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PLGA-based microparticles: Towards an elucidation of the drug release mechanisms

Microparticules à base de PLGA : Vers une élucidation des mécanismes de libération de principes actifs

THESE

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LIST OF ABBREVIATIONS

API(s)	Active pharmaceutical ingredient(s)
DDS	Drug delivery system(s)
DL(s)	Drug loading(s)
DSC	Differential scanning calorimetry
EdX	Energy dispersive X-ray analysis
MP(s)	Microparticle(s)
o/w	Oil-in-water
PB	Phosphate buffer
PBS	Phosphate buffered saline
PLA	Polylactic acid
PLGA	Poly(lactic-co-glycolic) acid
PGA	Polyglycolic acid
PVA	Polyvinyl alcohol
SD	Standard deviation
SEM	Scanning electron microscopy
T_g	Glass transition temperature
TGA	Thermogravimetric analysis
X-ray μCT	X-ray microcomputed tomography

CHAPTER I - INTRODUCTION

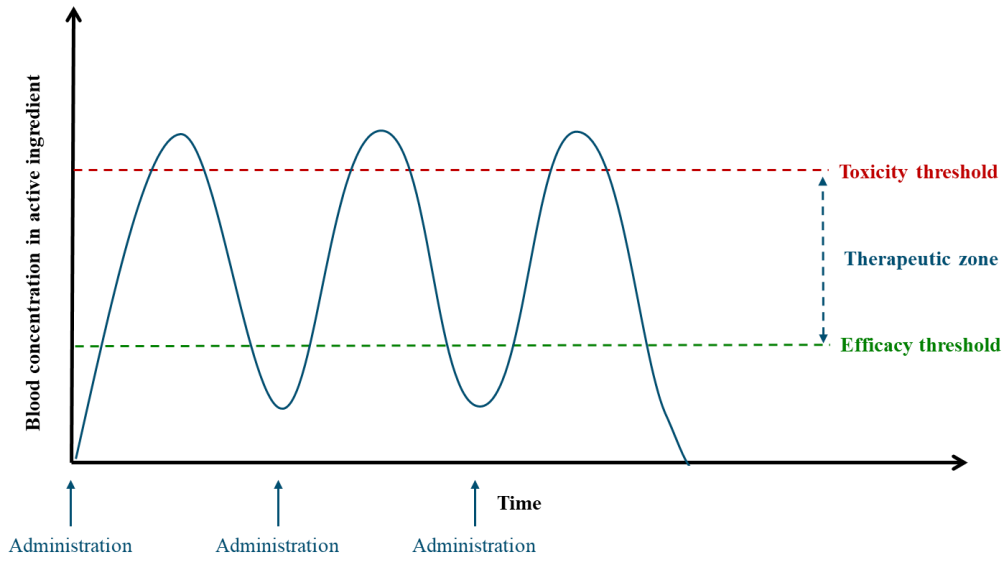
I. State of the art

Poly(lactic-co-glycolic acid) (PLGA) is a widely used polymer matrix former, especially for the development of sustained and controlled drug delivery systems (DDS) [1]–[5]. Indeed, its biocompatibility and biodegradability [6] make this polymer an interesting candidate for the pharmaceutical industry. Moreover, it is FDA (Food and Drug Administration) and EMA (European Medicine Agency) approved in drug delivery systems for parenteral administration, especially since the marketing authorization of Lupron Depot in 1989 [7] (sustained-release microspheres).

This promising polymer has the advantage of being suitable for many industrial manufacturing processes, such as 3D printing [8], [9], hot melt extrusion [10], [11], direct compression [12], [13], emulsion solvent-evaporation methods [14], [15], spray-drying [16], [17], etc.

Different types of PLGA can cover a large period of drug delivery rates, ranging from a few hours to several months [18], [19] (and even years in the case of Norplant [20]) in function of its composition. This kind of system offers the possibility of reducing the variability of drug concentrations in the bloodstream, limiting its fluctuations through precise control of the drug release. Indeed, the drug release must be in the so-called “therapeutic zone”, above the efficacy threshold but below the toxicity threshold. In the case of the administration of immediate release systems, there is a risk of excessive release causing toxic side effects, or on the contrary too low release leading to ineffectiveness of the active ingredient. The peak and trough effect induced by this type of administration is therefore not desired. In controlled drug delivery systems, the drug concentration remains constant in the therapeutic zone for a long time. Moreover, due to its long-term release, the PLGA DDS allows for a decrease in the frequency of administration and thus, patient compliance. The two types of release profiles resulting from immediate or controlled DDS are shown in Figure 1 a and b respectively.

a)



b)

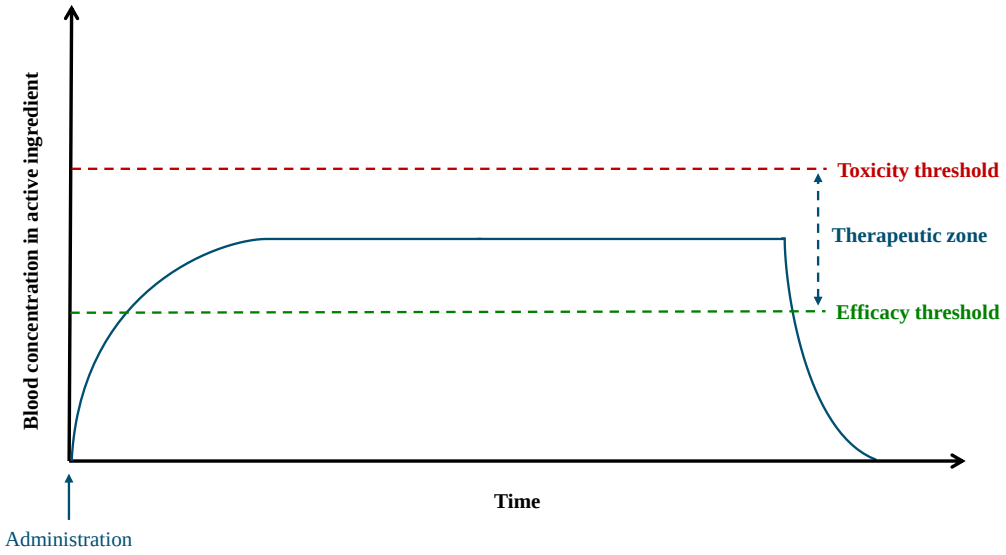


Figure 1. Drug release profiles in the case of (a) immediate or (b) controlled drug delivery systems

Besides the ability of these DDS to control the release of the active ingredient, they allow for specific organ, tissue, or cell targeting, which makes them interesting candidates in many therapeutic fields [21]. PLGA is already used in many formulations on the market, in the form of implants (Zoladex) [22], [23], in situ forming gel (Eligard) [24]–[26], films [5], [27], or nano- and microparticles [28], [29].

Amongst these types of drug delivery systems, microparticles are attracting increasing attention, because of the many advantages they bring, namely their relatively simple manufacturing process and scale-up [30], [31]. This interest is also due to the wide range of

molecules they can encapsulate [32], [33], and the large range of materials allowing for fine control of the release of the active pharmaceutical ingredient (API). This type of drug delivery system is suitable for intramuscular (Lupron Depot, Decapeptyl) or subcutaneous injection (Enantone, Eligard, Zoladex), for different indications, as the prostatic or ovarian cancers, endometriosis etc. The list of some of the marketed PLGA microparticles is presented in Table 1.

