Evaluation and comparison of analytical methods for monitoring polymer depolymerization: application to poly(bisphenol A carbonate) methanolysis.

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Abstract

The enhancement of polymer depolymerization represents a significant challenge to reduce the environmental impact of plastic pollution and the utilization of raw materials. Recently, there has been a demand to optimize degradation analysis approaches, which have been observed to be sometimes used inappropriately or incompletely. This article proposes an analysis strategy for monitoring the depolymerization of poly(bisphenol A carbonate) (PBPAC), using methanolysis as model method. It is based on five analytical methods, which our study attempts to combine and compare according to their ideal use case: size exclusion chromatography, liquid chromatography and FT-IR, NMR and MALDI-TOF spectroscopy. This strategy allows for both a qualitative approach to be taken, whereby the depolymerization products can be identified, along with a quantitative one, whereby the percentages of polymer degradation can be determined. As a result, the range of applications for each method is assessed, and a guide to the minimum methods to be used to qualify and quantify degradation is proposed, in relation to the progress of degradation and the yields obtained. This has enabled us to characterize and propose a new quantitative FT-IR based methodology, compatible with high-throughput screening, to the degradation of PBPAC, allowing to quantify degradation from 10 % onwards.

Introduction

The ever-increasing production of plastics and its consequences in terms of waste, the release of microplastics and pollutants into the natural environment, has led to the urgent need to examine the end-of-life of polymers. The global percentage of recycled plastic is estimated to be of only 16 %, and largely depends on the polymer considered, as well as its form of use.[1] Polycarbonates are one of the so-called "commodity polymers", with a global annual market estimated at 4.7 million metric tons, making them the fifth-largest heteroatom-containing polymer market.[1] This family of polymers, largely dominated by poly(bisphenol A carbonate) (PBPAC), is used for its optical properties, impact resistance, flame retardancy, etc., and is found in a wide variety of fields, including daily objects (plastic windows, compact disc, etc.), many electronic devices, or even larger objects such as in the automotive sector.[2] A good understanding of the degradation and fate of the polymers making up these objects, a large proportion of which unfortunately still end up in the environment, and in particular infinitely in the oceans, is essential in order to study the environmental risks. Especially since bisphenol-A is known to be an endocrine disruptor, and is now well recognized as being particularly toxic to many ecosystems.[3] Understanding the degradation of this polymer and its associated products, as well as the development of suitable and effective recycling routes, therefore seems inevitable if we are to contain its negative impact on the environment.

Examples of PBPAC degradation studies are numerous in the literature, starting with hydrolysis and methanolysis, with complete depolymerization or yields in excess of 80 %, analyzed mainly by infrared spectroscopy and gas chromography.[4–6] Other studies have focused on the biodegradation of polycarbonates, but this time with highly variable yields. If when it comes to aliphatic polycarbonates, they can sometimes exceed 50 %, or even 70 % in rare occasions, in the case of PBPAC, degradation hardly reaches 30 %, leaving considerable room for improvement in this type of approach.[7–9] Unlike for chemical methods, degradation products are then analyzed by a wider variety of methods, including mass loss, infrared spectroscopy and size-exclusion chromatography (SEC). Finally, a certain number of studies have focused on weaker degradation processes, such as heat treatment or UV irradiation, using analytical methods such as SEC, MALDI-TOF, yellowness index, FT-IR, SEM...[10–13] This very wide range of analytical methods is often used inconsistently between different studies, or even within the same study, creating a lack of clarity in understanding the phenomena involved. In particular, some analysis methods used are actually not suitable for the degradation percentages studied, and can lead to misinterpretation of the actual level of degradation.[14] Generally speaking, this heterogeneity makes it particularly difficult, if not impossible, to compare degradation methods, as no standardized experiment have been carried out, which further limits the scope of such studies.

In 2023, Tian *et al.* published a pioneering review in which they attempt to cover the usefulness of each analytical method, trying to propose an optimal set for characterizing polymer aging as a function of polymer properties and degradation stage.[15] The numerous analytical methods are discussed using two main characteristic types: a macro approach focusing on polymer properties, and a molecular approach focusing on degradation products. However, this review remains theoretical, based solely on the results of other publications, and is very general because it applies to the wide range of polymers described in the literature. As a result, the authors do not propose any concrete application cases, enabling the panel of analytical methods to be effectively compared under uniform conditions. So, in order to put these initial conclusions into practice, and to offer a concrete case study, our work proposes a deeper focus on PBPAC, and concentrates on the definition of an optimal strategy to study its degradation through a molecular approach, selecting, as in the review, the ideal panel of analytical methods for each level of degradation. The strategy implemented is based on five of these commonly used for PBPAC degradation studies: size exclusion chromatography, an indispensable analytical tool for measuring the evolution of molecular weight distribution (MWD), which provides an overall understanding of the evolution of chain length distribution as a function of the extent of conversion.[1, 15] HPLC, which enables the identification of small mass products. Infrared spectroscopy and NMR to identify chemical groups modified during depolymerization. Finally, MALDI-TOF, to characterize end-of-chain (called end groups) modifications. This study is the first to our knowledge to compare these five analytical methods applied to the depolymerization of PBPAC, or even any type of polymer. The output provided by each analysis method are discussed, along with their respective sensitivity and range of effectiveness, and finally the problems associated with each. In order to cover the various stages of PBPAC depolymerization, methanolysis was used here as a degradation method, based on the work of Bhogle *et al.*,[5] allowing both rapid total and slower very partial degradation. This makes it an ideal model approach for covering all the levels of degradation encountered by both the biological catalysis community (with low to very low levels), and the chemical catalysis community (with generally much higher levels). Finally, we hope this study will serve as a common base for the scientific community in the study of PBPAC depolymerization, helping in the choice of methods according degradation method selected.

Experimental

Materials

Polybisphenol A carbonate (PBPAC) was purchased from Merck and its mass-average molecular weight is approximatively 45,000 $g.mol^{-1}$ according to the manufacturer datasheet. Bisphenol A (BPA) 99 %, trans-*2*-[*3*-(*4*-tert-Butylphenyl)-*2*-methyl-*2*-propenylidene]malononitrile (DCTB) > 98 % and sodium trifluoroacetic acid (NaTFA) 98 % were purchased from Sigma. THF 99.6 % stabilized with BHT was purchased from Thermo Fisher Scientific. Methanol LC-MS grade > 99.9 % was purchased from Honeywell. All the chemicals were used without any further purification.

PBPAC methanolysis

The protocol used in the study is similar to the one proposed by Bhogle *et al.*[5] For complete depolymerization, in 8 mL glass vials, 30 mg of PBPAC are added (for a final concentration of 5 $mg.mL^{-1}$) in 6 mL of a THF:MeOH mixture (2:1 v/v). NaOH was added in catalytic quantity (3 mg). Controls were made in the absence of NaOH and MeOH. Samples were placed in a Bandelin Sonorex

ultrasonic bath (35 kHz) at room temperature for 1 h. The reaction was stopped by precipitating the polymer and inactivating the base in an acid bath (HCl 0.15 %). The reaction products were purified by evaporation of the solvent using miVac Quattro (Genevac) at 50 °C until total evaporation (about 1 h). For kinetic monitoring of partial depolymerization, 30 mg of PBPAC were added to 8 mL vials (for a final concentration of 5 mg.mL^{-1}) in 6 mL THF. 0.5 eq of MeOH were added (4,8 µL) and the samples were placed in a Bandelin Sonorex ultrasonic bath (35 kHz) at room temperature for 2 h. A 400 µL sample was withdrawn after 5 min, 15 min, 30 min, 1 h and 2 h. For each sample, the reaction was stopped by precipitating the polymer and inactivating the base in an acid bath (HCl 0.15 %). The reaction products were dried by evaporation of the solvent using miVac Quattro (Genevac) at 50 °C until total evaporation (about 1 h). The blanks are done in the same conditions as previously described. However, without the addition of methanol and NaOH, the balance of the reaction volume is completed with THF.

Size Exclusion Chromatography (SEC) analyses

SEC chromatograms were recorded on a Shimadzu HPLC-IR liquid chromatograph, equipped with an LC-30AD SP pump, a SIL-20AC TH autosampler, a CTO-20A column oven coupled with an SPD-20A UV detector. Analyses were performed using a GPC KF-805L column. THF HPLC grade was used as isocratic elution solvent, with a flow rate of 1 mL.min^{-1}, an acquisition time of 14 min, and the polymer detection was performed at 260 nm. The temperature of the column oven was set at 30 °C. 1 µL of polymers solution in THF (5 mg.mL^{-1}) were injected into the column. Calibration was done using Polystyrene standard with theoretical Mn of 2000 g.mol^{-1}, 5000 g.mol^{-1}, 9000 g.mol^{-1}, 17500 g.mol^{-1}, 30000 g.mol^{-1} and 50000 g.mol^{-1} purchased from sigma.

Fourier Transform Infrared (FT-IR) analyses

FT-IR spectra were recorded on a Fourier Transform Infrared (FT-IR) spectrometer (Tensor 37, Bruker), equipped with an HTS-XT compartment for use with multi-well plates. The device is equipped with a pyroelectric detector (DTGS) operating in transmission mode. Spectra were recorded by accumulating 32 scans with a resolution of 4 cm^{-1}. 8 µL of samples were deposited on a silicon multi-well plate and evaporated before analysis.

Matrix Assisted Laser Desorption Ionization - Time of Flight (MALDI-TOF) analyses

MALDI-TOF mass spectrometry analyses were performed on an Autoflex Speed™ (Bruker Daltonics). The molecular mass measurements were performed in the range of 1000 to 30000 m/z and in automatic reflectron mode using FlexControl™ 3.4 software. The equipment parameters were as follows: voltage values of ion sources #1 and #2 set as 19.00 and 16.95 keV, respectively; voltage values of reflectrons #1 and #2 set as 21.00 and 9.50 keV, respectively; lens tension 7.70 keV; pulsed extraction 450 ns; laser intensity between 70 and 80 %; smartbeam parameters set to ultra and sample rate; and digitizer settings set to 0.50 GS/s. The MS signals were acquired by summing 5,000 laser shots per spectrum. Prior to each analysis, the spectrometer was calibrated using PBPAC solution with the peaks corresponding to the cyclic polymer for DP 8, 10, 11, 12, 13, 14, 15, 25 with the respective masses (g.mol^{-1}) of 2057.23, 2565.79, 2820.07, 3074.35, 3328.63, 3582.91, 3837.19, 6379.99. Samples were prepared by mixing 6 µL of polymer solution at 5 mg.mL^{-1} in THF with 20 µL of a 20 mg/mL DCTB matrix in THF and 2 µL of NaTFA solution at 6.8 mg.mL^{-1} in THF and 1 µL was then spotted on a Polished Steel 384 MALDI target (Bruker). Mass spectra were visualized using FlexAnalysis software (version 3.4; Bruker) and Polytool software (version 1.18; Bruker)

Nuclear Magnetic Resonance (NMR) analyses

The 1H spectra were recorded at room temperature in $CDCl_3$ on a Bruker Advance 300 spectrometer (Bruker). Coupling constants were measured in Hertz (Hz) and multiplicities for ^{1}H NMR coupling were presented as s (singlet), d (doublet), t (triplet) and m (multiplet). Chemical shifts are reported relative to the sodium trimethylsilylpropionate reference.

Figure 3 (PBPAC): ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 7.25 (d, J_3 = 8.9 Hz, J_4 = 2.2 Hz, 4H, $H_{1,3,9,13}$), 7.16 (d, J_3 = 8.9 Hz, J_4 = 2.2 Hz, 4H, $H_{4,6,10,12}$), 1.68 (s, 6H, $H_{15,14}$).
Figure 7 (BPA): ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ = 7.09 (d, J_3 = 8.8 Hz, J_4 = 2.2 Hz, 4H, $H_{1,3,11,15}$), 6.72 (d, J_3 = 8.9 Hz, J_4 = 2.2 Hz, 4H, $H_{44,6,12,14}$), 4.61 (s, 2H, $H_{16,17}$), 1.68 (s, 6H, $H_{15,14}$).

High-Pressure Liquid Chromatography with Diode Array Detector (HPLC-DAD) analyses

HPLC-DAD liquid chromatograph from Shimadzu equipped with diode array detector (DAD) SPD-M20A (λ=190-800 nm) and binary pumps LC-30AD were used to detect Bisphenol A. Products separation was carried out using a Luna® Omega (Phenomenex) 1.6 µmx150x2.1 mm Polar C-18 100 Å column, operated at 22 °C. For elution, 2 mobiles phases, composed of respectively water with 0.1 %vol. formic acid (phase A) and acetonitrile with 0.1 % formic acid (phase B), were mixed according to the following table. Concentration Injection volume was of 1 µL of sample solution prepared in water/acetonitrile:75/25 (vol./vol.), and products detection was carried out at λ = 277nm. BPA concentrations in the samples were calculated from a calibration using BPA solutions in water/acetonitrile:75/25 (vol./vol.), at concentrations of 16 mM, 8 mM, 4 mM, 2 mM and 1 mM.

