Design of Polyhydroxyalkanoate (PHA) Microbeads with Tunable Functional Properties and High Biodegradability in Seawater

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Abstract
Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) and poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) were used to prepare microbeads, with diameter ranging from 50 to 100 µm, by an emulsion-evaporation process. The emulsification-evaporation process enables the formation of spherical polyhydroxyalkanoate beads, with crystalline rates similar to the ones of the former polymer and with important surface roughness as compared to amorphous polylactic acid smooth beads. The mechanical properties of the different PHA beads are also found to be intimately linked with their crystalline content, with modulus varying between 1 and 7 GPa. The degradation behavior of these PHA microbeads was tested under marine environment and revealed a rapid degradation, similar to cellulose, and a degradation rate correlated with the crystalline content. These results emphasize the possibility and interest in developing PHA materials with tunable functions and degradation properties.

Keywords Polyhydroxyalkanoates · Microbeads · Surface properties · Biodegradability · Seawater

Introduction
Microplastics are defined as solid plastic objects smaller than 5 mm in size, insoluble in water and not biodegradable [1]. Personal care products (PCPs) have been identified as a potential source of environmental pollution due to their high content in primary microplastics, with typical number and mass content about 2162 particles/g or 0.04 g g⁻¹ [2–4]. According to the PCPs’ consumption, approximatively 1500 tons/year of micro plastics leach into the global aquatic environments, which account for 0.1–0.8% of the annual global release of primary microplastics in the world oceans [2]. Until recently, PCPs products are mainly formulated with conventional microplastics such as polyethylene, polypropylene, poly(ethylene terephthalate) or poly(methylmethacrylate) [5–8, 13, 14]. These additives have the benefit to being natural and biodegradable materials compared to conventional polymers [15, 16], they nonetheless also present drawbacks because of their irregular shapes and sizes. They are also generally colored, not or poorly stable in aqueous medium, with an inadequate hardness and their commercial quantity and availability are rather limited [17]. The cosmetics industry is therefore interested in developing biodegradable particles, in the micrometer size range, which are gentler in action than ground natural ingredients [18, 19] for applications such as facial cleansing or scrubbing [5–8, 13, 18, 19].

Polyhydroxyalkanoates (PHA) which are bacterial polyesters especially biodegradable in marine environment [20] are natural candidates for applications in cosmetic meeting environmental constraints. Depending on the bacteria, stress conditions and substrates, a broad variety of PHA can be produced with different monomer units, leading to a variety
of chemical and physical properties [21]. These properties can be modulated by the chemical composition or length of the lateral chain, the proportion of the monomer units and their distribution all along the polymer chain [22]. For example, Lemechko et al. [23] obtained poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBHV) macromolecules by optimizing and choosing the nature of carbon source (agro-resources effluents) and the amount of valeric acid to produce a range of polymers with a controlled proportion of each monomer. Thermal analysis showed a decrease of the glass transition in the hydroxyvalerate unit (HV) content increased. By increasing the proportion of HV monomer (from 5 up to 20%), the PHBHV polymer becomes more ductile, its glass transition temperature \( T_g \) and crystalline rate [24] are also decreasing. Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBHHx) is another kind of commercially available PHA. The substitution of the hydroxyvalerate unit by an hexanoate unit (HHx) also modifies the polymer properties. This polymeric material becomes more ductile than PHB [25, 26]. The \( T_g \) temperature is also affected by a variation of the chemical unit of the copolymer [26] or the rate of HHx units within the copolymer. For example, increasing the proportion of HHx from 7 to 18% slightly decreases the glass transition temperature from \(-5 \) to \(-8 \) °C [27–29]. Consequently, it has been reported that the degree of crystallinity of PHBHHx is lower than that of PHBHV, explained by a greater steric hindrance generated by the HHx unit [29]. The amount of HHx unit within a PHA is also influencing the crystalline rate as highlighted by some authors who have measured a decrease of about 20% of the crystalline rate when the percentage of HHx unit increases from 4 to 20% [29].