Table 1. Non-exhaustive list of marketed PLGA microparticles

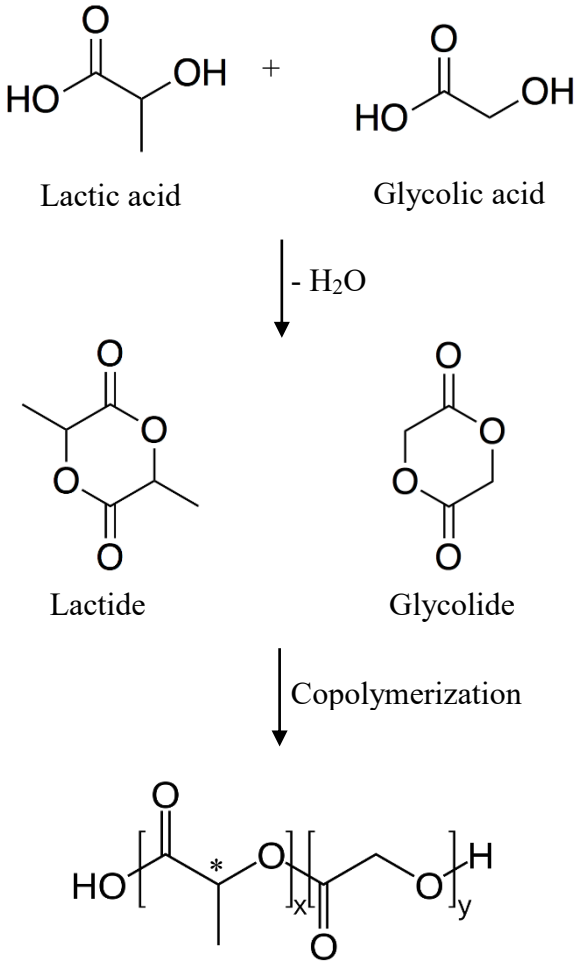
Commercial name	Active ingredient	Administration route	Therapeutic indication	Reference
ARESTIN®	Minocycline HCl	Periodontal	Periodontal disease	[34]
BYDUREON®	Exenatide	Subcutaneous	Type II diabetes	[35]
DECAPEPTYL® LP	Triptorelin	Intramuscular	Prostate cancer, endometriosis	[36]
ELIGARD®	Leuprolide acetate	Subcutaneous	Prostate cancer, endometriosis	[37]
ENANTONE® LP	Leuprorelin	Subcutaneous, intramuscular	Prostate cancer, endometriosis	[38]
GONAPEPTYL® LP	Triptorelin acetate	Subcutaneous, intramuscular	Prostate cancer, endometriosis	[39]
LUPANETA PACK®	Leuprolide acetate & norethindrone acetate	Intramuscular	Endometriosis	[34]
LUPRON DEPOT®	Leuprolide acetate	Intramuscular	Prostate cancer, endometriosis	[34]
PROFACT®	Buserelin acetate	Subcutaneous	Prostate cancer	[40]
RISPERDALCONSTA® LP	Risperidone	Intramuscular	Schizophrenia	[41]
SALVACYL® LP	Triptorelin embonate	Intramuscular	Deviating sexual behavior	[42]
SANDOSTATINE®	Octreotide acetate	Intramuscular	Acromegaly	[43]
SIGNIFOR® LP	Pasireotide diapsartate	Intramuscular	Cushing's disease	[44]
SOMATULINE® LP	Lanreotide acetate	Intramuscular	Acromegaly	[45]
TRELSTAR®	Triptorelin pamoate	Intramuscular	Prostate cancer	[34]
TRIPTODUR®	Triptorelin pamoate	Intramuscular	Central precocious puberty	[34]
VIVITROL®	Naltrexone	Intramuscular	Alcohol & opioid dependence	[34]
ZILRETTA®	Triamcinolone acetonide	Intra-articular	Osteoarthritis	[34]
ZOLADEX®	Goserelin acetate	Subcutaneous	Prostate and breast cancers	[22]

Despite the fact that PLGA is the subject of many promising studies, the mechanisms governing the release of APIs from these PLGA-based systems are not yet fully understood, which leads to complex industrial development in terms of regulation, amongst others [34]. As a matter of fact, numerous release mechanisms can be involved, due to a large variety of physico-chemical phenomena occurring when the systems release the drug [46]–[51]. The latter are themselves strongly influenced by different parameters, including the physiological environment in which the systems are [52], the type and the quantity of drug they contain, the type of polymer (molecular weight, monomer ratios, end groups), their porosity [46], [53] or their preparation method [54]–[56],… Amongst the release mechanisms of PLGA-based systems can be found: The polymer erosion, its degradation, drug dissolution, drug diffusion, water penetration, physical water- and drug-polymer interactions (e.g., plasticizing effects) [57]–[59] pore formation and closure [53], [60], osmotic effects [61], the autocatalytic phenomenon [62]–[64] or polymer swelling [52], [65]–[67].

II. General information about poly(lactic-co-glycolic) acid (PLGA)

II.1. Physicochemical characteristics of PLGA

Poly(lactic-co-glycolic) acid (PLGA) is a linear statistical copolymer, composed of a variable ratio of lactic acid and glycolic acid. This synthetic polymer is synthesized through ring-opening copolymerization from the lactide and glycolide molecules. These last two molecules are cyclic dimers obtained by the dehydration of lactic acid and glycolic acid respectively. This reaction is represented in Figure 2.



Poly(lactic-co-glycolic) acid (PLGA)

x represents the number of lactic acid units (PLA)

y represents the number of glycolic acid units (PGA)

Figure 2. Synthesis of PLGA from lactic and glycolic acid monomers

This synthesis process allows for a fine control of the monomer ratio and molecular weight of PLGA. This will be a major asset regarding the tunability of this polymer to the different drug-release rates desired.

The structure of PLGA has an asymmetric carbon (indicated by * in Figure 2) on the lactic acid part, which makes the molecule chiral and allows the formation of two enantiomers:
- D- lactic acid, for dextrorotatory lactic acid
- L- lactic acid, for levorotatory lactic acid

Generally, the D and L forms are found in equal proportion in the PLGA molecule, but if this proportion varies, we can suppose that this could have an impact on the physicochemical properties of PLGA [68].

The ending group of the PLGA chains will strongly influence the hydrophilicity or hydrophobicity of the polymer and thus its degradation, depending on whether it is a carboxylic acid or an ester.

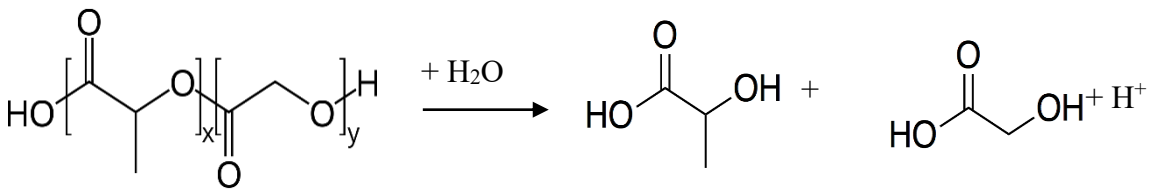
The physicochemical properties of PLGA, namely its glass transition temperature, its hydrophilicity/hydrophobicity, its mechanical strength, etc, and as a result, the drug-release rate, are directly linked to the lactic acid:glycolic acid ratio, the molecular weight and the ending chain group of PLGA, amongst others [69].

II.2. Biodegradation and biocompatibility of PLGA

II.2.1. PLGA biodegradability and impacting factors

Biodegradable qualifies a material which undergoes an *in vivo* physical or a chemical breakdown of its structure into low-weight compounds, either enzymatically or non-enzymatically, leading to non-toxic by-products [70].

The degradation of PLGA consists in a cleavage of the ester bonds by water molecules, leading to the formation of oligomers and then monomers of lactic and glycolic acid units as degradation products. These products are non-toxic for the organism and are mainly eliminated through urine or metabolized into CO_2 and H_2O after incorporation into the Krebs cycle. However, as the degradation products are acidic, they can potentially have negative effects on certain tissues if they are not rapidly eliminated. The PLGA degradation reaction is represented in Figure 3.



Poly(lactic-co-glycolic) acid (PLGA) Lactic acid Glycolic acid

x represents the number of lactic acid units
y represents the number of glycolic acid units

Figure 3. PLGA degradation by hydrolysis of its ester bonds

The PLGA degradation kinetic is known to follow a pseudo-first order kinetic [71]. Indeed, the PLGA concentration remains constant during the degradation reaction, and the global degradation rate depends only on the degradation of PLGA. This describes a first-order reaction. However, PLGA also acts as its own degradation catalyzer, as its degradation products catalyze its degradation; in this case, the reaction follows a pseudo-first order kinetic.

The first step of the PLGA degradation involves the penetration of water into the system, which interacts with the polymer forming hydrogen bonds and inducing the disentanglement of the chains. This mechanism leads to a decrease in the glass transition temperature of the PLGA due to the plasticizing effect of water.

The second phase of the degradation consists of the cleavage of covalent ester bonds in between the monomers, leading to a decrease in the molecular weight.

The third step is a more important degradation of PLGA due to the autocatalytic effect induced by the carboxylic end groups, which catalyze the cleavage of the backbone covalent ester bonds, leading to an important molecular weight loss.

The last part of the degradation process is the solubilization in the aqueous environment of the monomers of lactic and glycolic acids, obtained after the complete cleavage of the corresponding oligomers.

Many factors can impact the degradation process of PLGA, and the main ones are shown in the list below. The most significant of these will be described in the following.

Factors impacting the PLGA hydrolytic degradation behavior:

- Chemical composition
- Molecular weight
- Physicochemical factors (ion exchange, pH)
- Additives (acidic, basic, drugs)
- Porosity
- Device dimension and shape
- Mechanism of hydrolysis (non-catalytic, autocatalytic, enzymatic)
- Water permeability and solubility (hydrophilicity/hydrophobicity)
- Morphology (crystalline, amorphous)
- Glass transition temperature (glassy, rubbery)
- Sterilization
- Site of implantation

II.2.1.1. Effect of the PLGA chemical composition

As previously mentioned, the ratio of lactic acid:glycolic acid can be modulated to tailor the degradation rate; The more lactic acid there is, the more hydrophobic the polymer is and therefore the slower the degradation will be. The drug-release rate will thus be delayed and slowed down. On the contrary, the more glycolic acid monomers there are, the more the degradation will be accelerated and the faster the drug-release [1], [72]. Indeed, glycolic acid units are more hydrophilic, and more prone to attract water than lactic acid units which has an additional methyl group.

The terminal group of the polymer chain will also have a strong impact on the degradation rate, which can then potentially impact the release rate of the API. Indeed, a chain ended by a carboxylic acid group will be less resistant to hydrolytic degradation than a chain ended by an ester. This will result in a faster degradation of the polymer and therefore a faster release of the API [1], [73]. The acidic termination of the chain will also lead to a catalytic effect more pronounced than for chains ended by ester functions [74], [75].

