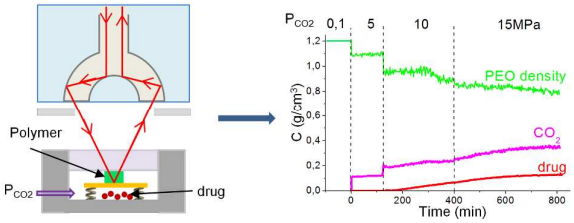

High pressure FTIR micro-spectroscopy to follow the kinetic of the drug loading during the supercritical CO_2 assisted impregnation process