Several methods have been described for the production of biopolymers microparticles [30, 31] such as emulsification, gelation, drying, coacervation or precipitation. The choice of the method and then of the proceeding and formulation parameters influence the physico-chemical characteristics of the particles such as the porosity, sphericity, size, dispersity, surface appearance or shape [32–37]. The type of polymer, in particular its botanical origin, affects the size of the so-formed microparticles. For example this has been demonstrated for starch-based particles obtained by nanoprecipitation [32]. The process of elaboration of micro and nanoparticles is also a fundamental parameter that governs their final properties. For example, it has been shown that ultrasound process allows to obtain small PHB microbeads in comparison with a stirring process, with diameter of 0.14 \( \mu \)m versus 32 \( \mu \)m respectively [33]. The organic solvent used also strongly affects the final shapes and sizes of the particles [31]. It has been shown that, dichloromethane allows to obtain more spherical PHBHV microbeads than chloroform [34]. When dichloromethane is used as solvent, particles with a wide range of diameters, between 0.8 and 7 \( \mu \)m, can be obtained whereas a narrow distribution, between 0.1 and 0.4 \( \mu \)m, is observed when chloroform is used. Other authors have shown that the size of PHBHV microbeads can be tuned by adjusting the surfactant concentration, obtaining a range of microbeads from 389 to 39 \( \mu \)m for 0.5 to 4% of poly vinyl alcohol surfactant [36]. The porosity of PHB-based microbeads is also found to be dependent on the amount of aqueous phase, used in an emulsification process [33]. The final process of microbeads fabrication is the solvent evaporation process which is also an important step in determining the final properties of the particles [33]. The biomedical domain is certainly the one that has contributed to the most significant results in the elaboration of PHA microbeads [38]. In a majority of studies on PHA particles, the emulsification process was developed to elaborate micrometer size particles made from PHBHV or PHBHHx polymer. Table 1 sums up some of the main parameters used in these processes.

The objective of this work is to synthesize PHA microbeads for cosmetic applications with the following goals: size of approximately 100 \( \mu \)m, spherical shape particle with tunable surface aspects and mechanical properties and biodegradable in the marine environment. Surprisingly, to the best of our knowledge, the development of PHA microbeads for such application is poorly described in the literature. We have used three commercial PHA, of different chemical structures, to prepare such microbeads by an emulsification-evaporation process. The physico-chemical properties of the different beads were then characterized, in terms of shape, crystallinity, surface, mechanical properties and biodegradability and compared with PLA microbeads, used as control. This latter polymer was chosen due to the large amount of studies on the elaboration and the characterization of PLA-based microbeads in the literature.

Among the three selected PHA, one (PHBHV) is readily commercially available while the two others (both PHB-HHx) are only sparingly distributed over the world due to their low available quantities. In this context, this prospective study will make it possible to consider expanding the range of applications of these PHA, particularly for the PHBHHx polymers.

### Materials and Methods

#### Materials

Poly(3-hydroxybutyrate-co-hydroxyvalerate) (PHBHV) with 3 mol% in HV was supplied by Tianan Biological Materials Co. Ltd. (China), under the trade name ENMAT Y1000P. Poly(3-hydroxybutyrate-co-hydroxyhexanoate) (PHBHHx) at 6 and 11 mol% in HHx were supplied by Kaneka Corporation (Japan), under the trade name Aonilex X131A and
| Type of polymer | Polymer solution | Surfactant solution | Stirring | Evaporation of the organic solvent | Microbeads size diameter | Applications | References |
|-----------------|------------------|---------------------|----------|------------------------------------|-------------------------|--------------|------------|
| PHBHV 5% w/v in chloroform | 2% w/v aqueous PVA | Mechanical stirring (3000 rpm) | Mechanical stirring (800 rpm) for 4 to 7 h | 21 µm | Encapsulation of andiroba oil | [39] |
| PHBHV 1.0% w/v in chloroform solution | 1.0–3.0% w/v aqueous PVA | Mechanical stirring | Mechanical stirring at 300 rpm or 900 rpm, continuously, for 24 h | 3–30 µm | Encapsulation of coumarin-6 (C6) or pyrene (Py) | [37] |
| PHBHV 0.88–5.12 g mL⁻¹ in chloroform | 0.38–4.60% g mL⁻¹ aqueous PVA | Mechanical stirring | Mechanical stirring at 1400 rpm and for 4 h | 4–19 µm | Encapsulation of flurbiprofen | [40] |
| PHBHV 2.5% w/v in chloroform | 1% (w/v) aqueous PVA | Mechanical stirring (1000 rpm) | Rotary evaporator | 9 µm | Encapsulation of annatto extract | [41] |
| PHBHV 1% w/v in chloroform | 2% (w/v) aqueous PVA | Mechanical stirring (1000–4000 rpm) | Vacuum distillation unit, and heated to 45 °C | 10–300 µm | Encapsulation of trypan blue dye | [42] |
| PHBHV 2% w/v in dichloromethane | 1% (w/v) aqueous PVA | Dropwise fashion | 900 rpm for 8 h at room temperature with a 3-bladed propeller in an off-centre position | 20–40 µm | Encapsulation of diazepam | [43] |
| PHBHV 0.05–0.20 g in 5 mL chloroform | 1.0–1.2% (w/v) aqueous PVA | Mechanical stirring (1000 rpm) | 12 h at 25 °C | 20–60 µm | Encapsulation of rifampicin | [44] |
| PHBHV 0.5% in chloroform | 0–4% (w/v) aqueous PVA | NS | 700 rpm and 50 °C for 6 h | 31–390 µm | Process effects on degradation in vitro | [36] |
| PHBHV 100 mg mL⁻¹ chloroform | 1–4% (w/v) aqueous PVA | Mechanical stirring (500 rpm) | Nitrogen atmosphere was created in the flask and the solution was stirred until all the chloroform was evaporated | 322–538 µm | Encapsulation of tetracycline | [45] |
| PHBHHx Dichloromethane | Aqueous sodium Docecyl Sulfate (SDS) | NS | Magnetic stirring at room temperature | 5–20 µm | Encapsulation of 5-fluorouracil or cyclosporin A | [46] |
| PHBHHx 0.1% w/v in acetone | ND | Vigorous stirring | Under vacuum at 35 °C overnight | 75 µm | Effect of PHA particles on cell growth | [47] |