II.2.1.2. Effect of the molecular weight

There are many commercial PLGA, with molecular weights ranging from a few kilodaltons (around 5 KDa) to about 150 KDa [76]. In the last few years, it has been known that the molecular weight of the polymer has a significant impact on the physicochemical properties of PLGA, and is in close relation to its degradation rate. Indeed, low-molecular weight polymers have small chains, which are less hydrophobic and then cleaved faster than high-molecular weight polymer chains. This results in a higher initial burst release and thus a faster drug-release, because of the facilitated diffusivity of the drug through the short polymer chains. On the contrary, the hydration process of the chains is longer for high-molecular weight polymers so the degradation occurs later, as well as the drug-release. These two-type release profiles, as a function of the polymer molecular weight, is represented in Figure 4.

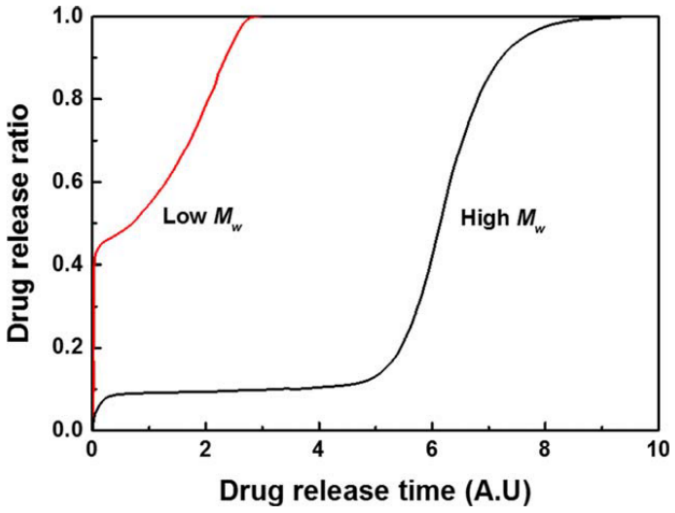


Figure 4. Theoretical drug-release profiles from high or low-molecular weight PLGA-based polymers, adapted from Xu et al., 2016 [76]

II.2.1.3. Effect of pH

The degradation of PLGA DDS will be impacted by the environment in which they are located. Indeed, the ester bonds of PLGA are sensitive to protons, which will catalyze the cleavage of the ester bonds. As described in Figure 3, the degradation process of PLGA produces the formation of lactic and glycolic acid monomers, which, as their name indicates are acidic, and the release of H^+ ions. This will contribute to acidify the release medium, and the protons will accelerate the cleavage of the ester bonds of the polymer chains, leading to a faster degradation. This phenomenon is called autocatalysis, due to the chain reaction induced by the degradation products of PLGA which increase the degradation of the polymer itself [62].

The catalysis of PLGA chains can occur not only in acidic but also in basic media. It has been demonstrated that the acidic degradation will lead to a physical degradation of PLGA, e.g. a loss of integrity of the DDS, whereas the basic degradation will lead to a chemical degradation of PLGA, releasing glycolic and lactic acids [77]. Basic media will also be capable of neutralizing the acidic degradation products and then slowing down the degradation process [46]. Moreover, the solubility of oligomers is increased when the medium is alkaline and very low at an acidic pH. Therefore, the molecular weight loss will be more important in a basic environment [78].

II.2.1.4. Effect of the drug

As seen previously, the acidic or basic environment in which the PLGA-based DDS is found has a strong impact on the degradation mechanisms. Accordingly, the acidic or basic nature of the active ingredient loaded in the DDS will also have an impact on the PLGA degradation. Indeed, an acidic drug will increase locally the pH of the DDS, increasing in turn the acidic hydrolysis of the PLGA ester bonds. A basic drug can accelerate the degradation of PLGA *via* basic catalysis, independently of the polymer end chain and molecular weight [79].

In addition, the physicochemical properties of each API can significantly affect the characteristics of the DDS and therefore its release behavior [80]–[82]. For example, if the drug acts as a plasticizer, the degradation of the chains will be facilitated by their lowest rate of entanglement. By decreasing the entanglement, the glass transition temperature of the polymer is lowered, allowing the polymer to change from a glass state to a rubbery state, which induces its fast degradation. Furthermore, the drug loading of the DDS will therefore have consequences on the intensity of the effects described above [50]. Hydrophilic drugs may also play a role in the PLGA degradation process by attracting water in the matrix.

II.2.1.5. Effect of porosity

The porosity of PLGA DDS is a characteristic to be considered regarding the penetration and diffusion of water through the matrix. In fact, a porous network will facilitate the penetration of water into the matrix, known to degrade the polymer, but it will also allow the diffusion of the acidic degradation products responsible for the decrease in the local pH. As a consequence, the autocatalytic effect will be more important in non-porous DDS, in which the acidic products cannot be eliminated from the matrix [46].

II.2.1.6. Effect of the matrix size and shape

The size and shape of the DDS strongly impact the degradation behavior of PLGA. The accessibility of water being the essential factor in the degradation mechanisms, these two aspects will inevitably influence it. It has been demonstrated several times that the larger the system, the longer the diffusion path from the core of the system to the medium for the acidic oligomers, and therefore the higher the internal pH of the system. This leads to an increase in the autocatalytic effect and therefore, to degradation [46], [83], [84]. According to this reasoning, degradation is hence more rapid at the core of the system than at the surface [85], [86].

II.2.1.7. Effect of enzymes

Concerning the potential enzymatic degradation of PLGA in biological fluids, controversial conclusions are given in the literature, but most would argue that there may be an effect of enzymes, even if minimal, on the degradation process. Research agrees that hydrolytic degradation of PLGA is predominant over enzymatic degradation, but the role of the latter is disputed. Some studies have shown that enzymes such as proteinase K, tissue esterases, pronase, or bromelain are involved in the degradation of lactic acid units [87], [88]. It has also been shown that the DDS access of enzymes degrading the polymer, which are hydrophilic, is facilitated if the water uptake is high [89]. The degradation could then be enhanced if the polymer is hydrophilic and the DDS is porous.

PLGA degradation is therefore a relatively complex phenomenon, influenced by several factors, which need to be considered to better understand the drug release from PLGA-based DDS.

II.2.2. PLGA biocompatibility

A biocompatible material is defined as a material performing its desired biological function in a specific application without causing any local or systemic toxic reaction, injury or rejection by the immune system [90], [91]. Biocompatibility does not characterize the intrinsic property of the material alone, but rather the property of the formulated material in a specific environment. Any degradation by-products of these compounds must also meet these criteria.

PLGA respecting all these aspects for several therapeutic applications, it has been approved by the FDA, making it a polymer with high potential in drug delivery.

III. PLGA in advanced drug delivery systems

PLGA-based drug delivery systems encompass a variety of formulations and structures designed to target the delivery of therapeutic molecules efficiently and with precise control. They have shown promising advances in improving drug efficacy, reducing side effects, and increasing patient compliance in various medical fields, such as cancer therapy, vaccine delivery, tissue engineering, biotechnology, theranostics, or even cosmetics.

PLGA-based systems can be distinguished into two subclasses, depending on the initial drug concentration in the system [92]:

- If the concentration is below the drug solubility: All the drug molecules are directly dissolved in the matrix or in the core (for matrix and reservoir systems, respectively), or they are dissolved when water has penetrated the system.
- If the drug concentration is higher than the drug solubility: A part of the drug molecules is dissolved in the system, but the excess drug is dispersed. The dispersed particles can be drug crystals or aggregates of amorphous drug. In that case, only the dissolved drug is able to diffuse when water penetrates the system.

Some of the most commonly developed PLGA-based drug delivery systems are listed in the following part.

III.1. Implants

PLGA-based implants are generally used as long-term treatments for chronic conditions. They are mostly implanted subcutaneously or intramuscularly, and allow the delivery of drugs over weeks or months. The advantage of PLGA in this type of DDS over other non-degradable polymers is that, as PLGA is a biodegradable polymer, the implant does not need to be removed once the active ingredient has been released, which improves patient compliance. Two types of implants can be found: *In situ* forming implants, or implants obtained by extrusion.

III.1.1. In situ forming implants

The special feature of this type of implant is that the initial formulation is not solid, but semi-solid or even liquid. It is only once this formulation is placed at the site of administration that, through solvent exchange with the biological tissues and fluids or a change in temperature inducing gelation, the implant is created by hardening. This type of DDS offers several advantages, including a facilitated administration (injection instead of implantation surgery), a

localized drug delivery, the avoidance of uncomfortable surgical implantation operations, and tunable properties [25], [93].

III.1.2. Implants obtained by extrusion

Drug delivery systems or medical devices can be proceeded by an extrusion manufacturing technique. PLGA and drug(s) are fed into a heated barrel that melts the polymer, and a totative screw pushes the molten PLGA through a specific shaped and sized die. This technique allows for the preparation of various-sized PLGA implant designs, which are tunable for specific applications with further processes [94].

III.2. PLGA conjugates

PLGA are biomedical materials that can be used as a backbone chemically linked to specific ligands or antibodies, creating targeted drug delivery systems that enhance drug uptake by specific cells or tissues. The degradation of PLGA over time allows for the progressive release of the attached molecules, prolonging the therapeutic effect and reducing the number of administrations. PLGA conjugates can enhance stability compared to the molecules alone, leading to a longer shelf life for formulated drug products [95]–[97].

III.3. Hydrogels

PLGA hydrogels are crosslinked networks, involving the polymer and a hydrophilic component (such as polyethylene glycol, hyaluronic acid, chitosan, or polyvinyl alcohol), which enhance water uptake and induce the creation of a water-swollen and gel-like structure. The drug can be directly added to the hydrogel matrix. The mechanical properties of hydrogels can be tuned by adjusting the composition and crosslinking density. This type of DDS is mainly used in tissue engineering due to its ability to mimic the extracellular matrix [98], [99].