Solvent flow control during analysis

Time (min)	% H_2O	% ACN
0	90	10
5	50	50
7	50	50
8	90	10
10	90	10

Data analysis

The limits of detection (LOD) were measured from calibration data according to the formula:

$$LOD = 3\sigma_0/S$$

With σ_0 the standard deviation at the origin and S the slope of the calibration curve.
The FT-IR relatives' errors in percentage are calculated according to the formula:

$$RE = \frac{\sigma}{R_A} * 100$$

With σ, the standard deviation and R_A the ratio of the area ratio of the pics.
Calculation of the degree of polymerisation (DP) by FT-IR:

$$DP = \left(\frac{1}{[BPA]_{eq\ PBPAC}}\right) + 1$$

With $[BPA]_{eq\ PBPAC}$ the concentration of BPA in molar equivalent of polymer repeating unit.

Results and discussion

Analysis of raw PBPAC

To start our study, we focused on the two extremes of PBPAC degradation, i.e. no complete conversion and total conversion, so that we could use them as reference points. In cases where no degradation has taken place, this obviously comes back to the study of the raw polymer. We consequently started with the SEC analysis of the latter as it allows for the determination of the molecular weight distribution and by extension the calculation of average molecular weights. In the case of the PBPAC studied, the starting polymer was eluted at 8.5 min in our conditions, with a chain distribution between 7.8 and 10 min (Figure 1). The average molar masses of the polymers could be estimated using a polystyrene standard to calibrate the measurement (Figure S1), although this is only an estimate as the separation power and retention time in SEC depends on the hydrodynamic volume of the polymer, which can be different between the two studied here. According to polystyrene calibration, the Mw of PBPAC was found of 47000 $g.mol^{-1}$, with a Mn of 31000 $g.mol^{-1}$, a polydispersity of 1.51 and a molecular weight at the highest point (Mp) of 42000 $g.mol^{-1}$. These results are similar to those given by the supplier, with a theoretical Mw of 45000 $g.mol^{-1}$. It is important to note that the mass separation profile of the column used is logarithmic, so as mass increases, mass accuracy decreases. Mp measurement uncertainties for PS standard values of 2500 $g.mol^{-1}$, 5000 $g.mol^{-1}$, 9000 $g.mol^{-1}$, 17500 $g.mol^{-1}$, 30000 $g.mol^{-1}$ and 50000 $g.mol^{-1}$ were measured to be of 59 $g.mol^{-1}$, 120 $g.mol^{-1}$, 206 $g.mol^{-1}$, 375 $g.mol^{-1}$, 884 $g.mol^{-1}$ and 1800 $g.mol^{-1}$ respectively (Figure S2). It is therefore important when interpreting the results to take into account this phenomenon, which obviously depends on the column and elution conditions, and results in variable uncertainty in the absolute measurement of average molar masses. This is why we think this type of error determination should be carried out and described systematically for each new case study, contrary to what is most often done at present. In complement, the liquid fraction of the prepared polymer suspension was also analyzed in HPLC to detect the potential presence of free BPA monomer, or additional additives, but none could be detected under the analytical conditions used (Figure S3). This demonstrates the absence of residual monomer, an important parameter for the study of monomer release in subsequent polymer degradation analysis.

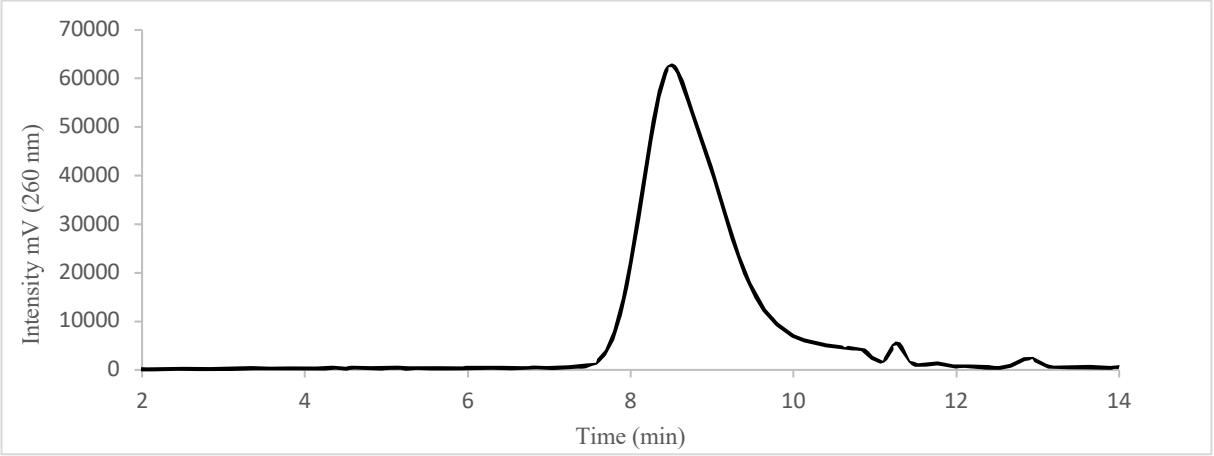


Figure 1 : Typical chromatogram obtained by SEC for PBPAC in standard conditions

Then, in order to fully characterize the polymer, and more especially its composition (chemical groups) in order to detect any potential subsequent modification, PBPAC was analyzed using three spectroscopic methods, starting with infrared spectroscopy, the spectrum of which is shown in Figure 2. The peaks between 3050 cm^{-1} and 2850 cm^{-1} correspond to the stretching of the C-H bonds of alkanes and alkenes, while the stretching of the carbonyl of the carbonate function is present at 1777 cm^{-1}. The peak at 1506 cm^{-1} is assigned to the stretching of the C=C bonds of aromatic rings. The peaks between 1350 cm^{-1} and 1100 cm^{-1} can be assigned to several functions, notably the stretching of the C-O-C bond of the carbonate bond at 1194 cm^{-1}, as well as a contribution from aromatic ring bonds.[16] Proton NMR spectroscopy provides further support for this identification. The spectrum obtained from the dissolution of our PBPAC sample in $CDCl_3$ is shown in Figure 3. The constituent monomer is composed of 14 protons, 6 belonging to the methyl groups and 8 to the aromatic rings. The former appears as a singlet at 1.68 ppm, which integrates for 6 protons. Regarding the chemical shift of aromatic hydrogens, it is found between 7.30 and 7.10 ppm, as doublet of doublet with a roofing effect, for which a coupling in J_4 can be observed. The first doublet at 7.16 ppm integrates for 4 protons and is attributed to the protons in *ortho* of the carbonate bond. The second doublet at 7.25 ppm is for his part assigned to the protons in *meta* of the carbonate group and integrates for 4.2 protons. Considering the overlapping between the latter and the $CDCl_3$ peak (singlet at 7.26 ppm), this may explain the slightly higher integration value than expected. The *ortho* and *meta* coupling constants are shown in Figure 3. These attributions are in line with those proposed by Kim *et al*.[17] As a result, FT-IR and ^1H NMR here form a complementary pair, allowing the observation of all the chemical functions available *i.e.* the carbonate bond, the aromatic carbons and the aromatic and methyl protons. NMR analysis of the carbons can be considered to obtain an even finer analysis of the polymer, as proposed by Kim *et al*., but this addition is not necessary since carbon's most valuable information is given by infrared.[17] These two methods only allow us to characterize the repeated units of the polymer, present in high concentration, and thus do not give access to the end groups, which are essential

elements to be determined with a view to potential degradation of the polymer, particularly with a mechanism in *exo*. In the case of our PBPAC, from the Mn estimated by SEC, it is possible to calculate the concentration of end groups to be close to 0.02 per repeating unit, which is effectively too low to be analyzed by conventional ^{1}H NMR with the 300 MHz magnetic field to which we have access.[17]

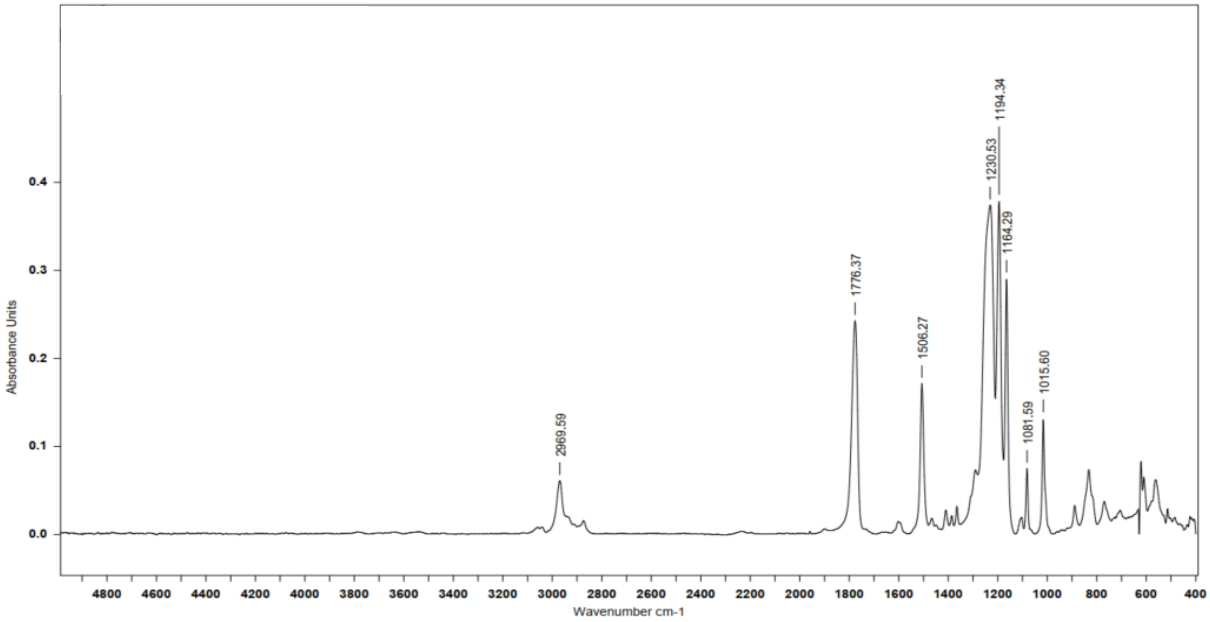


Figure 2: Fourier transform infrared (FT-IR) spectra of PBPAC, acquisition done in transmission.

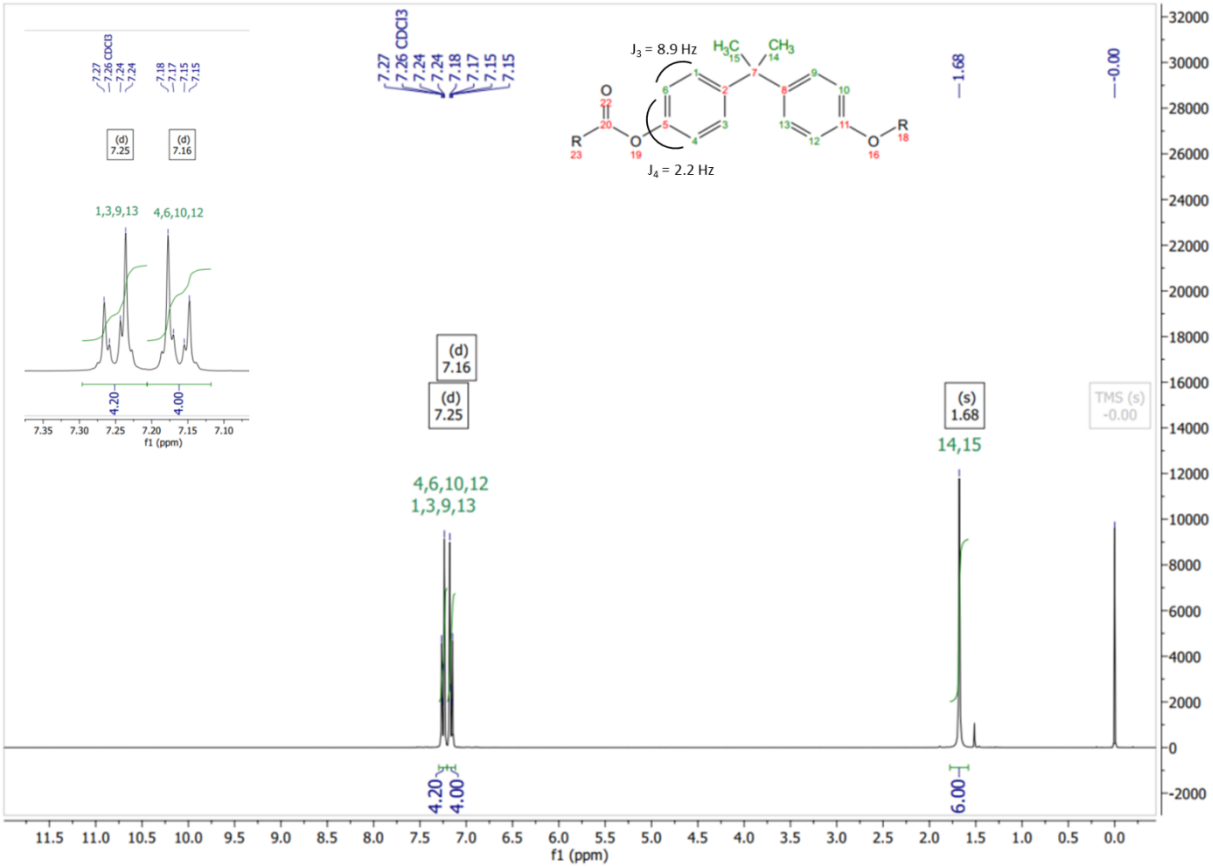


Figure 3: PBPAC ^{1}H NMR spectrum in $CDCl_3$ (300 MHz).