NS means not specified
Aonilex X151A, respectively. Polylactic acid (PLA) with 4% D-lactide was purchased from NatureWorks under the trade name Ingeo 7001D. The physico-chemical properties of the investigated polymers are given in Table 2. For clarification in the following text, the notation PHBHV, PHBHHx (6%) and PHBHHx (11%) and PLA will be used. All chemicals products and reagents used in these experiments were analytical grades and were purchased from Sigma-Aldrich. In addition to polymer materials, extra-pur powder of microcrystalline cellulose (also named micronized cellulose), purchased from acros organics (chromatographic grade, with diameter around 50 µm), was used as a reference polymer in the degradation test.

**Synthesis of Biopolymers Microbeads**

Microbeads of PHBHV, PHBHHx (6 and 11%) and PLA were elaborated by emulsification process. PHBHV was dissolved at 50 g L\(^{-1}\) in chloroform under reflux conditions (50 °C), PHBHHx and PLA were dissolved at the same concentration but in dichloromethane under reflux conditions (40 °C) during 24 h. These polymer solutions were slowly added in an aqueous solution of polyvinyl alcohol (PVA) (2%) under controlled mechanical stirring (4000 rpm). The emulsification time was fixed at 15 min. The particles were then dried by gently evaporating the solvent under continuous magnetic stirring during 24 h. The dried particles were finally collected by sequential sieving (Inox sieve with 250 and 50 µm mesh, Granuloshop, Chatou, France), washed several times with deionized water and lyophilized.

**Characterization of the Microparticles**

**Morphological Analysis**

The morphology of the microbeads was analyzed by a scanning electron microscope (JSM-IT500HR from JEOL). SEM observations were carried out with secondary electron detector at an acceleration voltage of 3 kV. The particles were stuck on an adhesive carbon tape and gold-coated with a sputter coater (Scancoat6 from Edwards). The determination of the microbeads diameter and their shape was extracted from SEM images by image analysis. For each sample, a minimum of 500 microbeads were analyzed using ImageJ software (version 1.52, NIH). The circularity factor (CF) was determined using Eq. (1):

\[
CF = \frac{4\pi \times \text{area}}{(\text{perimeter})^2}
\]

The surface topographies of the microbeads were also measured by atomic force microscopy (AFM). AFM images were obtained with a multimode 8 atomic force microscope (Bruker, Santa Barbara, CA) operated on the scanasyst@ mode (Bruker) under ambient conditions (23 °C, RH = 50%). Standard scanasyst tips (Bruker), with a resonance frequency of 70 kHz and a spring constant of 0.4 N/m were used. Images were analyzed using the Nanoscope analysis software (V1.80). To ensure a good reproducibility in the measurements, for each sample, a minimum of three areas were investigated for each microbead, and for each sample a minimum of five different microbeads were observed.

**Thermal Properties**

Thermal properties were determined by Differential Scanning Calorimetry (DSC). About 5–8 mg were introduced in

| Table 2 Physico-chemical characteristics of PHBHV, PHBHHx (6% and 11% HHx) and PLA pellets |
|---|
| **Polymer** | **Chemical structure** | **Average molecular mass \(\times 10^3\text{ g mol}^{-1}\)** | **Dispersity (D)** | **\(X_c\) (%)** | **Tm (°C)** | **Tg (°C)** | **References** |
| PHBHV (3% HV) | | 340–400 | 2.5–2.7 | 54–65 | 165–175 | 4–8 | [48, 49] |
| PHBHHx (6% HHx) | | 345–452 | 1.9–2.6 | 27 | 142–145 | 2 | [49] + Technical sheet from Kaneka |
| PHBHHx (11% HHx) | | 550–614 | 2.0–2.4 | 34 | 126–136 | 0–2 | [50–52] + Technical sheet from Kaneka |
| PLA (4% D-lactide) | | 174–220 | 1.6–1.8 | 5 | 150–160 | 55–60 | [53–56] + Technical sheet from NatureWorks |
standard aluminium pans, using a Mettler-Toledo DSC882 equipment. Samples were heated from 25 to 180 °C at a scanning rate of 10 °C min⁻¹ under nitrogen flow. Thermal characteristics were recorded such as transition temperatures (melting, crystallization and glass temperatures) and melting enthalpy (ΔHm). Each sample was analyzed in triplicate. As a rule, phase transitions of reference substances (pure indium and zinc) with known transition temperatures and transition enthalpies were used to check temperature and heating rate independence.