III.4. Nano-sized DDS

PLGA nanoparticles are the subject of growing attention due to their unique properties and versatile applications, allowing for targeted drug delivery [100], [101]. They enable the encapsulation of a wide range of molecules, such as hydrophobic and hydrophilic drugs, or drugs sensitive to heat or pH, including peptides, proteins, nucleic acids, amongst others [28]. Controlled drug delivery can be tailored from a few days to several months by easily adjusting their size and morphology, the PLGA composition and molecular weight, but also the drug loading [101].

III.4.1. Nanoparticles

The term nanoparticle can refer to nanospheres or nanocapsules, which are submicron-sized particles. Nanospheres are solid spherical nanoparticles, whereas nanocapsules are core-shell nanoparticles with a core containing the drug surrounded by a PLGA shell. In the case of nanospheres, the drug is combined with the polymer matrix, while in the case of nanocapsules, the drug contained in the core is protected by the PLGA shell, avoiding direct contact with the surrounding environment until the polymer degrades and releases the drug [102]–[104].

III.4.2. Nanofibers

Nanofibers are thin, long, and flexible structures mimicking biological tissues, and characterized by a high surface area-to-volume ratio, ideal for high drug loading of hydrophobic or hydrophilic APIs, and for cell interactions in tissue engineering applications [105], [106].

III.5. Microparticles

Microparticles are the most common PLGA-based DDS [29], [47]. They are widely used in various biomedical applications, and can provide systemic or targeted drug delivery. They are generally in the range of a few micrometers to several hundred micrometers. As other PLGA DDS, they offer a controlled drug release ranging from a few days to several months, thereby reducing administration frequency and improving patient compliance. In addition, they provide protection from the surrounding environment (degradation, enzymatic activity, etc.) for sensitive drugs, improve drug stability, and can encapsulate a broad range of APIs [107]. PLGA microparticles feature tailorable properties, including their size, shape, drug loading, other properties that will be engendered by preparation parameters (e.g. porosity), but also the degradation rate. The latter will be influenced by the type of PLGA selected, depending on its monomer ratio, its molecular weight, its terminal group, etc. PLGA microparticles can be administered through several routes, which can be invasive (e.g. intramuscular, subcutaneous injections) or non-invasive (e.g. oral, pulmonary, or transdermal delivery). Microparticles are found in several applications, such as oral, gene, nasal, intratumoral, buccal, ocular, gastrointestinal, colonic, vaginal, and transdermal drug delivery, amongst others [107], [108].

Microparticles can be divided into two categories [109]:

- Matrix systems: In this case, microparticles can be called microspheres, which are solid particles encapsulating drugs within their solid matrix. The drug is dissolved or dispersed homogeneously throughout the microsphere.
- Reservoir systems: In this case, microparticles can be called microcapsules, which are core-shell structures, where the core (liquid or solid) is surrounded by a protective shell. In this type of microparticle, drugs are encapsulated in the core, and not distributed uniformly within the entire microparticle.

These two microparticles' categories, based on their resulting drug delivery mechanisms, are schematically presented in Figure 5.

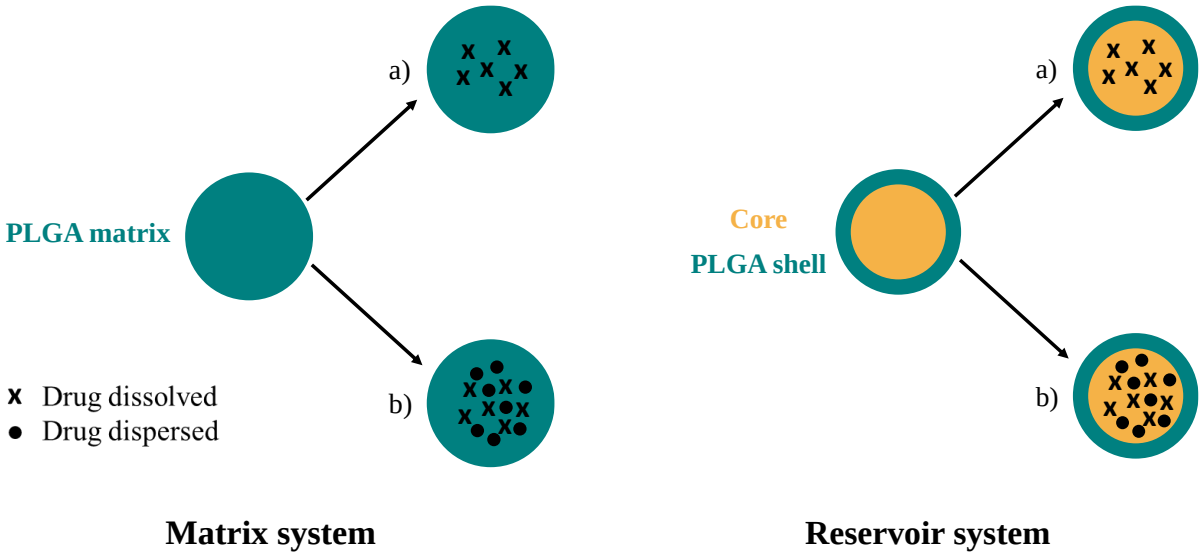


Figure 5. Schematic presentation of the microparticles' matrix and reservoir systems where a) the drug is totally dissolved in the system or b) a part of the drug is dissolved in the system and the other part is present in the form of crystals or aggregates of amorphous drug. (Adapted from Siepmann et al. 2008 [92]*)*

In matrix systems, the drug is homogeneously dispersed through the matrix; This type of distribution will be more favourable to the release of the drug by diffusion as the PLGA degrades, resulting in a controlled and sustained drug release profiles. The fact that the drug is uniformly distributed through the matrix will lead to consistent drug release rates. In the case of reservoir systems, the drug is only located in the core of the microparticle, giving it a higher drug loading capacity compared to the matrix system. This type of system offers protection for drugs sensitive to some surrounding environments. The drug is released mainly by degradation

of the PLGA shell or by diffusion through it. Due to the protective shell around the core filled with drug, the release is more sustained than in matrix systems, because of the time required for the shell to degrade before the drug is accessible to the release medium. The drug release mechanisms will therefore be different depending on the microparticle type [109]. Moreover, PLGA microparticles offer the possibility of associating them with other DDS (e.g. hydrogels [110], implants [111], etc.), or they can be produced by combining them with other materials (e.g. polymers [112]), thus providing additional ways for improving drug delivery control.

In this manuscript, the focus will be on the study of microspheres, e.g. matrix systems. Some of the most commonly used preparation methods for producing microspheres are presented in the following section.

IV. Preparation of PLGA microparticles

The microparticles can be prepared in different ways, depending on the desired final characteristics. Depending on whether the drug is hydrophilic or lipophilic, on their desired size, on the intended use, and on the type of drug release requested (e.g. more or less sustained), one method may be more suitable than another. However, the preparation methods must all respect some requirements [68], [113]:

- The drug's stability and biological activity should not be compromised during both the encapsulation process and in the final product
- High yields of microparticles within the desired size range (preferably less than 125 μm) and high drug encapsulation efficiency should be obtained
- Consistency in microparticle quality and drug release profile is essential, and they should be reproducible within specified limits
- The microparticles should present the characteristics of a free-flowing powder, exhibiting no aggregation or adhesion between them

A number of techniques are available for the microencapsulation of drugs into PLGA particles. The most commonly used are the emulsion-solvent evaporation/extraction methods, phase separation, and spray drying. However, promising new manufacturing techniques that can meet many of the previously mentioned requirements (particularly reproducibility) are emerging, such as microfluidics.

IV.1. Conventional emulsion-solvent evaporation/extraction technique

IV.1.1. Single emulsion method

This method is principally used for the encapsulation of hydrophobic or poorly water-soluble drugs, thanks to an oil-in-water (o/w) emulsification process [29], [32], [114]. PLGA is dissolved in a volatile organic solvent, which is immiscible with water. In this organic phase, the drug can be dissolved or suspended. The resulting mixture is emulsified in a large volume of water in which is present a surfactant. Upon solvent evaporation at elevated temperatures or by extraction in the large amount of aqueous phase, the microparticles are formed by precipitation. The final morphology and intrinsic characteristics of the microparticles are influenced by the rate of solvent removal. The latter is determined by several factors, such as the aqueous phase temperature and pressure, the nature and concentration of the surfactant, the

PLGA's solubility characteristics, the solvent, etc. The stirring speed and type of equipment, as well as the viscosity and volume ratio of the dispersed and continuous phases, can also impact the resulting microparticles [68], [115]. For example, faster solvent extraction will lead to more porous microparticles. It has to be noted that hydrophilic drugs may diffuse into the aqueous phase, resulting in a low encapsulation rate [114]. For hydrophilic drugs, the oil-in-oil (o/o) emulsification method or the double emulsion method are preferred. The latter will be described in the following part. Concerning the o/o method, PLGA and drugs are dissolved in water-miscible organic solvents. The continuous phase is composed of hydrophobic oils. As for the o/w process, the microparticles are hardened after the removal of the organic solvents through evaporation or extraction [116]. The single o/w emulsion method is schematically presented in Figure 6.