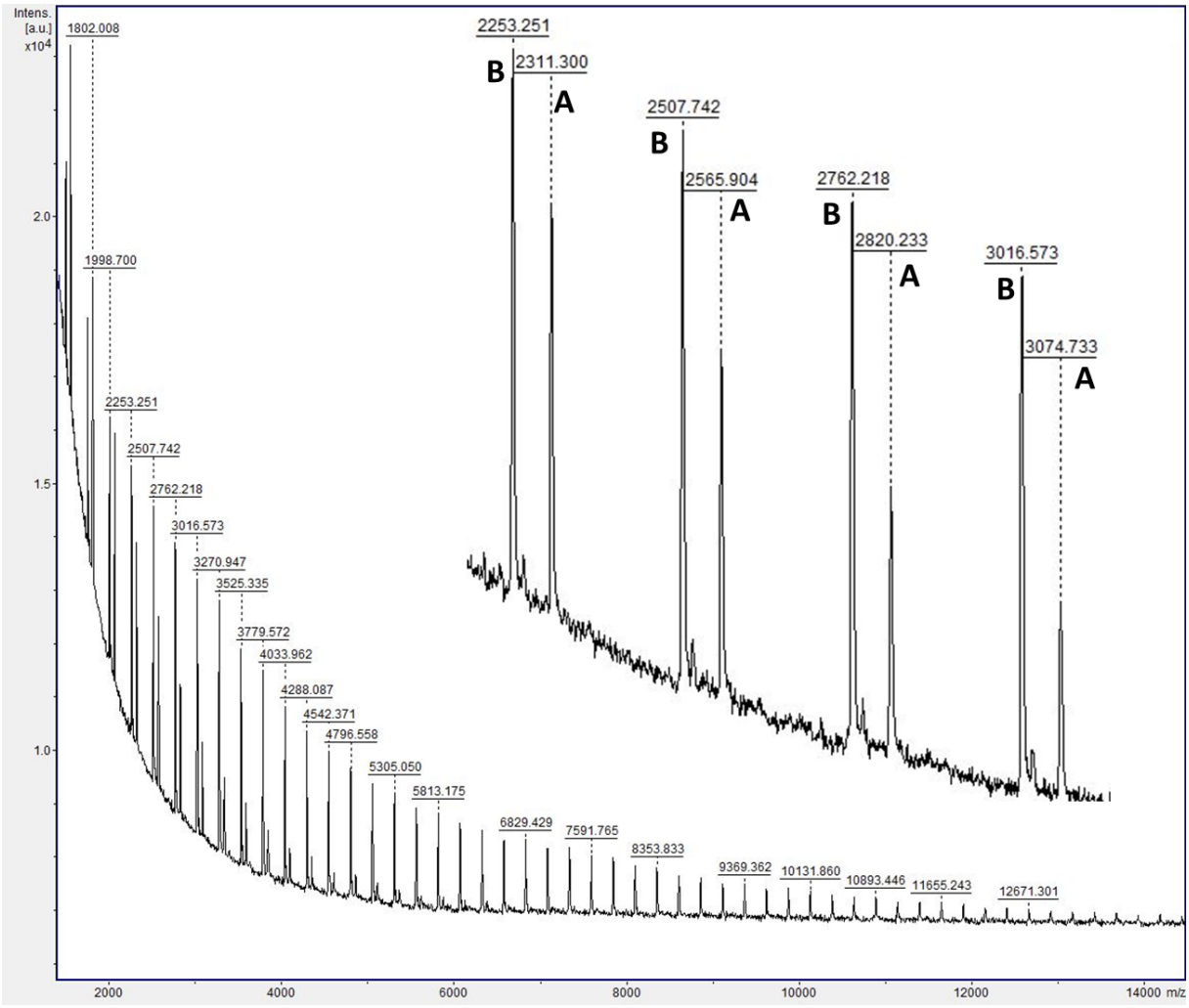


Figure 4 : MALDI-TOF spectrum of PBPAC with detailed view of degree of polymerization ranges 8 to 12 highlighting the two series A and B.

MALDI-TOF mass spectroscopy provides an answer to this need, by measuring the exact mass of the polymer chains and thus extracting detailed information about the masses of the repeating unit and the ends groups. The MALDI-TOF spectrum obtained with our PBPAC is shown in Figure 4.

It is important to remember that in the analysis of synthetic polymers, ionization is generally not achieved by direct excitation of the analyte, but requires the presence of a cationization reagent, usually a Lewis acid metal cation, which reacts with the soft Lewis base of polymers such as double bonds or phenyl rings to form an $[M+cation]^+$ adduct.[18] To takes into account the mass of these adducts and groups the peaks into series, based on the mass difference between the peaks, giving a linear function, the spectra were analyzed using Polytool1.18 software, which has been developed in this objective. In the PBPAC spectrum, two series of peaks can be detected, separated by the molar mass of the repeating unit (254.28 $g.mol^{-1}$), identified as the "A" and "B" series. The constant term of this affine function represents the residual molar mass of the adduct, *i.e.* the molar mass of the cation (Na^+) added to the molar mass of the end groups. The molar masses of the end groups calculated by Polytool1.18 for the A and B series are close to 0 and 196 $g.mol^{-1}$ respectively, and represent 38 % and 62 % of the polymer peak intensity. Series A therefore corresponds to cyclic PBPAC, as there is no additional end-group. The end groups in the B series can be identified as two *4*-cumylphenol units, with a molar mass of 450.6 $g.mol^{-1}$. However, Polytool1.18 groups the peaks according to the repeating unit, in which case a polymer of size *n* made up of these end groups is considered by Polytool1.18 as a polymer of size n+1, giving an end group with a molar mass of 196.3 $g.mol^{-1}$. Note that the presence of the monomer *4*-cumylphenol is expected, as it is a capping agent commonly used to stop the polymerization reaction.[9] Secondly, it is important to point out that the mass range taken into account by Polytool1.18 for spectra processing has an impact on the values returned. Indeed, the instrument is calibrated on the first 6000 Da of the spectrum, beyond which peak intensity is too low to be used for accurate calibration with Flex Control software in our conditions. When the spectra are processed over the calibrated range, on 20 samples, the average molar mass of the end groups is 196.49 ± 0.17 $g.mol^{-1}$. When the whole spectrum is then taken into account by polytool1.18, a shift in masses is observed, with the average molar mass of the end groups being 194.45 ± 0.32 $g.mol^{-1}$. Finally, when only the peaks that are beyond the calibrated range are considered, the shift is even greater, with an average end group molar mass of 193.08 ± 0.43 $g.mol^{-1}$, which shows how they effectively influence the calculated mass. It is therefore necessary when developing a polymer MALDI-TOF analysis method to precisely define the calibrated m/z range, a parameter which is often not specified,[19–21] and to focus mass analysis only on the latter. Finally, it is important to note that the mass range analyzed here by MALDI-TOF is lower than the molar mass distribution of the polymer, in fact only masses below 15000 m/z can be detected. In consequence we must state that

the results obtained are only partial. Nonetheless, it is widely accepted in the literature that high masses are discriminated in favor of low masses in MALDI mass spectrometry, particularly in the case of polymers with high degree of polymerization (DP) and polydispersity greater than 1.2 or 1.5.[22–24] As a result, high masses fall outside the instrument's analysis range, and quantification of the final groups based on peak intensity can only be partial, which remains one of the main limitations of this technique.

In conclusion, the analysis of raw PBPAC identified characteristic polymer responses for each analytical method, with results similar to those described by the supplier or in other literature studies. This can serve as a basis for our next steps, with any change in the responses obtained being taken as an indication that degradation has taken place, the most expected ones being a shift in the molecular weight distribution observed in SEC, as well as the appearance of new peaks in FT-IR, ^{1}H NMR and MALDI-TOF.

Total methanolysis of PBPAC

In opposition to the previous analysis, we have also characterized the complete depolymerization of our PBPAC to confirm that the degradation products are only bisphenol A (BPA) and dimethyl carbonate in the case of methanolysis as performed here. We should precise that for obvious reasons, the complete degradation of the polymer to CO_2 will not be discussed here as our conditions shall not lead to such drastic decomposition. Total degradation could then be achieved by methanolysis under conditions similar to those described by Bhogle *et al*. and the depolymerization results were compared with those of the monomer BPA.[5] First, we shall state that the release of dimethyl carbonate could not be observed as it is evaporated at the same time as the solvent during the post-treatment of our degradation sample before analysis. First SEC analysis of the resulting species is shown Figure 5.A and when compared with that of the polymer it shows a complete disappearance of the PBPAC chain distribution, in exchange for a narrow peak at 11.04 min. This retention time is equal to the one found for the BPA monomer (Figure S4), suggesting complete depolymerization in our conditions. However, under our analytical conditions, SEC is not sufficiently resolutive to confirm the existence of a single species represented by this single peak, nor that it actually corresponds to BPA. In fact, BPA dimers or even trimers may exhibit the same retention time and so cannot be discriminated here. To perform this confirmation, PBPAC methanolysis product was then analyzed by HPLC. Figure 5.B shows the chromatogram of the detected products, with a single peak observed at 6.59 min that corresponds to the BPA retention time (Figure S5), and that is not present in the blank reaction where no methanolysis occurred. This is in accordance with Bhogle *et al*., where the two main products being effectively BPA and dimethyl carbonate.[5]

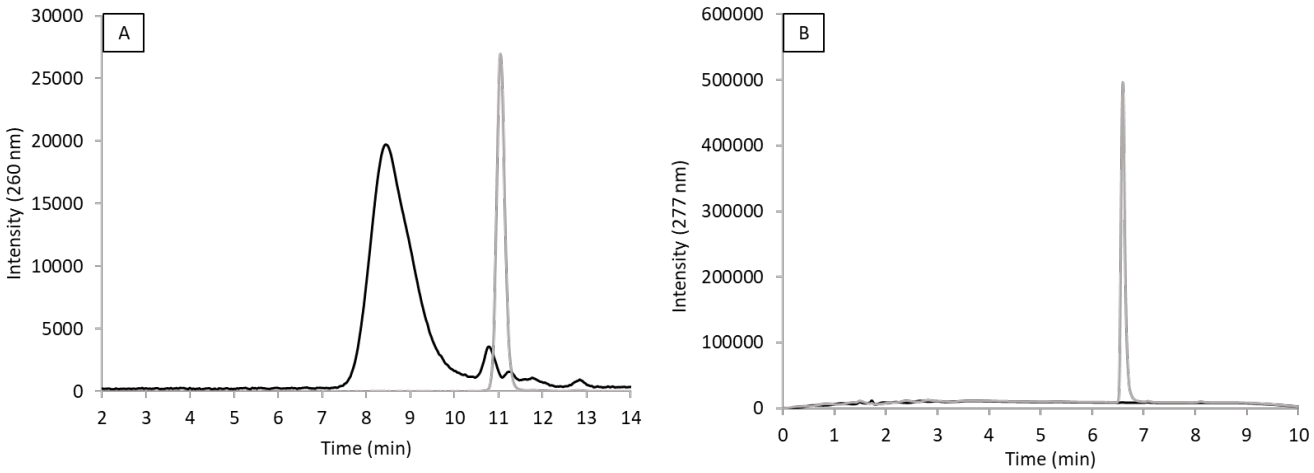


Figure 5: A - In grey, SEC chromatogram of total methanolysis products, in black, non-degraded PBPAC chromatogram. B - In grey, HPLC chromatogram of total methanolysis products, in black, non-degraded PBPAC chromatogram.