**Infra-Red Spectroscopy**

Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) spectroscopy (Vertex70v, Bruker) was operated in the range from 4000 to 600 cm⁻¹ with 4 cm⁻¹ resolution. Dried microbeads were deposited directly on to the diamond crystal. For each sample, a minimum of 16 scans were performed to ensure a good reproducibility in the signals.

**Nanomechanical Properties**

Nanoindentation experiments were performed with a Nanoindenter XP (MTS Nano Instruments) equipped with a three-side pyramid indenter (Berkovich) [57]. All experiments were conducted under ambient conditions (23 °C, RH 50%), using the continuous stiffness measurement (CSM) method with the following parameters: 3 nm amplitude and 45 Hz oscillations using a 0.05 s⁻¹ loading rate. Measurements were taken at depths to 1500 nm. A Poisson’s ratio of 0.35 was used in all modulus calculations as suggested by Young et al. [58] for polymers where the Poisson’s coefficient is unknown. For each sample, around 75 indents were performed with 5 x 5 matrix on different locations; average values of both elastic modulus and hardness were then calculated from curves according to the method of Oliver and Pharr [59]. Experiments were performed on polymer pellets embedded in an epoxy resin (Epolam 2020), dried at 50 °C overnight and then submitted to a careful polishing process (until paper grade 4000). Since the polymer pellets are agglomerated in dense particles, they appear in white in contrast with the transparent resin. Prior the indentation experiments, the sample is observed with an optical microscope (OlympusAX70), equipped with a 5 times BD objective (NA 0.15) to get a large image of the whole surface necessary to distinguish between the two materials and to choose an indentation area of interest.

The AFM technique was also used to probe the nanomechanical properties of the polymer microbeads using the peakforce quantitative nanomechanical measurements (PFQNM). In PFQNM, the piezo of the AFM is vertically oscillating at a frequency of 2 kHz, with an amplitude of 150 nm. While the piezo moves the sample beneath the tip in the X and Y direction, a force curve is recorded in every coordinate, allowing to extract mechanical properties from the materials. The spring constant of RTESP-525 commercial tips were evaluated using the Sader method (https://sadermethod.org) and the tip radius was determined using the relative calibration method. A polystyrene film of 2.7 GPa was used as a standard of calibration (PFQNM SPM kit-12M, Bruker). The peak force setpoint was set at 200 nN.

The indentation modulus was calculated using the Dejarguin–Muller–Toporov (DMT) model using the nanoscope image analysis software (V1.80). For each sample, a minimum of three areas were recorded and for each polymer, a minimum of three different microbeads were analyzed. The microbeads were simply fixed using double-sided tape to prevent them from moving during the measurement.

**Biodegradability**

Stability tests were carried out in aqueous solution, as adapted from personal care tests, since aqueous solution is the least stable medium for exfoliating particles studied in cosmetic formulation. Test solutions were prepared by introducing 2% (w/w) of microbeads in purified water containing 1% of phenoxyethanol. The stability of the dispersion was followed for solutions stored under three different conditions, ageing at room temperature in the absence of light, at room temperature in the presence of light, or in a solution maintained at 45 °C to mimic an accelerated ageing. The variations in pH of the different solutions were followed over a period of three months.

The biodegradability of the different microbeads was determined with the NF EN ISO 19679 test that measures aerobic biodegradation of non-floating plastics at the seawater/sediment interface. The amount of total organic carbon contained in each sample, \( m_{CO2_IR} \), was determined by elemental analysis and is respectively of 44.44 ± 0.02% for micronized cellulose, 55.18 ± 0.02% for PHBHV, 56.52 ± 0.25% for PHBHHx (6%), 57.08 ± 0.18% for PHBHH (11%) and 49.59 ± 0.17% for the PLA. These theoretical masses of carbon were then used to calculate precisely the mass of product (approximatively 5 g) needed to get the same amount of carbon per polymer in the degradation test.

Sample preparation was previously described for solid/liquid state, as an adaptation of the Sturm test [20]. Under continuous oxygenation in a sealed system, 5 g of microbeads were introduced in a mixture of seawater (100 mL) and sediment (30 g), placed in a first compartment, and the second compartment was containing an absorbing solution with a NaOH solution (20 mL, 0.2 mol L⁻¹) and distilled water (20 mL). A blank flask with no sample was also included in each test set-up to determine the blank respiration of the seawater, as well as a standard composed of micronized
cellulose to validate the results of the test. The test was carried out for 250 days in a water bath at 25 °C.