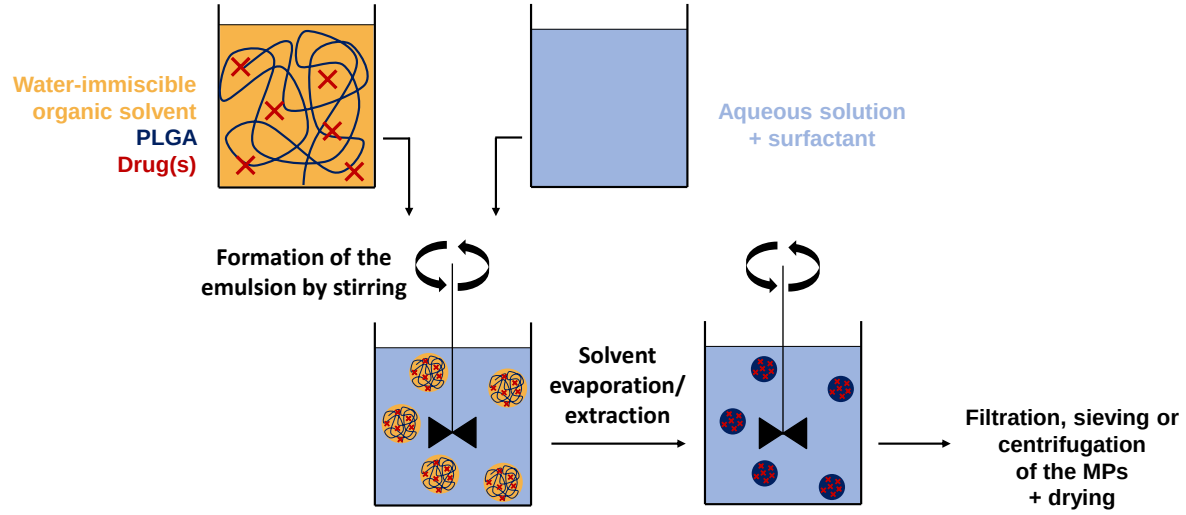


Figure 6. Schematic presentation of the single o/w emulsion technique

IV.1.1. Double emulsion method

This technique allows for the encapsulation of water-soluble drugs, thanks to the water-in-oil-in-water (w/o/w) method [68]. In this case, the drug is dissolved in an aqueous solution, while the PLGA is dissolved in a water-immiscible organic solution. These 2 phases are emulsified, leading to a primary emulsion, which is itself transferred to a large volume of another aqueous phase generally containing a surfactant, thus forming the w/o/w emulsion. The solvent removal occurs as for the single emulsion method, by extraction or evaporation [2]. The double w/o/w emulsion method is schematically presented in Figure 7.

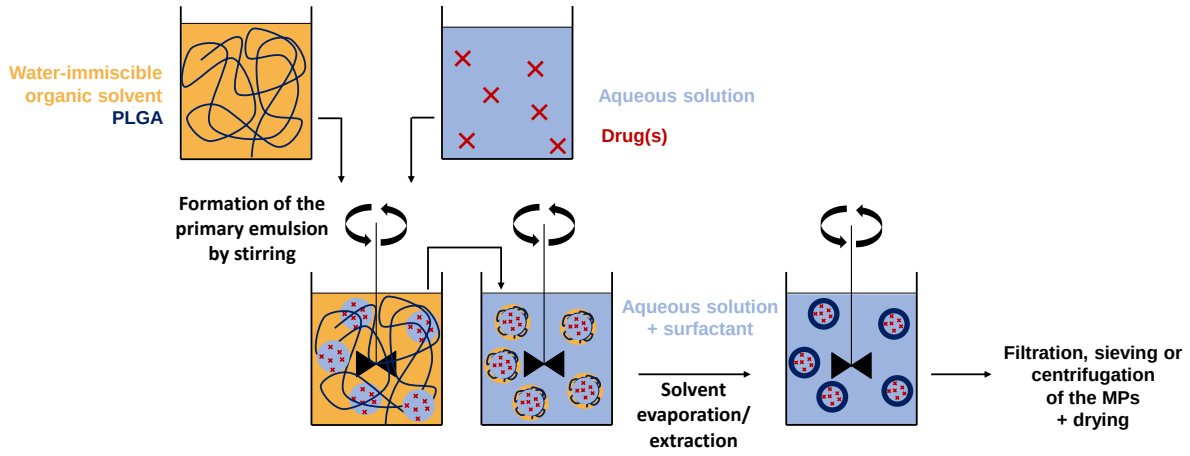


Figure 7. Schematic presentation of the double w/o/w emulsion technique

These emulsion-solvent evaporation/extraction techniques are simple to implement and allow the encapsulation of a wide range of drugs. In addition, the size and morphology of the microparticles can be tailored easily thanks to the many parameters that can be adapted [55]. However, the scale up is sometimes complex, and the quantity of components required is relatively high. Moreover, homogeneity in terms of microparticles' sizes as well as reproducibility are not always convenient, and these techniques involve a high stirring rate, which exposes the microparticles to a high shear stress which is damaging to fragile drugs. Other preparation techniques exist to try to overcome some of these issues.

IV.2. Phase separation (coacervation)

This other technique used for the microparticles' preparation involves the phase separation of a PLGA solution into two distinct phases: A polymer-rich phase (called coacervate) and a polymer-poor phase. The coacervation method is based on the decrease in the polymer solubility after the addition of a non- or anti-solvent (water or polar solvent) to the drug/polymer solution [68], [117]. The first step is phase separation: At some point, the process produces two liquid phases, i.e. the polymer-containing coacervate phase and the polymer-depleted supernatant phase. The second step is the adsorption of the coacervate around the drug particles. With the addition of a non-solvent under stirring, the PLGA solvent is gradually extracted from the droplets. The third step is the solidification of the microparticles, thanks to the addition of excess non-solvent. The non-solvent addition rate affects the rate of solvent extraction, and therefore the size of the microparticles and the drug encapsulation efficiency. The phase separation technique is schematically presented in Figure 8.

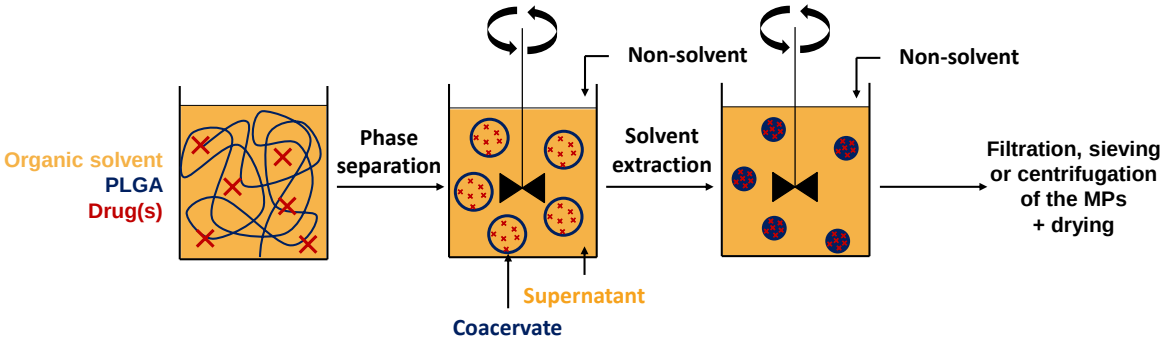


Figure 8. Schematic presentation of the phase separation method

These preparation conditions are more gentle for heat- and shear-sensitive APIs. It enables a high drug loading rate, water-soluble as well as water-insoluble drugs can be encapsulated *via* this technique. Microparticles' sizes [118] and release profiles [119] can be modified by adjusting formulation parameters (organic phase volume, stirring rate, addition rate, concentration, and type of the non-solvent), and the scalability is relatively simple. However, this technique requires optimizations for each specific drug/polymer combination and a large amount of organic solvents, which leads to difficulties removing it from the resulting microparticles, and aggregation may occur due to the absence of surfactant [68].

IV.3. Spray-drying

This widely used technique involves the atomization of a solution composed of the polymer (dissolved) and the drug (dissolved or dispersed) in an organic volatile solvent. The fine organic droplets created thanks to a nozzle are then dried by a gas stream and/or hot air, in order to evaporate the solvent, therefore hardening the droplets into solid microparticles. The latter are collected directly from the bottom of the drying chamber or, more often, through a cyclone separator. The spray-drying technique is schematically presented in Figure 9.