To finally confirm the structure of the BPA formed, we nonetheless analyzed the methanolysis product with FT-IR. Figure 6 shows the spectra obtained for BPA and the reaction product. When comparing the two, it is found that the spectrum of the methanolysis product is very similar to the one of pure BPA, with the appearance of peaks between 3600-3200 cm^{-1} and 1600 cm^{-1} and the disappearance of carbonate bond peaks at 1777 cm^{-1} and 1194 cm^{-1}, confirming the total depolymerization of the polymer. In addition, the spectrum of BPA differs from that of the non-degraded polymer (Figure 2) by the appearance of a broad peak at 3600-3200 cm^{-1} corresponding to the stretching of the hydroxyl bond of BPA and the appearance of a double peak centered around 1600 cm^{-1}, which can be attributed to the stretching of the alkene bonds.[25]

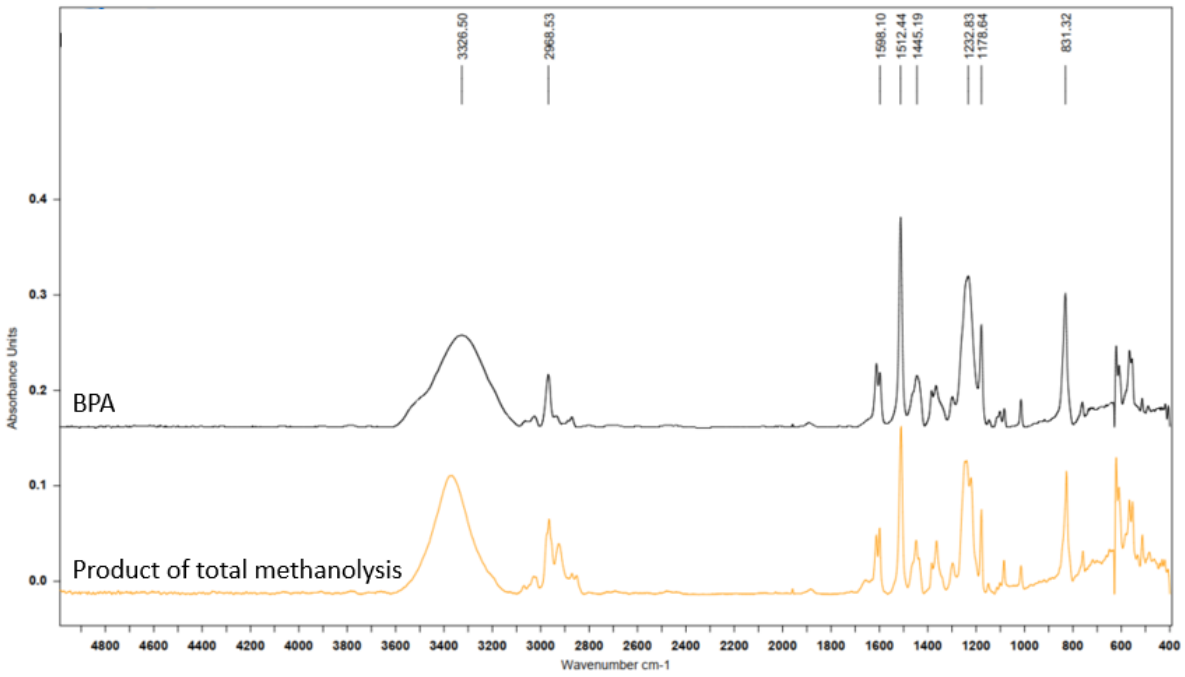


Figure 6: Infrared spectrum of the product of a complete PBPAC methanolysis in orange and pure BPA in black, obtained in transmission

Following the same logic as previously, ^{1}H NMR was used to further identify the chemical groups of the depolymerization products. The resulting spectrum is shown in Figure 7 and compared with that of BPA (Figure S7). Once again, the two spectra are very similar, with a singlet at 1.62 ppm integrating for 6 protons and that can be assigned to the two methyl groups of BPA. A singlet at 4.61 ppm integrating for 2 protons (located at 4.73 ppm for the reference BPA) can then be assigned to the hydroxyl group protons. Finally, the doublet of doublet corresponding to the aromatics protons with a roofing effect can be observed between 6.65 and 7.15 ppm. The first doublet centered at 6.72 ppm integrates for 4 protons and can be assigned to the protons in *ortho* of the alcohol group, while the doublet centered at 7.08 ppm is assigned to the protons in *meta* and also integrates for 4 protons as expected. The respective coupling constants are shown in Figure 7. As a result, the ^{1}H NMR spectrum of BPA can be distinguished from that of PBPAC by the stronger shielding effect of the monomer protons and the appearance of the singlet corresponding to the hydroxyl group protons at 4.7 ppm. These changes in the NMR and IR spectra can be considered as effectives markers to monitor depolymerization. To complete the analytical panel, the methanolysis product was finally also analyzed by MALDI-TOF, but BPA could not be detected, as expected, its mass falling in the mass range where matrix peaks are located. However, the combination of the other analytical methods presented here enables efficient confirmation of total depolymerization, and the identification of the methanolysis products, the latter being reduced, as expected, to bisphenol A alone.

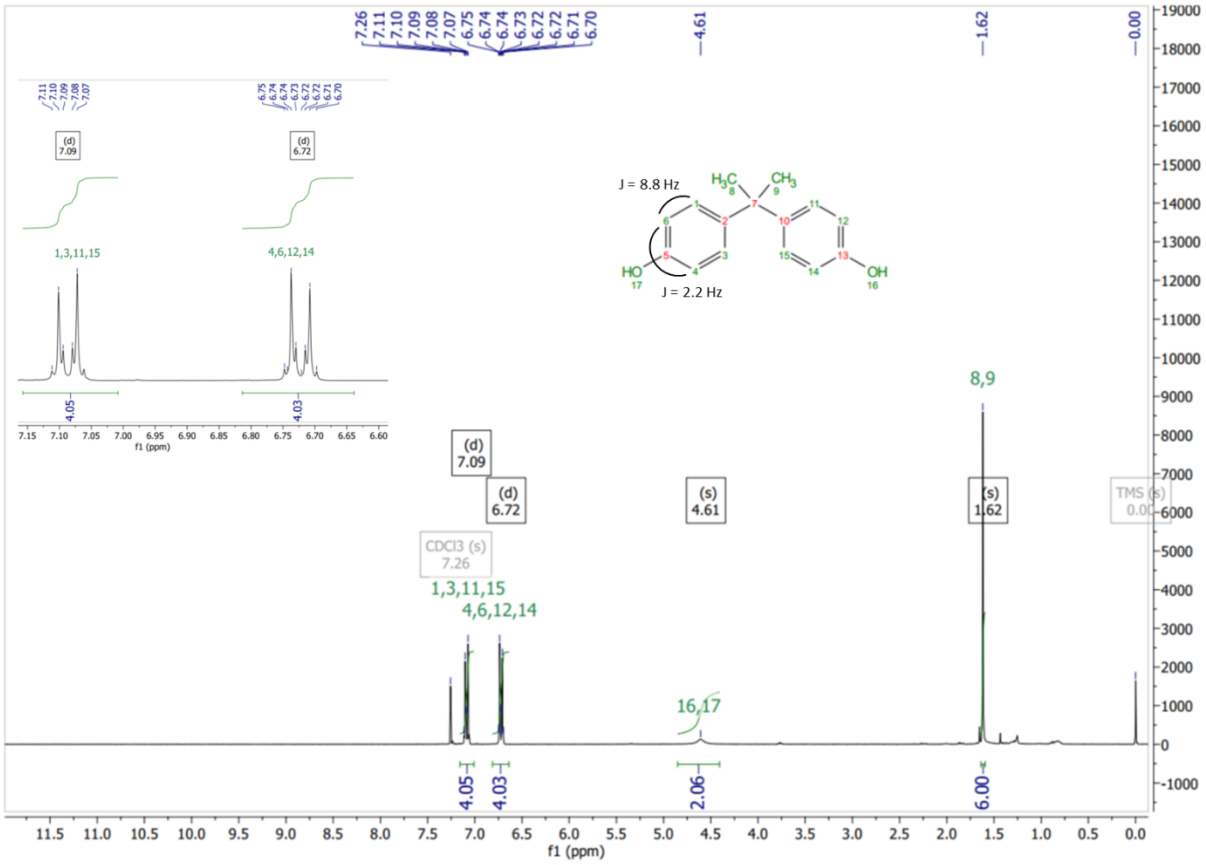


Figure 7 : BPA ^{1}H NMR spectrum in $CDCl_3$ from complete methanolysis of PBPAC (300 MHz).

Partial methanolysis of PBPAC

Having studied the two cases at the extremes of the degradation spectrum, the characterization of the different levels of partial polymer degradation was then carried out. These are, in fact, the most representative cases of what can be obtained in the case of catalytic degradation of PBPAC, particularly in the case of biodegradation, which is generally very limited. As such, the main question we sought to answer here was to determine the sensitivity and limit of quantification of each analytical method, in order to best define which one can be used for which type of catalyst/depolymerization stage. For this, the kinetic monitoring of depolymerization was performed, by adjusting the concentration of methanol and polymer in the reaction medium, to obtain a measurable progression of degradation over several hours. As before, we began by characterizing the degradation products by SEC. The resulting chromatograms obtained in these conditions at t = 0, 2, 5, 15, 30 and 120 min are shown in Figure 8 and the description of the molecular weight distribution of the associated depolymerization products are presented in Table 1.

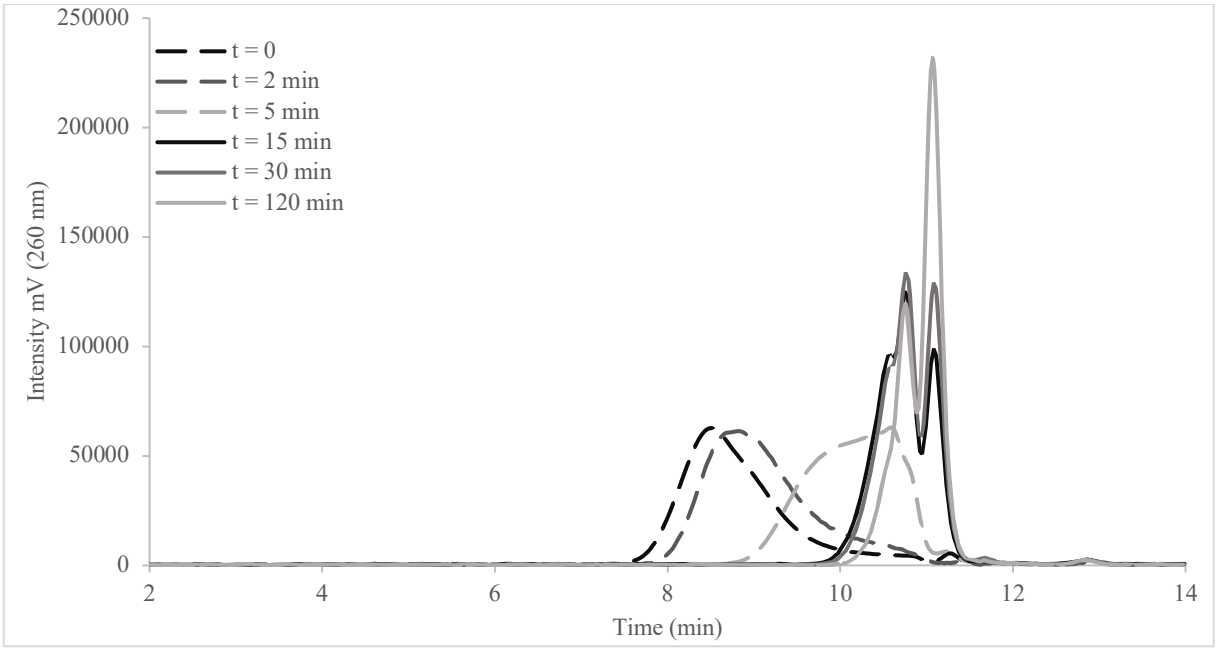


Figure 8 : Superposition of SEC chromatograms of partial methanolysis of PBPAC obtained from samples taken at t = 0, 2, 5, 15, 30 and 120 min.

After 2 min of reaction, the molecular mass distribution is eluted between 8 and 11 min, compared with 7.5 and 10 min initially found for the raw polymer, meaning that the average molar mass of PBPAC has slowly decreased, from approximately 32000 g.mol^{-1} to 23000 g.mol^{-1}. After 5 min of reaction, the molecular mass distribution is eluted between 9 and 11 min, indicating a further reduction in mass, reaching approximately 4000 g.mol^{-1}. After 15 min, elution times ranged from 9.7 min to 12 min. Finally, the 30 min and 2 h samplings lead to a similar mass distribution, ranging from 10 min to 12 min, reaching approximately 1000 g.mol^{-1}. The exact values are presented in the Table 1.