Since the produced carbon only comes from the biodegraded sample, the CO$_2$ trapped by the absorbing solution is proportional to the amount of carbon consumed. The product Na$_2$CO$_3$, by the reaction of CO$_2$ and NaOH is precipitated by a BaCl$_2$ solution. The remaining NaOH is titrated with hydrochloric acid (0.1 mol L$^{-1}$) to determine the CO$_2$ trapped by the absorbing solution. Rate of biodegradation (%CO$_2$) was determined from the Eq. (2):

$$\text{% CO}_2 = \frac{(m_{\text{CO}_2, \text{sample}} - m_{\text{CO}_2, \text{control}}) \times 100}{m_{\text{CO}_2, \text{theoretical}}}$$

where $m_{\text{CO}_2, \text{sample}}$ is the amount of CO$_2$ produced in the sample test, $m_{\text{CO}_2, \text{control}}$ is the amount of CO$_2$ produced in the blank test.

### Results and Discussion

#### Morphology of Microbeads

SEM images of the different microbeads are presented in Fig. 1. All the beads are spherical, as revealed by a circularity factor close to 1 (Table 3). The diameter of the beads (Table 3) is found to be dependent on the polymer nature, thus indicating that, for a similar elaboration process, using

| Microbeads    | Microbeads size distribution (µm) (mean diameter ± SD) | Circularity factor |
|---------------|-------------------------------------------------------|-------------------|
| PHBHV         | 94 ± 25                                               | 0.91              |
| PHBHHx (6%)   | 48 ± 20                                               | 0.93              |
| PHBHHx (11%)  | 75 ± 25                                               | 0.92              |
| PLA           | 111 ± 42                                              | 0.93              |

The average diameter and standard deviation were estimated on approximatively 500 particles
between 130 and 210 nm is obtained for the three PHA par-
of the different PHA beads. At such scan size, a roughness
topography of the PLA beads and the important roughness
their corresponding surface analysis, confirming the smooth
AFM images obtained of the top of the different beads and
obtained using a similar process and determined by SEM or
optical microscopy [16].

From the SEM images, the surface roughness can be
qualitatively observed. The SEM images at different reso-
lation (Fig. 1) reveals that the PLA bead is rather smooth
in comparison with the rough surfaces of PHBHHx or
PHBHV beads. The roughness was quantitatively estimated
by means of the AFM, on area of approximatively 25 µm². It
should be noted here that stable AFM images were difficult
to obtain on a larger scale (> 25 µm²) due large z devia-
tion measured on our samples may be associated with
the radius of curvature of the beads. Figures 2 and 3 pre-
sents AFM images obtained of the top of the different beads and
their corresponding surface analysis, confirming the smooth
topography of the PLA beads and the important roughness
of the different PHA beads. At such scan size, a roughness
between ~ 130 and 210 nm is obtained for the three PHA par-
ticles whereas a roughness of only 25 nm is obtained for the
PLA. This observation is well correlated with the maximum
height (Rmax parameter) measured on these images, show-
ing a large distribution in height for the PHA particles (up
to 1000 nm) and a smaller height distribution for the PLA
particle (Rmax up to 200 nm). The roughness of the PHA
particles can be related with the semi-crystalline character of
the polymer. Finally, it should also be mentioned, despite the
difference in crystallinity rate between PHBHV and PHB-
HHx samples, no difference in RMS (considering scan area
of 25 µm²) were measured between the different PHA beads.

Thermal Properties

The thermal properties of four types of microbeads are
reported in Table 4 and the thermograms presented in Fig. 4.
Characteristic values are determined from the first heating
to observe the effect of the elaboration process on the final
properties of the beads. PHA microbeads have a glass transi-
tion temperature around 0 °C, with the lowest Tg observed
for the 11% HHx content PHA beads. PHBHV exhibits a
melting temperature at about 173 °C. In the case of the PHB-
HHX beads, two melting peaks were detected, at 125 and
144 °C for PHBHHx (6%) and 109 and 137 °C for the PHB-
HHx (11%) respectively. These two melting peaks could be
attributed to the melting temperature of the PHB segment and
the PHHx segment, as reported in other works [26, 27,
40, 49]. Nevertheless, melting phenomenon could be very
complex; an apparent double peak could also be the result
of an exotherm peak superimposed on an endotherm peak
(crystalline perfection through melting/recrystallization)
works. The proposed interpretation of the double melting
peaks is not straightforward and can also be explained by
other complex phenomenon occurring in semi-crystalline
polymer such as crystal thickening, crystal perfecting or
recrystallisation that occur simultaneously with fusion and
influenced by the heating rate [60, 61]. Crystalline reorgan-
ization upon heating can only be excluded by comparing DSC
curves recorded on the same sample (same polymer with the
same microstructure) with sufficiently different heating rate.
This crystallization study would require much more work to
be more assertive about the mechanics involved.

PLA microbeads exhibits higher glass transition tempera-
ture, around 60–63 °C, and a melting temperature of 147 °C.
These results are also in agreement with those described for
amorphous PLA microbeads obtained by emulsion-evapo-
ration in the presence of dichloromethane [34].