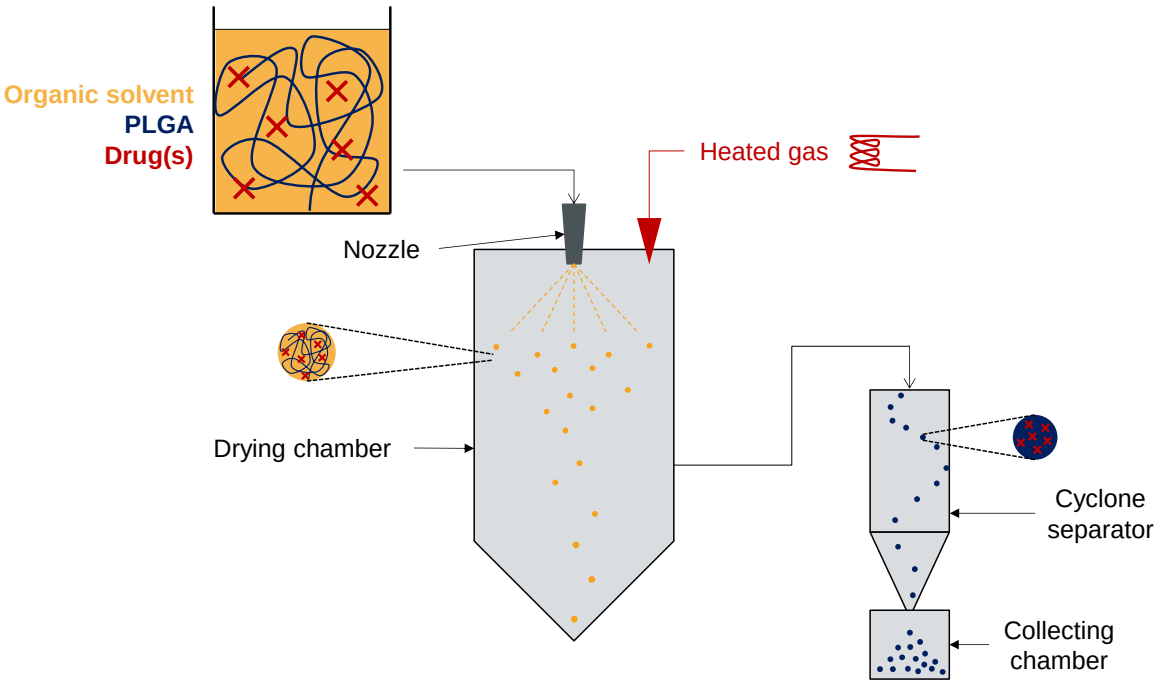


Figure 9. Schematic presentation of the spray-drying technique

This technique has the advantage of being rapid, suitable for both hydrophilic and hydrophobic drugs, easily scalable and reproducible. Spray-drying also allows for a high drug loading, is suitable for various drugs and polymers, and the microparticle characteristics (size, shape, etc.) can be controlled by adjusting parameters, including the nozzle size or the drying conditions, amongst others [120]–[122]. Nevertheless, thermally sensitive components cannot be used, the size homogeneity of the obtained microparticles is generally very low, and the yield is low as well due to an important loss of product during the process (particle aggregation and adhesion to the walls of the device).

IV.4. Microfluidics

Microfluidics is a promising method that has emerged in recent decades, rapidly evolving, which allows the preparation of microparticles, nanoparticles, emulsions, etc [123]. This technique is characterized by its capability to produce highly uniform particles in terms of size, shape, and composition [124], due to the precise and controlled production enabled by some device features, such as: Fine channels, chips with precise geometry, monitored flow rates on a microscopic scale, etc [125]. Microfluidics offers several approaches to produce PLGA microparticles, most of them are based on the formation of single, double, or multiple emulsions [126], [127], while others are based on polymeric precipitation [128].

To do so, different device geometries exist and are classified into four main categories:

- Cross-flow geometry: The dispersed and continuous phase fluids meet at a defined angle. Depending on the angle, the type of junction can be called a T- or Y-junction. These different cross-flow junctions are presented in Figure 10 a.
- Co-flow geometry: The dispersed and continuous phase fluids meet in parallel flows, either in a plan (two-dimension) configuration or in a coaxial (three-dimension) configuration. The 2D co-flow device geometry is presented in Figure 10 b.
- Flow-focusing geometry: The dispersed and continuous phase fluids meet in parallel flows, but they are hydrodynamically focused by flowing through a contraction pattern. As for the co-flow configuration, 2D and 3D configurations exist. The 2D flow-focusing geometry is represented in Figure 10 c.
- Membrane emulsification geometry: The dispersed phase is pushed through a porous membrane, after which the droplets formed are separated into the continuous phase. This membrane emulsification process is presented in Figure 10 d.

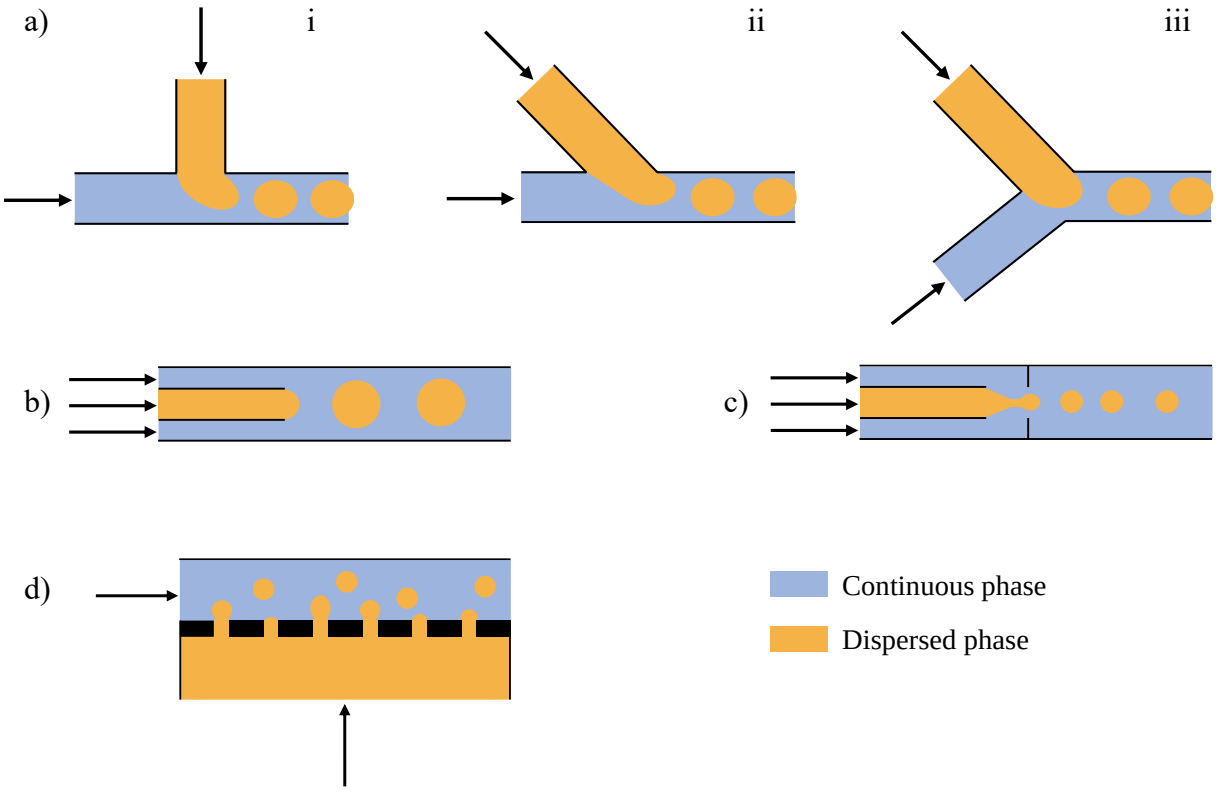


Figure 10. Schematic presentation of the mainly used microfluidics device geometries: a) Cross-flow geometries: i) Right-angle T-junction, ii) T-junction with an intersect angle <90°, iii) Y-shaped junction; b) Co-flow geometry; c) Flow-focusing geometry; d) Membrane emulsification geometry. Adapted from Zhu et al., 2016 [129].

It should be noted that the dispersed phase can be composed of a primary emulsion, leading to multiple emulsion droplets after passing through these elements. Geometries can be combined together, leading to an infinite possibility of microparticles' production. Moreover, the dispersed phase is mostly composed of a drug/polymer mixture dissolved in an organic solvent, while the continuous phase is an aqueous solution containing a surfactant. Nevertheless, depending on the desired microparticle type (microspheres, microcapsules, etc.) and characteristics (size, porosity, etc.) the dispersed and continuous phase compositions can be adapted. Tremendous other microfluidics types exist and are currently under development, only the most commonly used are described in this manuscript.

This technique is a continuous process which requires fewer reagents than conventional methods. Microfluidics is compatible with hydrophobic, hydrophilic, sensitive drugs, and diverse molecules, which allows for the highly reproducible and scalable preparation of complex structures (multi-layered, lipidic, Janus microparticles, etc [130], [131]). However, microfluidics can present some drawbacks: The production rate is quite slow, equipment can be complex and expensive, and the thin channels are susceptible to clogging, which can interrupt the continuous process.

V. Process parameters affecting microparticles' characteristics

All the microparticle manufacturing processes discussed in the previous section result in microparticles that may be intrinsically affected by certain parameters inherent in the preparation method. In this section, the focus will be on microparticles produced by the most popular method: The o/w emulsion-solvent evaporation/extraction technique. In addition, this was the manufacturing process used for the microparticles investigated in this study.

The most critical parameters, which have the greatest impact on microparticles' characteristics and on their release mechanisms, are listed in the following sections.

V.1. The blade shape and stirring speed

The shear forces produced by the stirring process will break the emulsion droplets into smaller ones, which will become microparticles after hardening. The higher the speed of the stirrer, the smaller the resulting microparticles [54], [132]. Moreover, the shape of the blade used can create more or less shear during the droplet's formation, impacting the microparticles' sizes.