Table 1 : Molar mass distribution obtained from SEC monitoring of PBPAC kinetic partial depolymerization

Time	Mn (g.mol^{-1})	Mw (g.mol^{-1})	Mp (g.mol^{-1})	Average degree of polymerisation	% of degradation
0	31716 ± 651	47366 ± 107	42113	125 ± 2,5	0
2 min	22792 ± 374	36503 ± 32	26235	90 ± 1,4	0,39
5 min	3635 ± 913	8046 ± 1789	1632	14 ± 3,6	7,72
15 min	1570 ± 178	1932 ± 213	1375	6.2 ± 0,7	19,20
30 min	1280 ± 128	1584 ± 167	1210	5.0 ± 0,5	23,79
2 h	925 ± 88	1067 ± 135	747	3.6 ± 0,3	33,28

Interestingly, the elution profile is found to slowly shift from the classical mass distribution centered around the average DP, towards a massif with two main peaks (at 10.76 min and 11.09 min respectively), reflecting the appearance of two majority species, or families of species. Note that, as observed before, the peak that elutes at 11.09 min is close to the retention time of BPA. However, for the same reason as mentioned previously, the products formed cannot be confirmed based on SEC analysis only. Also, after 30 min, it is no longer the elution time that varies, but only the width and intensity of these two peaks, showing that degradation seems to converge towards these two majority species in the allotted time. It should be noted, however, that it is the peak at 11.09 min that is increasing the most, and thus seems to be benefiting most from the continuing degradation as it can be expected from our previous findings with total methanolysis. Also, regarding the peak at 10.76 min, it should be noted that as its intensity seems to vary very little, it could suggest either the creation of a transition species, which is consumed at a rate equivalent to that of its formation, or a species produced at a given moment in the de-polymerization process and which proves to be fairly stable over the measurement time. However, given that the methanolysis conditions have been modified very little, and that in the case of the total methanolysis only the peak at 11.09 min was again observed at the end of the reaction, we can logically assume that this transitory peak would effectively disappear if the deterioration were allowed to continue over a longer period. Interestingly too, the absence of any low-mass peaks before 10 min of degradation suggests that the depolymerization mechanism is most probably *endo* and not *exo*, which supports the previous hypothesis, the peak at 10.76 min being probably an oligomer, or family of oligomers, of a specific polymerization degree that serves as an intermediate species towards more complete degradation into BPA. This hypothesis can be confirmed by the expected increase in polydispersity measured in the first few minutes, reflecting the random cleavage of polymeric bonds. Indeed, the increase in polydispersity rose from 1.5 to 1.6 after 2 min, then to 2.2 after 5 min. From this, we can then calculate the percentage of depolymerization, since the method for calculation

effectively depends on the type of cleavage. In our case, the depolymerization percentage (1) corresponds then to the number of polymeric bonds cleaved (2) compared to the number of polymeric bonds in the initial polymer.

$$(1)\quad \%_{Depolymerisation} = \frac{n*100}{P_0}$$

$$(2)\quad n = \frac{P_0}{P} - 1$$

For the calculation of the number of cleaved bonds, the Mn of the polymer was taken into account. The results obtained for the depolymerization percentages calculation are listed in **Erreur ! Source du renvoi introuvable.**. As it can be observed, the percentage of degradation increases from just 0.4 % after 2 minutes of reaction, to 33 % after 2 hours. This indeed correlates to the decrease in Mn and Mw. In addition, the example shows that in the case of *endo* degradation, from as little as 0.4 % depolymerization, SEC is able to confirm that degradation has taken place. Using the results presented in Table 1, the depolymerization detection limit can then be calculated to 0.06 %, which makes SEC an extremely sensitive analytical technique in our conditions. It then appears to be an indispensable analytical method for confirming that degradation has taken place, especially when the percentage of degradation is low, and for determining changes in MWD during depolymerization. However, as in the case of total depolymerization, SEC still struggles to identify the products formed, and in our case, also the transitional species.

To help identifying the latter, the partial methanolysis products were then analyzed by MALDI-TOF (Figure 9). Keeping in mind that the response of each polymer to ionization is not known, absolute quantification of the different series in the analyzed sample cannot be possible. The spectrum at t = 0 min shows the presence of two series of peaks named A and B, corresponding respectively to the cyclic polymer and the presence of *4*-cumylphenol end group (linear polymer), as described before. The profile of the spectrum at 2 min is similar to the initial time, with the main difference being the appearance of a series of peaks, with a measured mass of residual units of 212 g.mol^{-1} – C series. After 5 minutes of reaction, this profile evolves with the almost complete disappearance of the B series peaks and the appearance of three new series of peaks, named D, E and F, with measured residual unit masses of 32 and 90 and 228 g.mol^{-1} respectively. From 15 min the almost complete disappearance of the A series can be observed, with the C, D and E series representing now the majority of the peaks detected. The spectrum profile of the samples taken at 30 min is rather similar to that at 15 min. Finally, for the 120 min sample, the disappearance of peaks above 2000 m/z is clearly observed. This confirms the results obtained by SEC *i.e.* the presence in the sample of only small-sized oligomers. In this case, the degree of polymerization is calculated to less than 7. In order to identify these end peak series with their corresponding end groups, it is first worth remembering that methanolysis is carried out by the action of methanolate in the form of a double transesterification at the carbonate level. This can lead first to the formation of a hydroxyl end group and methyl carbonate followed by formation of a second hydroxyl end-groups. The molar masses of these expected structures are as follows: 212.1 g.mol^{-1} for PBPAC with end groups that are composed of a *4*-cumylphenol unit and a hydroxyl group (corresponding to series C here); 228.1 g.mol^{-1} for PBPAC with two hydroxyl end groups (corresponding to series D here); 32.0 g.mol^{-1} for a polymer with hydroxyl and methyl carbonate end groups (series E); and 90.0 g.mol^{-1} for PBPAC with two methyl carbonate end groups (series F). The corresponding product structures are shown in Table 2. The correspondence between the masses of the measured and theoretical end groups clearly demonstrates MALDI-TOF efficiency for oligomers' structure determination, and more especially end groups. When plotting peak area for each peak series in function of time now (Figure 10, sum of the area of all the peaks in a given series divided by the sum of the areas of all the series at a given time), it is possible to estimate the degradation rate evolution, but it also allows to identify which type of depolymerization has occurred. At 2 min, the SEC chromatogram (Figure 8) shows that Mn has decreased from 32 000 g.mol^{-1} to 22 000 g.mol^{-1} while at the same time Figure 9 shows that series A and B make up the majority in the sample. We can therefore conclude that the cleaved polymer chains make up only a small part of the mixture (10 %). The concordance of this information with the significant decrease in Mn leads us to believe once again that this is very likely a case of an *endo* degradation. Additionally, at this point we can also observe a decrease in the relative intensity of the series B (linear polymer) compared to series A (cyclic polymer). The direct consequence of this is an increase in the relative intensity of the series A from 0 to 2 min, as A and B make up the majority of the composition. This therefore seems to indicate a preferential depolymerization of the linear polymer over the cyclic polymer. This preference seems to be confirmed by the almost total disappearance of the series B peaks after 5 minutes, whereas series A only disappears after 15 min. This is further confirmed by the rapid appearance of the peaks of series C at 2 min without the appearance of series E. Indeed, when trans-esterifying polymers of series B, we end up with series C population, on the side where the hydroxyl group is released. We shall note that the other population formed, with a *4*-cumylphenol chain end and a methyl carbonate, with a theoretical residual unit mass of 270.1 g.mol^{-1}, was not observed under our conditions, even though we cannot yet explain why. Conversely, when the cyclic polymer (series A) is trans-esterified, we obtain series E as the first intermediate, with a hydroxyl group and a methyl carbonate at the chain ends. More generally, the combined appearance of the series D, E and F and the gradual disappearance of the cyclic polymer confirm the overall depolymerization of the polymer. In addition, the presence in equivalent proportions of the series D, E and F at the end of the reaction confirms the complexity of the mixture previously analyzed by SEC, and validates the impossibility of using SEC to identify the diversity of polymers present in the sample unlike MALDI mass spectrometry. Moreover, the appearance of signals attributed to the reaction intermediates as early as 2 min, accompanied by a decrease in the signals corresponding to the raw polymer, suggests that the detection limit for confirming depolymerization by MALDI-TOF under these conditions can be estimated at 0.5 % degradation, by correlation with SEC results. Therefore, despite the fact that it is not directly quantitative, MALDI-TOF is clearly a key analytical method for studying the depolymerization of PBPAC, and more generally of many polymers, in order to identify decomposition intermediates, especially as its use is now adaptable to high throughput, as we recently demonstrated for another application.[26]

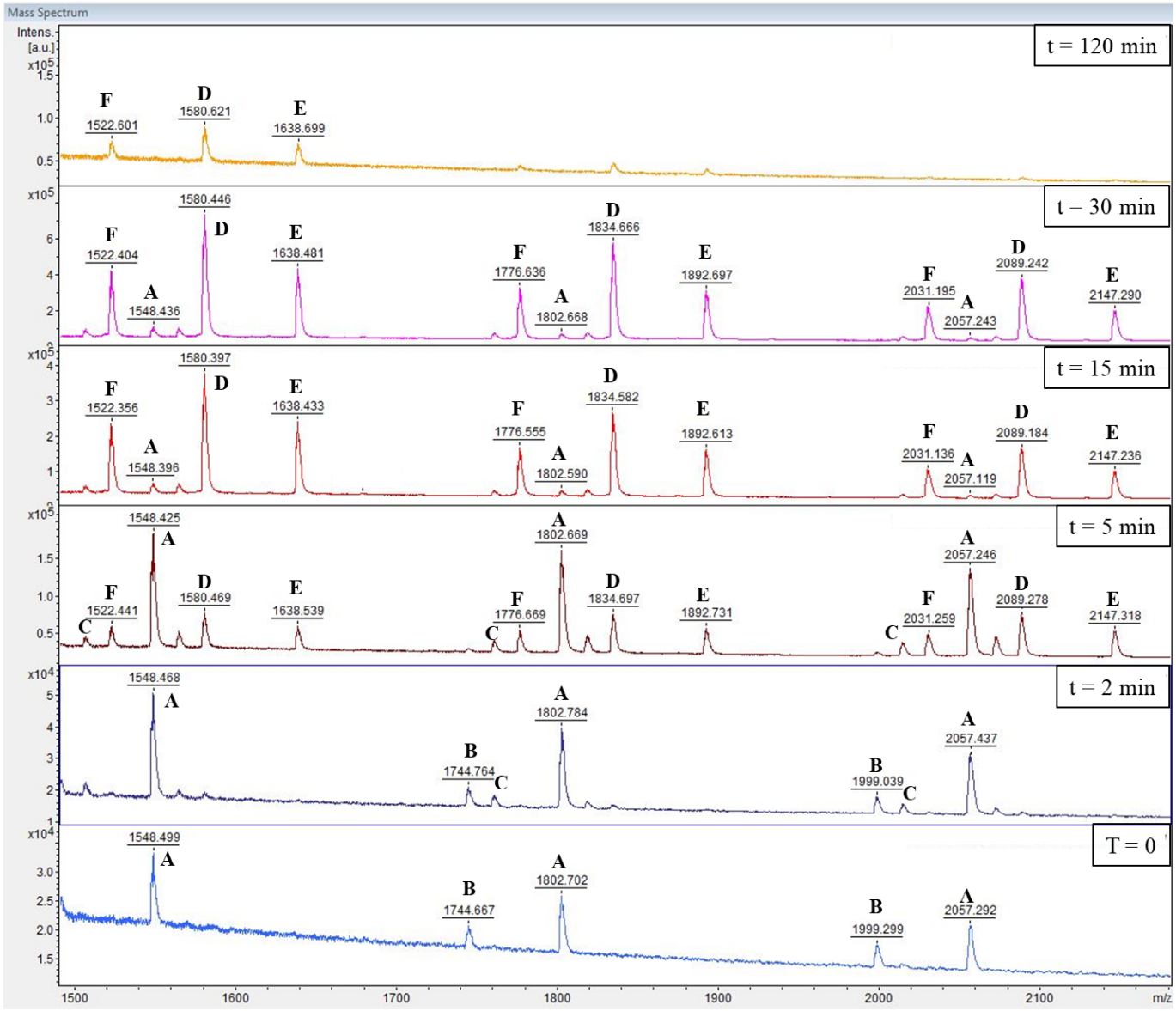


Figure 9: Stacking of MALDI-TOF spectra of partial methanolysis of PBPAC, detailed view of 1400 m/z to 2200 m/z, samples taken at t = 0, 2, 5, 15, 30 and 120 min. Peaks corresponding to polymers from the same series are identified with the letters A, B, C, D, E and F, the corresponding structure are shown in table 2.