From these data, it appears that PHBHV sample gives the
most crystalline beads. A difference in crystalline content can
also be observed between the two PHBHHx samples, with the
most crystalline bead being the 6% in HHx content.
These data emphasize the important role of the chemical
nature of lateral chain (HV vs. HHx) in the thermal proper-
ies of the polymer material. The increase of the lateral chain
size also contributes to slightly decrease the glass transition
temperature from 2 °C for PHBHV to − 1 °C for PHBHHx
(11%). This is in agreement with the literature data since it
has been demonstrated that increasing the fraction of HV
monomers in PHBHV leads to a decrease in the glass transi-
tion temperature proportionally [62]. Similarly, it has been
shown that, increasing the percentage of HHx from 7 to 18% results in a decrease in the crystallization rate and sup-
pression of the spherulitic growth rate [27].

FTIR spectroscopy analysis was performed in order to
get access to the surface crystallinity of the different beads.
This technique is highly complementary with the DSC tech-
nique that gives an overall estimation of the crystallinity,
independently of the structural organization of the polymer
within the bead and from the center of mass. FTIR is sensi-
tive to the polymer organization on its periphery and within
a thickness of a one micron approximatively. FTIR spectra of
PHA microbeads are presented in Fig. 5. The bands at 1230,
1380 and 1724 cm⁻¹ are assigned to the crystalline part of
the PHA sample whereas those at 1186 and 1741 cm⁻¹ are
representative of the amorphous part (see Table 5). By com-
paring the intensity ratios of some of the characteristic bands
of PHA, some authors were able to evaluate a crystallinity
index and established a correlation between FTIR and
DSC measurements [63, 64]. For example, in the work of Xu
et al. [63], the crystallinity index was calculated as the ratio
Fig. 2  AFM height image of the different particles (5 µm × 5 µm), with a section profile for each bead. The mean roughness (Rq value) and the maximum height (Rmax) are also given to provide a comparison between the surface topographies of each bead, for scan areas of 25 µm².
of the crystalline band at 1380 cm$^{-1}$, assigned as the conformational band of helical chains in the crystalline phase, over the amorphous reference peak at 1453 cm$^{-1}$, assigned as methyl (CH$_3$) or methylene (CH$_2$) deformations [64]. In other studies, the crystallinity index of PHA samples was also evaluated by calculating the ratio of the absorbance peak at 1227 cm$^{-1}$ over the absorbance peak at 1184 cm$^{-1}$ [65], between the absorbance peak at 1724 cm$^{-1}$ over the absorbance peak 1453 cm$^{-1}$ [64], or between the absorbance peaks at 1380 cm$^{-1}$ over the amorphous band at 1186 cm$^{-1}$ [66].

Intensities of these ratio were calculated and reported in Table 6. It can be observed that the highest intensity ratios are obtained for the PHBHV beads, followed by the PHB-HHx at 6 and 11% respectively. These results are in agreement with the previous DSC results and confirms that the crystallinity on the surface of the particles is well correlated with their crystallinity content. The roughness of the different PHA beads is also well correlated with the crystalline aspect of the periphery of the beads, as probed by FTIR.

### Table 4 Thermal characteristics of the different microbeads determined by DSC (scanning rate of 10 °C min$^{-1}$ under nitrogen flow)

| Microbeads  | T$_g$ (°C) | T$_m$ (°C) | ΔH$_m$ (J/g)$^a$ |
|-------------|------------|------------|------------------|
| PHBHV       | 3 ± 2      | 173 ± 1    | 97 ± 2           |
| PHB-HHx (6%)| 2 ± 1      | 125 and 144 ± 1 | 58 ± 2        |
| PHB-HHx (11%)| − 1 ± 1 | 109 and 137 ± 1 | 40 ± 6       |
| PLA         | 61 ± 2     | 147 ± 1    | 22 ± 1           |

$^a$Values of melting enthalpy, ΔH$_m$, are given as raw data and not converted into crystalline content because of the absence of reference for a PHA with 100% crystallinity.

### Mechanical Properties

Nanoindentation measurements directly on the microbeads, with the MTS equipment were not successful since the beads were not clearly identified within the epoxy resin. Thus, nanoindentation test have been performed here directly on aggregates of pellets, more distinguishable within the
epoxy resin due to their large size (few millimeter) and white color. In parallel, PFQNM experiments allow a more direct analysis of the mechanical properties of the beads since indentation experiments are conducted directly on the top of the different microbeads. Nanoindentation measurements on pellets were performed in order to have a reference for comparison.