V.2. The type of solvent

PLGA can be more or less soluble in organic solvents. However, the polymer solubility is a parameter affecting the precipitation process and then the encapsulation efficiency: The higher the solubility of PLGA in organic solvent, the longer it will take to precipitate, and hence, decreasing the encapsulation efficiency [133], [134]. The drug solubility in the solvent will also have an impact on the encapsulation efficiency and the physical state of the drug in the microparticle: The higher the drug solubility, the higher the drug loading, and the greater the chance that the drug will be in amorphous form [135]. The choice of solvent will also have an impact on the interfacial tension between the organic and aqueous phases, which will strongly influence the droplet formation process and therefore the characteristics of the MPs obtained [136].

V.3. The organic phase addition technique

There are several ways to perform the organic phase addition into the aqueous phase, such as an addition in a single operation, or drop by drop with a syringe and a needle. With the latter, the initial droplets are smaller and can be easily broken by the shear forces of the stirring,

leading to smaller microparticles than in the method where all the organic phase is poured directly. The size of the needle used and the rate at which the organic phase is added might also impact the final size of the microparticles. A needle with a fine diameter, and/or a fast addition rate will lead to smaller microparticles. The spreading time of the organic droplet is also a critical parameter in the microparticle preparation process [137].

V.4. The organic phase volume

The less important the solvent volume, the more the organic phase is concentrated in PLGA, and the larger will be the resulting microparticles [54], [55]. The increase in viscosity can also lead to better encapsulation of the drug [138]. Moreover, a high viscosity and a fast precipitation process, induced by a high polymer concentration, can also lead to less porous microparticles, thus decreasing the burst release effect compared to highly porous microparticles [133]. In addition, a highly concentrated organic phase, i.e. with less solvent, will induce a faster precipitation of the microparticles, thus increasing the encapsulation efficiency because the drug has less time to diffuse in the aqueous phase.

V.5. The aqueous phase volume

The aqueous phase will have an impact on the solvent extraction speed from the organic droplets. The more important the volume, the fastest the extraction, and then the quickest the precipitation of the microparticles. This will result in larger microparticles and better encapsulation efficiency, as the fast precipitation process will not allow the microparticles to be sheared and reduced in size before solidification, and less drug will have time to diffuse into the aqueous phase [133], [138], [139].

V.6. The aqueous/organic phase ratio and their solubility

In a study by Mao et al. (2008) [138], the effect of the aqueous/organic phase ratio was investigated. The size of PLGA microparticles was not really affected by this parameter, however, the drug loading increased with the increase in the aqueous/organic phase ratio. Furthermore, the solubility of the organic solvent in the aqueous phase is not to be neglected. Indeed, a high miscibility of the two phases will allow for a fast mass transfer between them and lead to rapid PLGA precipitation, improving the encapsulation efficiency [133], [140].

V.7. The surfactant agent type, pH and concentration

In the same study [138], the authors showed that slightly bigger microparticles were observed at low aqueous phase pH, but while increasing the pH, no more differences in sizes were noted; However the drug loading was improved for higher pH. No significant differences in the release profiles were reported. The drug loading was slightly higher for higher concentrations in emulsifier (0.1 and 0.5% of polyvinyl alcohol). However, if the surfactant concentration is higher than the critical micellar concentration, micelles will potentially solubilize the drug and lead to a diffusion of the drug to the micelles, thus decreasing the final drug loading [141]. High concentrations of surfactant reduce the interfacial tension between the two phases, resulting generally in smaller microparticles [54], [132], and this higher stability also leads to a more homogeneous drug distribution in the microparticles, decreasing the burst effect [142].

V.8. The initial drug loading and type of drug

As the drug loading increases, the viscosity of the organic phase changes, due to the decreasing PLGA concentration and the rise in drug concentration. The encapsulation efficiency and resulting release will also depend on the nature of the drug (hydrophilic, hydrophobic, ability to interact with PLGA, etc.). In general, a good solid-state solubility between the drug and the polymer will result in higher drug loadings [138], [141]. In addition, a drug soluble in the aqueous phase will diffuse easily in it, and the resulting encapsulation efficiency will be lowered.

V.9. The temperature

Temperature has an impact on viscosity, and as previously mentionned, a lower organic phase viscosity will lead to smaller microparticles. It should be noted that the polymer and drug solubility, as well as the solvent evaporation rate, might be altered by a change in temperature, inducing once again a change in microparticles' size according to what has been described above. It has recently been demonstrated that microparticles prepared at higher temperatures than room temperature exhibited fewer and smaller pores [143].

V.10. The pressure

In many cases, it has been demonstrated that reduced pressure during microparticles' manufacturing accelerates solvent evaporation and enhances drug encapsulation efficiency. Microparticles produced under pressure exhibit smoother surfaces [144].

V.11. The drying process

Even though this process takes place after the manufacturing process, it is a necessary step in the production of microparticles to enhance their stability during storage. The drying process, such as freeze-drying (one of the most used drying processes for microparticles), will remove residual water or solvent traces, both outside and inside the microparticles. After their removal, pores can appear within the microparticles, subsequently impacting the drug release. Even more significantly, freeze-drying can cause cracks in the microparticles during the sublimation process. It has been reported that the freeze-drying process can also potentially redistribute the drug within the microparticles due to the convection phenomenon during the drying step [133]. Indeed, when the water flows from the center of the microparticle to the edges, it can carry some of the drug closer to the surface, leading to an increase in the burst release [145]. The development of the porous network combined with the redistribution of the drug along the edges of the microparticles by freeze-drying is a process impacting the release mechanisms, by facilitating drug diffusion [146].

VI. Drug release from PLGA-based microparticles

VI.1. Drug release mechanisms

The term "release mechanism" has been employed to describe how drug molecules are transported or released, and how the release rate is controlled [47], [147]. Understanding the release mechanisms and the physicochemical interactions which have an impact on the release rate is crucial for the development of controlled-release PLGA-based DDS. To this date, it has not been possible to fully elucidate these release mechanisms, given the complexity of the processes involved. However, three main ways for drug molecules to be released have been reported:

- Transport through water-filled pores [27], [60]: This is the most common way of release. The most frequent mode of this transport is diffusion [47]. Convection, driven by the osmotic pressure, can also be found as a transport way through water-filled pores.
- Transport through the polymer matrix [148]: This phenomenon is most common for small and hydrophobic drug molecules.
- Transport due to the polymer degradation [48], [149].

Many other release mechanisms have since been identified, greatly complicating our understanding of the release of APIs. Indeed, several mechanisms can simultaneously occur, preventing from a clear determination of the predominance of one mechanism over another, for different PLGA-based DDS. Most of these release mechanisms will be described in the following sections.

VI.1.1. Diffusion through water-filled pores

Diffusion through water-filled pores plays a major role amongst the release mechanisms, significantly influencing the drug release kinetics [83], [150], [151]. Those pores can either initially exist in the MP or can be created after the degradation of the polymer matrix. The porous structure, sometimes resulting from PLGA hydrolysis upon contact with water, allows water molecules to penetrate, generating a gradient that favors drug diffusion. Nevertheless, there have been cases where the drug has been completely released before erosion has even begun [152], [153]. The initial burst release phase is occasionally attributed to diffusion-related mechanisms [154]. Furthermore, pores have to be larger than the drug molecules pass into the release medium, for this mechanism to occur. With this kind of

mechanism, the release rate will be governed by the rate of pore formation, the latter being itself governed in great part by the rate of polymer hydrolysis (erosion). The diffusion rate is influenced by several parameters including the diffusion coefficient of the API in the release medium, the initial and evolving porosity, and the tortuosity of the pore network [58]. This type of drug release mechanism is schematically presented in Figure 11.

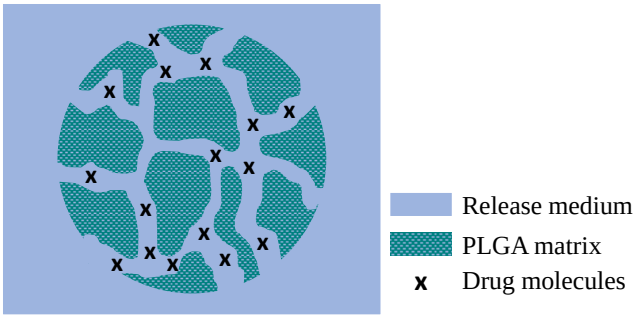


Figure 11. Schematic presentation of the diffusion through water-filled pores drug release mechanism (adapted from Fredenberg et al., 2011 [47]*)*

VI.1.2. Osmotic pumping

The osmotic pumping phenomenon shows similarities with the previously mentioned release mechanism. Indeed, as PLGA undergoes degradation, water molecules penetrate the matrix, creating an osmotic pressure gradient. PLGA degradation products can also increase the osmotic pressure within the microparticles [61], [67], [155]. If the pressure becomes too high, the microparticles can undergo cracks in their structure, leading to a significant drug release. The final mechanism also relies in the diffusion through water-filled pores, but it has been amplified by this osmotic pressure. The nature of the drug transport is then no longer called diffusion but convection. Moreover, the osmotic transport is only channel length dependent, while the diffusive transport depends on the length and area of the channels. It should be noted that some osmotic agents (such as polyethylene glycol) can be added to the formulation of PLGA-based microparticles, in order to promote water penetration and increase the osmotic force [156]. A prerequisite for osmotically-driven transport is the equilibrium between water influx and efflux, following an initial phase of water content adaptation. The osmotic pumping release mechanism is schematically presented in Figure 12.