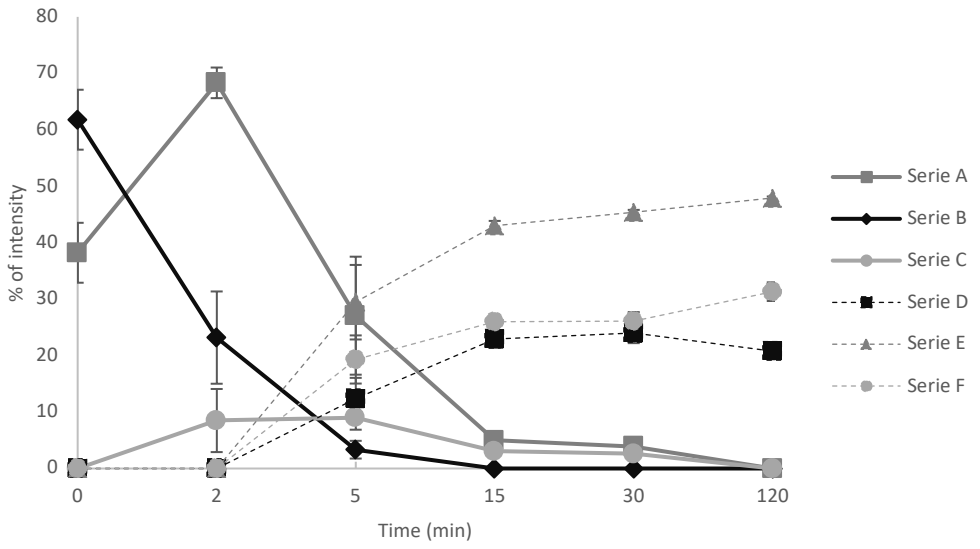
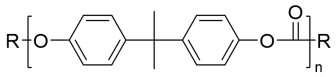
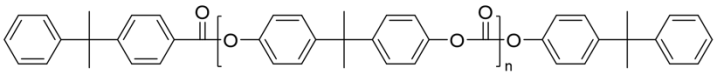
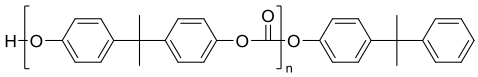
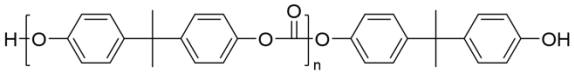
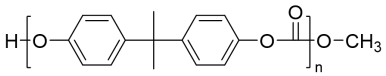
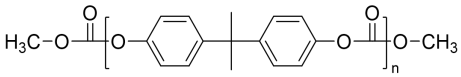


Figure 10 : Evolution of the relative percentage intensity of series peaks assigned to PBPAC analyzed by MALDI-TOF during kinetic monitoring of PBPAC methanolysis

Table 2: Proposed structure of ends groups and their associated theoretical molar mass

End group identification (series)	End group identification	Theoretical mass of the end group (g.mol^{-1})
A		0
B		196.3
C		212.1
D		228.1
E		32.0
F		90.0

To further analyze the degradation, but also to add another analytical technique to the comparison, samples issued from the partial PBPAC methanolysis were analyzed by FT-IR (Figure 11). Here, depolymerization is first manifested by the appearance of the peak corresponding to the hydroxyl group between 3600 and 3200 cm^{-1} after 15 min. In parallel, the intensity of the peak at 1776 cm^{-1}, corresponding to the carbonyl bond of the carbonate moiety, gradually decreases, revealing a doubling of the peak at higher degradation percentage after 30 min. The appearance of a pic at 1612 cm^{-1} and peak doubling at 1235 cm^{-1} can also be observed. Finally, the intensity of the peak at 1194 cm^{-1}, which can be assigned to the C-O bond of the carbonate moiety, also decreases. Interestingly, the appearance of doubling in certain peaks could be the result of intermolecular hydrogen bonding between the polymer chain and the BPA released, and more specifically between the oxygen from the carbonyl of the carbonate and the hydroxyl group of BPA, resulting in a blue shifting of the peak.[27, 28] Nonetheless, these observations clearly generally confirm that the decrease in molecular weight observed in SEC is effectively the result of the carbonate bond cleavage in favor of the appearance of a hydroxyl group. But also, in agreement with the SEC and MALDI-TOF results, depolymerization is found to be uncomplete after 120 min, as evidenced by the remaining presence of the carbonyl carbonate bonds.

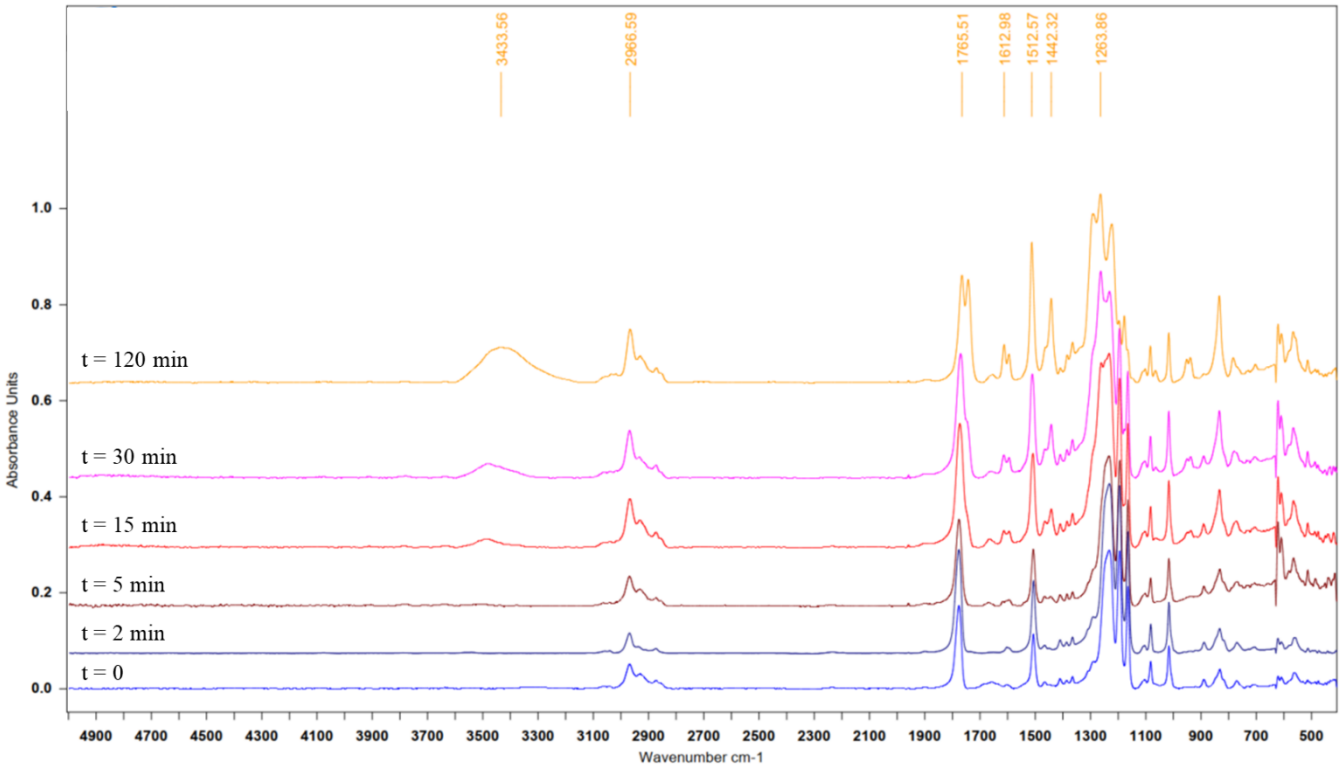


Figure 11: Stacking of FT-IR spectra for partial methanolysis of PBPAC after t = 0, 2, 5, 15, 30 and 120 min, acquisition done in transmission.

Even more interestingly, the detailed analysis of these spectra revealed the possibility to develop a quantitative approach to PBPAC methanolysis based on FT-IR. It is largely inspired from the study by Ferreira *et al.* which performs the quantification of the BPA units in a copolymer PBT/PBPAC using this analytical method.[29] In our case, we focus on quantifying the BPA end groups formed during depolymerization, which relies on the area ratio between two carefully selected peaks belonging only to BPA and to the PBPAC. For the polymer, the peak at 1777 cm^{-1} corresponding to C=O carbonate stretching was chosen for its high resolution and no overlapping with other peaks. For BPA quantification, two choices are possible: the first is to the hydroxyl group that can be detected between 3600 and 3200 cm^{-1}, while the second is the peak corresponding to the stretching of BPA C=C bonds between 1646 cm^{-1} and 1583 cm^{-1}. While the first remains the indicator most used in the literature to confirm PBPAC degradation, it should be pointed out that in real-life situations, depending on the type of catalysis being studied, this choice could prove problematic, particularly in cases where, for example in biological samples, other hydroxylated products are present in the mixture (sugars, glycerols, etc.), which is often the case. In such cases, it may be worth considering the use of the C=C stretching peak. That's why we've tested both here, to effectively compare the sensitivity and error that can be obtained with each. To determine the equation for the correlation function linking the ratios of the two selected peaks in each case, we carried out a calibration curve mixing BPA and PBPAC in increasing proportions, from 5/95 to 90/10 molar ratios (*i.e.* 0.053 to 9 BPA equivalent to PBPAC polymeric unit). The results are presented in Figure 12, Table 3, Figure S8. For the peak area ratio between the O-H from BPA and C=O from PBPAC, we can first clearly observe a linear correlation starting from the 20/80 ratio (*i.e.* 0.25 eq, Figure S8). In the case of the area ratio between the C=C from BPA and C=O from PBPAC, the response is also linear but starting at a lower 5/95 ratio (*i.e.* 0.053 eq), showing how the choice of the peak to be considered can drastically influence the range of application of the method. To discriminate further between the two ratios, the relative errors (RE %) of the area ratios were measured (Table 3), and it clearly appears that the values are smaller in the case of the C=C and C=O peak area ratio, with errors between 2 and 8 % compared to 7 and 13 % for the ratio of the O-H and C=O bonds. Additionally, relative error measured in the case of the 90/10 ratio (BPA/PBPAC) being 20.5 and 16.0 % for the two peak couples, this point was excluded from the calibration curve. Finally, the molar ratio LODs for the two peak couples were calculated, with as a result a minimum ratio of 25/75 (0.35 eq) for the O-H and C=O bonds and 10/90 (0.11 eq) for the C=C and C=O bonds, which once again shows the superiority of the latter in our conditions. It should also be pointed out that under our current conditions, this corresponds to a detection limit for depolymerization confirmation of 10 %. To conclude, this study shows that it is possible to effectively quantify the proportion of BPA in a BPA/PBPAC mixture through the ratio of characteristic peak areas of the polymer and monomer, at least up to 4 BPA molar equivalent to PBPAC. In addition, this also confirms that the O-H bond from BPA may not be the most is not the ideal one to consider for quantitative monitoring of PBPAC degradation, as its sensitivity is lower than that of other characteristic peaks of the monomer such as C=C. This may be due to the fact that it is a poorly resolved peak, strongly influenced by the presence of other hydroxylated molecules, starting with water, despite being located in a region of the spectrum that contains no other peaks.

Table 3: Calibration data obtained from the ratio of the areas of the two selected peaks for BPA and PBPAC as a function of the ratio of the molar concentration of BPA to PBPAC.

	$Area_{3400\ cm-1}/Area_{1777\cdot cm-1}$		$Area_{1600\ cm-1}/Area_{1777\cdot cm-1}$	
BPA concentration in PBPAC equivalent (molar eq)	**Average value of $Area_{3400\ cm-1}/Area_{1777\cdot cm-1}$**	**Relative error (%)**	**Average value of $Area_{1600\ cm-1}/Area_{1777\cdot cm-1}$**	**Relative error (%)**
0.053	-	-	0.052 ± 0.004	7.063
0.075	-	-	0.057 ± 0.002	3.827
0.111	-	-	0.066 ± 0.004	6.111
0.250	0.290 ± 0.038	13.356	0.095 ± 0.007	6.896
0.667	1.399 ± 0.109	7.809	0.193 ± 0.004	2.153
1.000	2.261 ± 0.154	6.833	0.253 ± 0.006	2.410
1.500	4.073 ± 0.521	12.784	0.384 ± 0.030	7.891
4.000	12.417 ± 1.542	12.417	0.909 ± 0.071	7.826

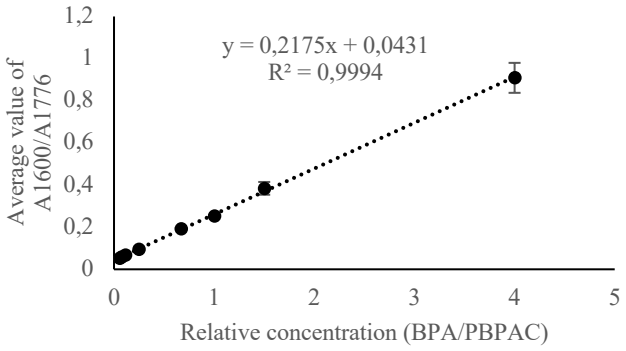


Figure 12: Calibration curve of BPA concentration against PBPAC as a function of the ratio of peak area at 1600 cm^{-1} (C=C from BPA) over peak area at 1776 cm^{-1} (C=O from PBPAC), obtained by FT-IR.