Fig. 4 DSC curves of the different types of microbeads during first heating. PHBHx (blue), PHBHx 6% (black), PHBHx 11% (red) and PLA (green) (Color figure online)

Fig. 5 A FTIR spectra of the different PHA bead surfaces showing the different peaks of interest. PHBHx (blue), PHBHx 6% (green) and PHBHx 11% (yellow) and B zoom showing the variation of the intensities of the 1230 cm⁻¹ peaks for the different PHA, used to calculate the crystallinity index (Color figure online)

Table 5 Identification of the representative FTIR peaks for PHA

| Frequency (cm⁻¹) | Description                                                                 | References                  |
|-----------------|------------------------------------------------------------------------------|-----------------------------|
| 1741            | Stretching vibration of the amorphous carbonyl group                         | [63–65, 67, 68]             |
| 1724            | Stretching vibration of the crystalline carbonyl group                        | [63–65, 67, 68]             |
| 1453            | Methyl (CH₃) or methylene (CH₂) deformations in the amorphous phase          | [64]                        |
| 1380            | Symmetric wagging of CH₃ in the crystalline phase                            | [63–65]                     |
| 1227–1230       | Absorption of helical (α-type) crystals (C–O–C stretching modes of the crystalline components) | [63, 65, 69]                |
| 1184–1186       | Asymmetric C–O–C stretches (amorphous phase)                                | [63–65]                     |
modulus given the absence of PFQNM measurements on PHA polymers in the literature, to the best of our knowledge. Table 7 reports the values of the modulus for the different polymers pellets, as measured by nanoindentation. At first, the Young’s modulus for PLA is found to be around 5.5 GPa, in agreement with the literature value [70]. If we compare the three PHA samples, the two PHBHHx samples give a lower Young’s modulus as compared to the PHBHV. For the PHBHV polymer, a Young’s modulus of 7.5 GPa has been found, similar to the results of Chick et al. on injected PHBHV polymer [48]. The highest modulus found for the PHBHV sample can be related with its high crystalline content. In contrast, for the PHBHHX samples, a lower Young’s modulus was found for the 11% content in HHXmonomer, in comparison with the 6% content. If this observation is opposite with the crystalline content of both samples, it gets along with the work of Voyiadjis et al. [71], who studied the mechanical properties of PEEK semi-crystalline polymers. They observed a softer response of the amorphous polymer part as compared to the crystalline regions.

Table 7 Young’s modulus and hardness of the different polymer materials (pellets) as measured by nanoindentation

| Microbeads | Modulus (GPa) | Hardness (GPa) |
|------------|---------------|----------------|
| PHBHV      | 6.05 ± 0.8    | 0.26 ± 0.06    |
| PHBHHx 6%  | 2.46 ± 0.5    | 0.10 ± 0.02    |
| PHBHHx 11% | 1.49 ± 0.2    | 0.07 ± 0.01    |
| PLA        | 4.94 ± 0.2    | 0.31 ± 0.02    |

Finally, the hardness of the different samples, extracted from the nanoindentation measurements using Oliver and Pharr [59] theory, are shown in Table 7. The results reveal that both PHBHHx samples have the lowest hardness in contrast with the PHBHV or the PLA samples. The observed trend for PHA samples is also well correlated with the crystalline state of the different samples, the more crystalline being the tougher. For the PLA sample, which is less crystalline than the PHA sample, the mechanical properties can be explained by its high glass transition in comparison with the PHA samples. It has been reported that the intrinsic stiffness of glassy polymers below the T_g may lead to microhardness values larger than those obtained for semi-crystalline polymers [74].

Stability in Aqueous Medium and Biodegradability in Marine Environment

The stability of these particles in aqueous medium, with various conditions to mimic ageing conditions for cosmetic products (room temperature, room temperature in the presence of light and accelerated ageing at 45 °C) was observed by measuring the pH of the solution during a period of 3-months storage. Measurements of pH versus times may provide information on the stability of biodegradable polymers in aqueous media. The hydrolysis of polyesters (PLA, PHA for example) results in the formation of carboxylic acid molecules and alcohol. A decrease of pH (acidification), due to the formation of carboxylic acid functions, can thus be a direct measurement to follow the hydrolytic degradation of these polyester chains. For a stable polyester in an aqueous medium, the pH should not change over time. A blank sample, without beads, is used as a control to ensure a stable pH over the period and conditions of investigation.

The comparison between the PHA and PLA microbeads allows to observe different trends. On the one hand, the pH variation for the solution containing the PHA microbeads was only slightly decreasing, with a pH variation inferior to 1 pH unit. For example, after 3 months at room temperature, a pH of 3.9, 3.7 and 3.8 for PHBHV, for PHBHHx (6%), for PHBHHx (11%) were measured respectively while the starting pH of all solutions was measured at 4.2. Similar results were obtained when the PHA microbeads suspensions were
exposed to the 45 °C aqueous solution (with a pH of 3.8, 3.6 and 3.7 for PHBHV, PHBHHx (6%) and PHBHHx (11%) respectively). A more pronounced decrease of the pH, but still less than 1 pH unit, was recorded after the immersion of the microbeads in the solution at room temperature but exposed to light (pH of 3.5, 3.6 and 3.6 for PHBHV, PHBHHx (6%) and PHBHHx (11%) respectively). On the other hand, the pH of the PLA suspension is decreasing of 1 pH unit for the aqueous solution stored under normal conditions and for the aqueous solution, stored at room temperature with light exposure conditions (from a pH = 4.4 down to a pH = 3.4) and of 2 pH unit for the 45 °C aqueous solution conditions (from pH = 4.4 to 2.4). The more important acidification of the solution containing PLA microbeads can be correlated to a more important degradation state. We can thus deduce from this test that PHA microbeads are less degradable and consequently more sable, under the observed conditions, than PLA.