This quantitative determination method in hand, we then used it to determine the degradation percentage in the samples from the methanolysis, and to extrapolate from it the average polymerization degree all along the reaction progress. Data are presented in Table 4, in comparison with the values calculated from the SEC analysis. Based on our calibration curve, we could first observe that the first two points (t = 0 and 2 min) fall outside the calibration range. The depolymerization percentage at 2 min being of 0.39 % according to the SEC analysis, this clearly shows that FT-IR is not suitable for such low degradation rate, unlike the latter. Then, from 5 min to 120 min, the average polymerization degree ranged from nearly 11 to 2.5, values that are closed to those derived from the SEC analysis (14 to 3.6 respectively), despite the latter being only estimates as stated before. We should point out, however, that as our calibration curve was made only with a mixture of BPA/PBPAC, it can only serve as an approximation for the analysis of methanolysis, which also generates products with methyl carbonate-bisphenol A chain ends, which are not taken into account in the analysis of peak ratios detected in FT-IR. That said, the correlation between the two methods shows that these approximations are actually quite small compared with the overall monitoring of the degree of depolymerization, in particular as long as we place ourselves in percentages of degradation higher than the LOD of the two approaches. As a consequence, we may conclude that it is therefore be possible to use FT-IR to obtain a somehow quantitative idea of the PBPAC depolymerization, with a minimal detection limit of 10 %. In such sense, it could represent a good alternative/complement to SEC as, unlike FT-IR, the latter is fairly complex and expensive to perform, requires important post-processes depending on the reaction mixture, is quite solvent-intensive, most importantly, quite low throughput. With only a few seconds per spot, the need for a few µL droplet of sample and the possibility of using it in a 96-well plate format compatible with a liquid handler, FT-IR could be much more suitable for screening campaigns where large collections of catalysts and sets of reaction conditions need to be rapidly evaluated. Especially, for biocatalyst research for example, given the difficulty of finding enzymes or strains capable of acting on this family of polymers, this type of approach seems to be essential for exploring biodiversity to the maximum, or optimizing the catalytic capacity of species by rational design, for example.[9] As a proof of concept, we have recently succeeded in fully automating sample deposition on FT-IR targets with a Biomek i7 robot (Beckman Coulter), thanks in particular to the development of an adapter making the target compatible deck of the latter (Figure S10). Nevertheless, it is not advisable to rely solely on FT-IR when the depolymerization rate does not reach 10 %, and this must be considered in advance of test campaigns, in anticipation of the yields expected for the catalysts and conditions tested.

Table 4 : Results of FT-IR quantification of PBPAC methanolysis and comparison with SEC values

Time (min)	**average value of $Area_{1600\ cm-1}/Area_{1777\cdot cm-1}$**	**Ratio BPA/PBPAC**	**DP**	**DP measured by SEC**
t = 0	0.023 ± 0.012	-	out of calibration range	125

t = 2 min	0.032 ± 0.005	-	out of calibration range	90
t = 5 min	0.065 ± 0.030	0.101	10.86	14
t = 15 min	0.079 ± 0.012	0.164	7.09	6.2
t = 30 min	0.103 ± 0.014	0.276	4.62	5.0
t = 120 min	0.191 ± 0.044	0.678	2.47	3.6

Complementary to FT-IR, we have also analyzed our PBPAC depolymerization reaction by ^{1}H NMR to assess its efficiency and compare it to the one of the former, particularly in terms of its minimum detection limit, as well as the different levels of information it can potentially offer. The resulting spectra for each time point are shown in Figure 13, with a focus on the 1-7.7 ppm chemical shift region (full spectra are available in Figure S11-16). First of all, in the region where the protons of the two methyl groups are located, we can observe depolymerization through the progressive decrease of the singlet at 1.68 ppm, until its virtual disappearance at 120 min. In addition, from 5 min of reaction, the appearance of a new singlet at 1.64 ppm can be observed. For the rest of the study, we used this region as a proton-equivalent reference for the integrations. We therefore set the integration of the sum of peaks at 6 (i.e. 2 methyl groups with 3 protons each). Notably, the integration for these two peaks at 5 min was respectively 1.2 and 4.6 proton equivalents. This corresponds actually to 23.4 % of methyl groups that are not located inside the polymer chain, which is within the expected range according to previously calculated yield, as for 10 % degradation, we can expect 20 % of these protons since a cleaved bond produces 2 chain ends. At 15 min, 5 new singlets are observed at 1.62 ppm, 1.64 ppm, 1.65 ppm, 1.66 ppm and 1.67 ppm. Based on the previously determined ^{1}H NMR spectra, the singlets at 1.62 ppm and 1.68 ppm could be assigned respectively to the methyl protons of BPA and the PBPAC repeating unit. In 2008, Kim *et al*. were able to use ^{13}C NMR to observe the carbons assigned to the methyl at the end of the chain by the appearance of slightly changed chemical shift signals from the methyl in the repeated unit.[17] This is explained by the fact that the end unit does not contain a carbonate bond on both sides, unlike the repeated unit, leading a slightly different shielding effect. Similarly, when applied to our samples, the appearance of singlets at 1.64, 1.65, 1.66 and 1.67 ppm can be assigned to the methyl protons of the different end groups of the reaction intermediates, identified by MALDI (Table 2), although each individual peak cannot be precisely attributed. A second revealing region is of course that of the aromatic protons, between 6.5 to 7.5 ppm. The first changes in this region can be seen after 5 min of degradation, with the appearance of new signals as well as the decrease in intensity of the protons of the repeating unit, which continues until 120 min. The chemical shifts of these new peaks were between 6.67 and 6.75 ppm and between 7.03 and 7.20 ppm. Unfortunately, the overlapping of the signals makes it impossible to determine the multiplicity, neither the exact integration of each signal. However, keeping the same reference as for the methyl protons, the sum of the integrations of all the signals in the aromatic region is equal to 8 equivalent protons, as expected. Also, as in the previous case, these new signals can be attributed to the aromatic protons at the end of the chain of intermediates, even though they don't help to know how many new species are effectively produced. Finally, the region around 3.8 ppm corresponds to the methyl protons of the various methyl carbonates generated from the methanolysis. We can thus see 2 singlets with almost identical chemical shifts (3.892 and 3.894 ppm respectively). Given the population of methyl carbonate chain ends detected by MALDI-TOF (series E and F peaks), it is possible that at least one of these two singlets corresponds to the latter. Note that it is very likely that both populations have the same chemical shift for these protons, especially as the number of polymeric units is large, as the environment is very similar for each end of the chain. On the other hand, it could be that the second singlet corresponds to the methyl protons of the methyl carbonate attached to a released BPA molecule, which could have a slightly different chemical shift, although it's not really possible to confirm this from these results alone. Finally, note that the triplet at 3.76 ppm corresponds to THF remaining after evaporation, as can be seen from t = 0 in very small proportions. As a main conclusion to this analysis, we can first confirm once again that depolymerization has indeed taken place, as observed with the other analytical methods, and that ^{1}H NMR offers several markers to track it correctly. On the other hand, since most of the signals here come out of the same zone, it's rather difficult to distinguish between the degradation products, let alone identify each of them precisely. As with the other techniques, here too we can calculate the minimum detection limit. If we refer to the percentage of degradation determined in SEC, and taking into account the fact that the first new signals are only distinguishable in ^{1}H NMR from 5 min onwards, this would amount to a limit of around 8 % depolymerization. This makes ^{1}H NMR a slightly more sensitive approach than FT-IR. However, in addition to the limitation sported regarding the determination of the intermediate products, NMR is like SEC quite limited by certain technical constraints (low sample throughput, expensive deuterated solvents, etc.), As such, it would only be an interesting alternative or complement if we were to use a more advanced analysis of the spectra, perhaps by carrying out long-distance coupling experiments to better identify and characterize the species present. In this case, it should be used at the end of the analysis to confirm certain by-products that are difficult to identify, or to validate results obtained with high-throughput approaches, for example. Finally, as for FT-IR, NMR is also is highly dependent on the purity of the analyte, requiring an important sample post-processing if used on complex degradation media such as fermentations.

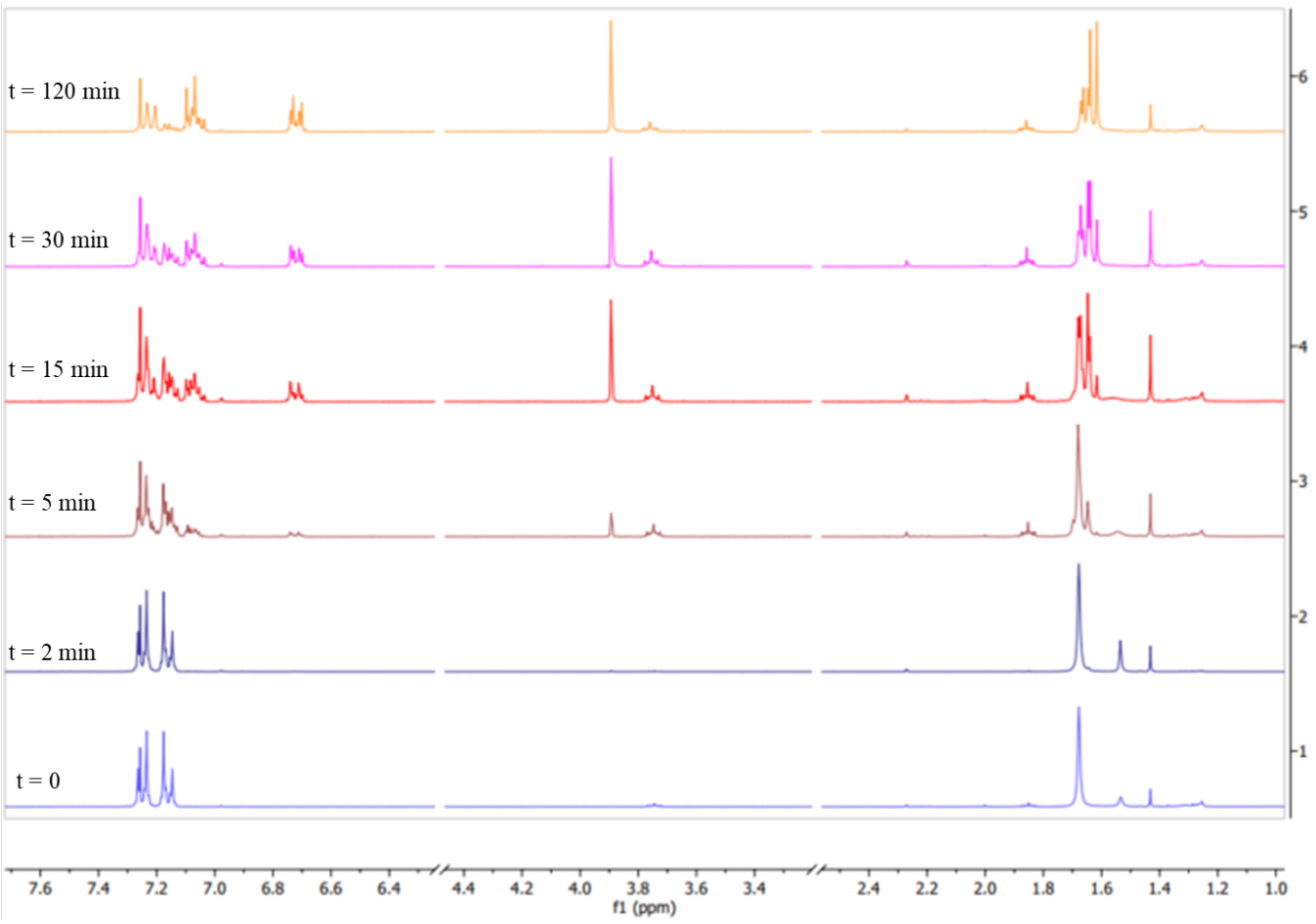


Figure 13 : Stacking of NMR spectra of partial methanolysis of PBPAC. with detailed view of peaks assigned to the polymer. samples taken at t = 0, 2, 5, 15, 30 and 120 min.

Finally, as already mentioned, BPA release into the reaction medium can be quantified easily by HPLC, in order to confirm the actual yield of conversion, but also to assess if the degradation appears to be complete or stops at a certain degree of polymerization. The chromatograms obtained from the different samples are shown in Figure 14. Unlike the other techniques used previously, the first changes are detected only after 15 minutes of reaction, with two peaks with respective retention times of 6.59 and 7.67 min. However, if the depolymerization mechanism does indeed take place *endo* as we hypothesized, this seems quite logical as the chances of breaking a random bond within the polymer chain, producing oligomers, are much higher than those of hydrolysis of the polymer ends which would release BPA. Concerning the product attribution, the first peak appears to be BPA monomer, in accordance with the first measurement done and the standard chemicals. However, the second peak could not be identified with certainty, notably due to the absence of oligomer standards in our possession. Nonetheless, its retention time being higher, significantly longer than that of BPA, implying a lower polarity of the molecule compared to BPA. Consequently, while we can it could correspond to the more hydrophobic methyl carbonate derivative of BPA, a product in which one of the chain ends has not undergone double transesterification as identified by MALDI-TOF. Using our calibration curve, we could however estimate the concentration of BPA to be of 0.22 mM after 15 min, which corresponds to a molar conversion yield of 1.1 % of polymer to pure monomer. The fact that no monomers could be detected before this time also leads us to be able to determine a minimum detection limit for polymer degradation of 20 %, by correlation with the SEC results. Although quite high in comparison with other limits measured before, this is again quite relevant under our conditions, since HPLC here cannot allow us to detect anything other than total depolymerization of the polymer. In consideration of the extent of degradation that could be quantified by HPLC, it is notable that the maximum concentration of BPA recorded after two hours of reaction was 3.9 mM, which corresponds to a final molar conversion of 19 ± 4 % of PBPAC to pure BPA. As a result, without additional standards, that can be particularly hard to obtain for some polymers, including PBPAC, HPLC analysis provides only partial results on depolymerisation. Nevertheless, this approach remains highly accurate for quantifying the release of BPA into the medium, with a detection limit of 6 µM for the monomer as measured by the device. Consequently, this method would be much better suited to studying the degradation of PBPAC, or any other polymer, under conditions that favor an action in *exo*, as can quite often be observed with enzymes. This is even more true when, instead of focusing on the level of polymer degradation, the emphasis is placed on the production of a specific species (*i.e.* monomer or degradation product) for its subsequent valorization.

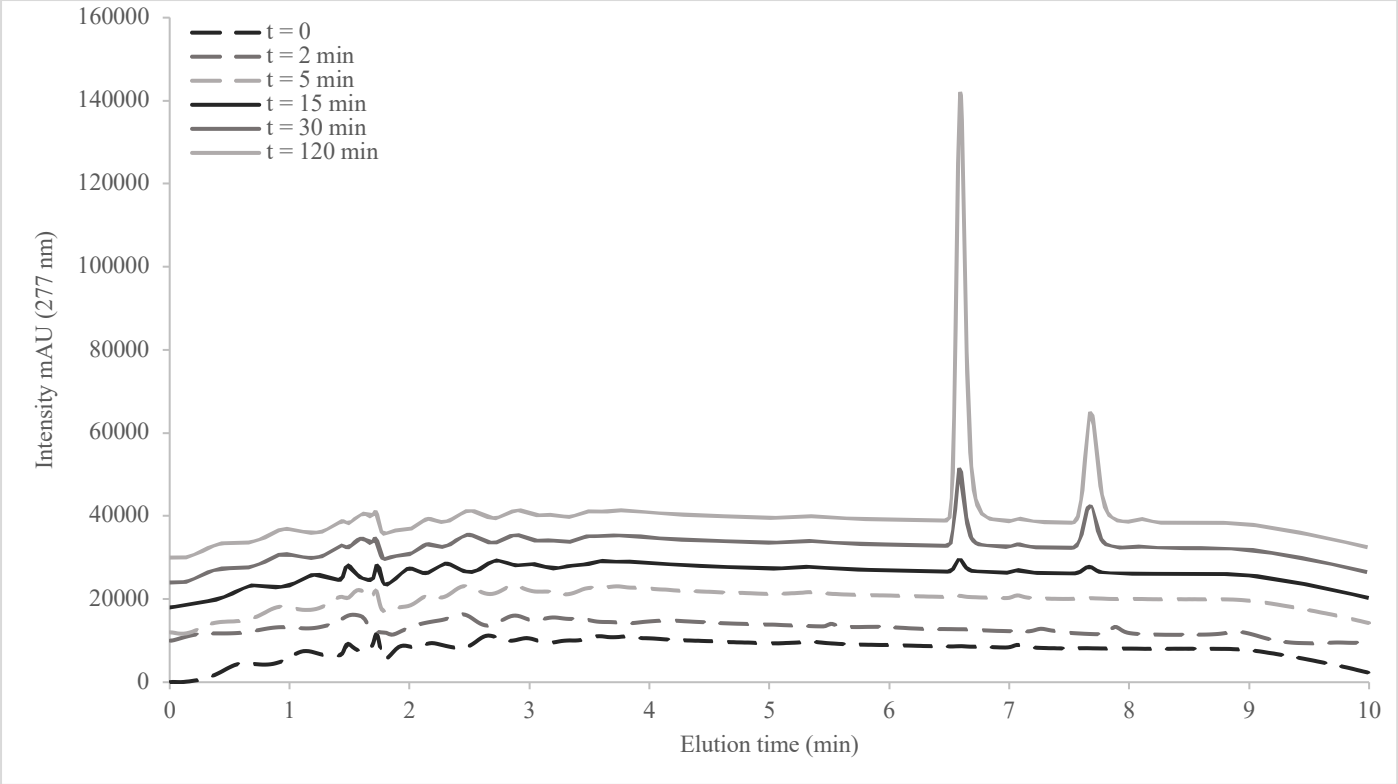


Figure 14 : HPLC chromatograms obtained from PBPAC methanolysis of after 0, 2, 5, 15, 30 and 120 minutes of reaction.

If we now take stock of all the results from this analytical panel, and put them into perspective with what's commonly in use in the community, starting with the recommendation from Tian *et al.*,[15] we can firs state that our observation are very much in line with theirs. As these authors point out, SEC is the main technique for monitoring changes in molar mass distribution as polymer degradation proceeds. NMR and HPLC are used to identify the intermediates and end products, respectively, that result from this degradation. MALDI-TOF, on the other hand, is primarily used to identify the end groups formed. However, contrary to the authors' conclusions, the latter does not appear to be suitable for the measurement of average molar masses, since from our observations in the present study, but also when reading the bibliography dedicated to this technique, it appears that MALDI favors low masses to the detriment of high masses.[24, 30] This results in a biased representation of the populations, and it can therefore neither be used quantitatively, nor even really allow the degree of polymerization of the polymer to be calculated at a given time. Finally, our study has interestingly also shown that infrared spectroscopy can not only identify broken and formed bonds, but also quantify the degree of polymer degradation, with different minimum detection levels depending on the peaks used to identify the species involved. The advantages and disadvantages, as well as the detection limits that we were able to determine for these 5 analytical methods are summarized in

Table 5 in order to offer a guide to the selection of the most appropriate according to the anticipated depolymerization/degradation yield as well as other parameters such as the complexity of the reaction medium or the need for high throughput.

In short, when the degradation is limited (*i.e.* between 0.5 to 10 %), both SEC and MALDI-TOF analyses can be employed to confirm the occurrence of degradation. SEC can be used to quantify precisely the extent of degradation, while MALDI-TOF analysis can be used to identify the products formed, and in particular the end groups generated. Note that if the depolymerization medium is rich in salts, samples must be desalted before MALDI analysis, which is highly sensitive to the presence of ions. Conversely, when there is a need for high-throughput analysis, MALDI can be used in the first instance to rapidly observe degradation, without however being able to quantify it, before carrying out a quantitative SEC analysis on the most interesting samples. In the special case where depolymerization takes place in *exo*, HPLC can also be considered as a complement to these two techniques, in order to precisely identify the lower molecular weight products formed, provided they are available as analytical standards. If depolymerization now exceeds 10 %, NMR and FT-IR can be added. Interestingly, the latter may be sufficient on its own initially to quantify degradation, if a calibration curve can be made beforehand. As this technique requires very little sample processing before analysis, and as we have succeeded in adapting it to high throughput, it could prove to be a very good approach for large-scale screening, particularly when studying microorganisms or enzymes. Finally, we generally recommend considering coupling at least one method for identifying reaction products with a method for quantifying the progress of depolymerization. MALDI-TOF is an effective approach for identifying each end group and, consequently, the various reaction intermediates, and can also be adapted for high-throughput screening. Coupled with the low sample quantity and solvent requirements, it is certainly an interesting first option, if the polymer can be analyzed in this way, as is the case with PBPAC.

Table 5 : Summary table of the 5 analytical methods in the panel studied, detection limit for confirmation of depolymerisation/degradation, recommendation and warning discussed for each method

Method	Minimal detection limit polymer degradation	Advantages	Drawbacks
SEC	0.06 %	- Very sensitive; - Provides a complete set of information on the remaining polymer (degree of polymerization, dispersity, etc.); - Quantitative method.	- Quantification only approximate because relative to the standard used for calibration; - No identification of the products formed.
MALDI-TOF	0.5 %	- Identification of reaction intermediates - end groups; - Compatible with high-throughput screening.	- Not quantitative; - Analysis of the whole MWD depends on the polymer studied (lower masses favored over higher masses); - Small oligomers and monomers not detectable.
FT-IR	10 %	- Quantitative method (from 10 % up to 80 %); - Identification of formed and broken bonds; - Compatible with high-throughput screening.	- Spectrum quality depends on sample purity; - Quantitative only if degradation products are available for the calibration curve; - Quantification potential is highly dependent on the peaks selected to characterize the different species.
^{1}H RMN	8 %	- Confirmation of the presence of both polymer and monomer; - Possibly quantitative.	- Signal superposition is possible, especially when product diversity is high, which can make it impossible to identify reaction intermediates.
HPLC	20 %*	- Highly sensitive method in the case of an *exo* degradation, with a BPA detection limit of 6 µM; - Quantitative, and allows the identification of all degradation products as long as analytical standards are available.	- Product identification limited to those for which standards are available; - Impossible to determine and quantify all depolymerization products, especially when the degree of polymerization remains high.

[1]Limit of detection calculated for an *endo* degradation. In case of an *exo* degradation the actual limit of detection is the minimal concentration of monomer detected by HPLC.

Conclusion

The aim of this study was to propose the minimal, or ideal, panel of analytical methods to be used to study polymer depolymerization, with a focus on poly(bisphenol A carbonate). To this end, we carried out methanolysis of the latter at various stages of advancement, in order to generate the intermediates and reaction products that appear during depolymerization. The various samples were then systematically analyzed using 5 analytical methods, each offering a different level of characterization of the reaction medium: size exclusion chromatography (SEC), high performance liquid chromatography (HPLC), infrared spectroscopy, MALDI-TOF mass spectrometry and proton NMR. For each, the limits of quantification of degradation were calculated, and compared, along with the various advantages and disadvantages that each present. As expected, SEC proved to be an indispensable choice for analyzing depolymerization at any rate. In addition to its very low detection limit (0.06 % under our conditions), it is also the only method that gives access to the molecular weight distribution of the sample at each instant. However, it does not allow us to identify the products formed, a shortcoming compensated for by MALDI-TOF analysis, which allows us to identify the chain ends generated, and which also has a fairly low limit of quantification (0.5 % under our conditions). HPLC has also proved useful for identifying degradation products, but only when standards are available, which severely limits its use in the case of unconventional depolymerization methods, or with unusual polymers or co-polymers (specialty polymers, for example). In addition, when the depolymerization rate exceeds 10 %, infrared spectroscopy and NMR, which enable direct analysis of the chemical groups present, or even identification of the individual molecules, may prove complementary for the purpose of analyzing unknown transient species. Furthermore, infrared spectroscopy appears to be a particularly interesting analytical method for estimating the

average degree of polymerization of oligomers, as we have shown that it can be used quantitatively, provided that a calibration range can be established beforehand by mixing the various species expected in solution. What's more, unlike SEC or NMR, this method is particularly solvent-efficient, does not require complex sample processing or a large sample volume for analysis, and above all can be used at high throughput thanks to its analysis speed and compatibility with robotized platforms. It is therefore particularly interesting for screening campaigns when the expected depolymerization rate is not too low. This is also the case for MALDI, which can also be performed at high throughput with small sample quantities. Together, these two methods form a particularly relevant tandem for quantifying and identifying PBPAC depolymerization, especially when the latter involves mechanisms more complex than the simple release of a monomeric unit in *exo*.

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Competing interest

No conflict is to declare

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