Figure 7 shows the biodegradability of the different microbeads in seawater. The biodegradation has been performed on marine environment (seawater + sediments) at 25 °C, according to the NF EN ISO 19679 standard. As expected, PLA microbeads are poorly biodegradable in these conditions since their biodegradation degree is only about 20% after 250 incubation days. It has been previously demonstrated that PLA is relatively stable, due to its glassy state at 25 °C, as long as the medium temperature does not exceed the PLA glass transition temperature, i.e. about 55 °C [75]. Concerning the PHA series, significant differences on the biodegradability can be noted depending on the chemical
PHA structure. The PHBV turns out to be the most biodegradable as its biodegradability percentage reaches 90% after 250 days of immersion. For the PHBHHx, the composition of monomer units also influences the biodegradability since the PHBHHx with 11% in HHx biodegrades faster than the PHBHHx with 6% in HHx. The biodegradation rate is 80% for the first and 62% for the second. It is very interesting to note in Fig. 7 that all these PHA present a biodegradability greater than, or close to cellulose, the reference sample. The beginning of the biodegradation is even faster for the 3 PHA, as compared to cellulose.

This spectacular PHA biodegradability is explained by the action of some marine microorganisms such as bacteria which excrete extracellular PHA degrading enzymes, i.e. PHA depolymerases, that hydrolyze water-insoluble PHA chains into water-soluble forms [76]. The resulting products are finally metabolized into the cells and utilized as nutrients [20]. At this temperature, the microbead degradation is therefore managed by an enzymatic degradation which is a heterogeneous surface reaction. Previous studies have revealed the presence of two PHA degradation mechanisms occurring in parallel (enzymatic degradation and chain scission by hydrolysis) but the enzymatic degradation is largely predominant at 25 °C in marine ageing conditions [75]. This process, whatever the sample shape, takes place in the presence of PHA depolymerase involving two steps: the first step involves the adsorption of the enzymes on the surface by the binding domain of the enzymes and the second step involves the enzymatic cleavage of polymer chains by the active sites of the enzymes [77].

The slight differences in terms of biodegradation between the three PHA studied are more complicated to explain since the biodegradation is a combination of physical, chemical and biological phenomena leading to the material dissolution by enzymatic action of microorganisms. More experiments are needed in order to understand the role of the extrinsic (correlated to the medium) and intrinsic (relative to the polymer) parameters influencing the biodegradation process. It might be relevant to identity and quantify the microorganism population which specifically colonizes the microbeads surface during the test [78]. The diversity of microorganisms associated with these different stages of biodegradation is not yet characterized but this is being further explored in order to better understand the mechanisms. Likewise, the surface morphology of the different PHA has also to be studied in order to correlate the surface properties with the colonization and then the biodegradation. In this study, the PHBV with 3% in HV is the most biodegradable while being the most crystalline compared to the two other PHBHHx. This could a priori constitute a surprising result even if some bibliographic data show that the substrate binding domain of the enzyme is capable to bind to the crystalline
PHA material. PHA depolymerase being an enzyme made up of a catalytic domain and a substrate-binding domain, both these domains are connected by a linker domain. Subsequently, the catalytic domain starts to cleave the single crystals which can be enzymatically hydrolyzed [21]. Other parameters relative to PHA will have to be explored as the hydrophilic/hydrophobic balance of the surface as well as the surface porosity. Understanding the mechanism of PHA degradation and the factors that affect its degradation will help the researcher in designing suitable material for the specific needs.

**Conclusion**

The emulsification-evaporation method allowed the preparation of spherical micrometer PHA beads with tunable materials properties, surface morphologies and related degradation behavior. Using different chemical PHA structures, we showed that the different beads have properties and surface morphologies that are governed by the crystalline organization of the polymer chains within the beads, thus able to provide suitable abrasive and mechanical properties for cosmetics applications. Since pollution of aquatics systems by microplastics should be stopped, the degradation behavior of these PHA microbeads were further tested in marine environment. Biodegradation experiments reveal that the degradation rate and kinetic were faster than those of cellulose polymer, considered as the most biodegradable polymer materials. They also suggest the crucial role of the crystalline content in the degradation process of PHA beads. Very interestingly, these PHA particles are stable in aqueous media commonly used in cosmetics while being rapidly biodegradable in the marine environment. By combining these two behaviors, they thus offer ideal characteristics for the development of microbeads in cosmetics. In addition, the use of different PHA structures allows to tune the surface morphologies, the mechanical and biodegradable properties of PHA beads. This possibility provides an effective and promising approach to replace conventional plastic beads from formulation in cosmetic products.

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