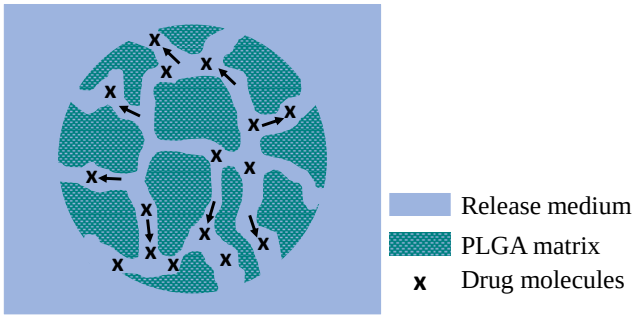


Figure 12. Schematic presentation of the osmotic pumping drug release mechanism (adapted from Fredenberg et al., 2011 [47]*)*

VI.1.3. Diffusion through the polymer matrix

As mentioned in *Section VI.1.* of this part, this release mechanism is only possible for small hydrophobic drug molecules with low molecular weights [157], [158]. The drug diffusion through the polymer matrix is not governed by the porous network but by the physical state of the PLGA. Indeed, under physiological conditions, at 37°C, PLGA, which has a glass transition temperature of around 44°C, is in a rubbery state. This will allow for the polymer chains to increase the molecular mobility, facilitating drug diffusion through the matrix. Moreover, once the microparticle is exposed to the aqueous release medium, water will act as a plasticizing agent of the polymer, decreasing its glass transition temperature, and transforming it from a glassy to a rubbery state. As a consequence, the polymer chains are even more disentangled and more flexible, inducing easier diffusion of the drug molecules [32]. For matrix-type DDS, drug release tends to be primarily governed by Fickian diffusion, which is influenced by factors such as concentration gradient, diffusion distance, and the extent of polymer swelling [92]. The diffusion through the polymer release mechanism is presented in Figure 13.

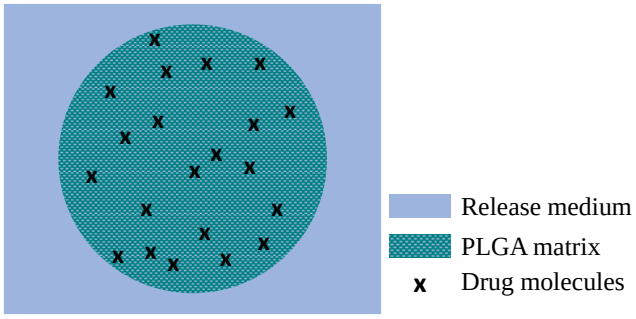


Figure 13. Schematic presentation of the diffusion through the polymer drug release mechanism (adapted from Fredenberg et al., 2011 [47]*)*

VI.1.4. Polymer degradation / matrix erosion

Biodegradable polymers such as PLGA undergo bulk erosion. Bulk-eroding polymers refer to polymers which are primarily degraded throughout the material after hydration, in the interior of the DDS [114], [159]. This term is opposed to surface erosion, where the degradation occurs from the exterior of the DDS. In the case of bulk-eroding polymers, the erosion process occurs after exposure to the release medium, during the hydrolysis of the polymer. Indeed, water molecules permeates the matrix, initiating the ester bond cleavage of PLGA chains, and leading to the gradual breakdown of the polymer into smaller fragments, i.e. oligomers and monomers of PLA (polylactic acid) and PGA (polyglycolic acid). As the polymer degrades, voids are created within the matrix, leaving space for drug molecules to diffuse out into the release medium [160]. Erosion is influenced by parameters, such as the polymer molecular weight, composition (e.g. lactide/glycolide ratio), the rate of water uptake, or the specific conditions of the surrounding environment (pH and composition of the release medium, for example) [161]. The way in which a polymer will erode will depend on the diffusivity of water through the matrix, the solubility of the degradation products in the release medium, the size and shape of the matrix, and the PLGA ending group (a hydrophilic group will facilitate water diffusion, and erosion will be promoted) [159], [162]. A schematic presentation of the erosion process is presented in Figure 14.

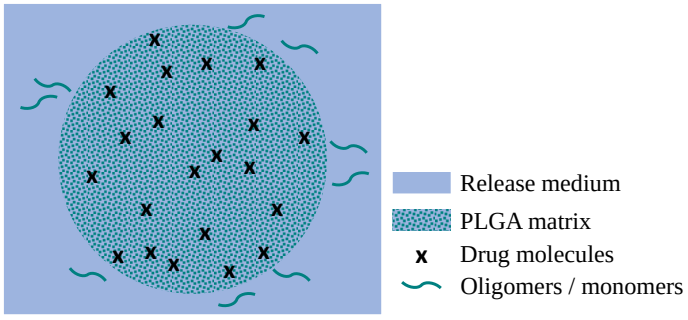


Figure 14. Schematic presentation of the erosion drug release mechanism

VI.1.5. Swelling of the polymer

Swelling is a drug release mechanism resulting from previous water penetration into the polymeric system. This mechanism is usually followed by polymer chain hydrolysis. Swelling cannot take place if the polymer chains are too entangled together. Once a critical polymer molecular weight has been reached, PLGA is able to swell, resulting in an increase in its volume and the formation of a hydrated gel-like structure [65], [67]. This phenomenon can thus occur after water penetration through a porous network, helped or not by osmotic pumping. The

resulting drug release rate can vary according to the type of polymer, or DDS. Indeed, by swelling, the length of the diffusion pathways increases, inducing a slower drug release. However, recent studies showed an increase in the drug release when the polymer exhibits substantial swelling [52], [65]–[67], [163], due to a higher mobility of the PLGA chains, resulting in an enhanced diffusivity of the drug. The swelling rate and subsequent drug release can be influenced by factors such as the polymer's composition, molecular weight, the hydrophilicity of the drug, and the development degree of the porous network. A scheme presenting the swelling release mechanism is presented in Figure 15.

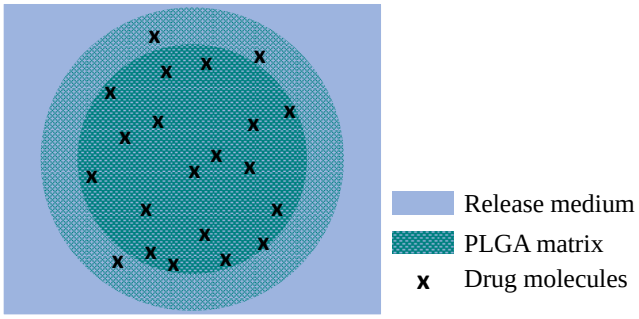


Figure 15. Schematic presentation of the swelling drug release mechanism

VI.1.6. Pore closure

Amongst the mechanisms mentioned above, polymer degradation and/or swelling can have unexpected effects on release mechanisms. Polymer degradation can cause an initially porous structure to collapse, closing the pores and slowing down release. Similarly, swelling of PLGA can cause the pores to be filled with swollen polymer, closing off access to the pore network and also limiting drug release [114]. This pore closing has been identified as the reason for the burst release stopping [164]. It has been noted that the more mobile the PLGA chains are, the greater the rate of pore closure [53], [165]–[167]. Fredenberg *et al.* (2011) [47] proposed that the closure of pores was driven by a hydrophobic effect, which can be attributed to the increased hydrophobicity of PLGA with a lower degree of carboxyl acid dissociation within the polymer at low pH. Nevertheless, as pores are also formed throughout the release process, this rate of pore formation must be set against the rate of closure of other pores before the effects on release kinetics can be determined. Fredenberg *et al.* (2011b) [165] showed that pore closure predominated at either a low or a medium pH (3 and 7.4), while pore formation was dominant at intermediate pH (5-6). The structure collapse and pore filling with swollen polymer pore closure mechanisms are represented in Figure 16.

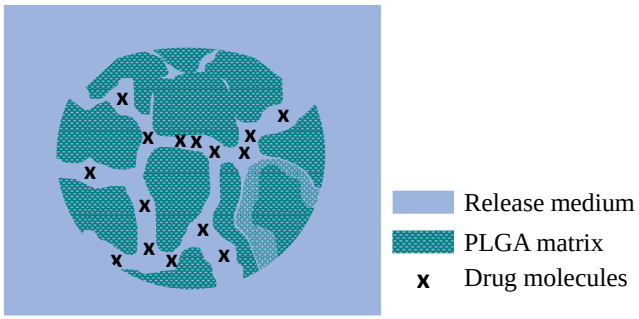


Figure 16. Schematic presentation of the pore closure mechanism

VI.1.7. Autocatalysis

Autocatalysis, in the context of PLGA-based DDS, refers to a self-accelerating process where the degradation of the polymer matrix releases acidic byproducts (polylactic and polyglycolic acids) that further catalyze the degradation of the polymer. In other words, as the polymer breaks down, it generates degradation products containing carboxylic acid functions that decrease the local pH and accelerate the degradation of the polymer itself. The autocatalytic effect is strongly dependent on the system's size. Microparticles undergo cleavage of their backbones into alcohols and shorter acid chains. Concentration gradients allow for the diffusion of these acids out of the microparticles into the surrounding fluid, while bases from the release medium (which is often a phosphate buffer) seep into the microparticle. These diffusion processes, either through pores or the polymer chains, help to neutralize the acids generated. Porous microparticles will thus be more likely to present a reduced autocatalytic effect [46]. However, due to the relative slowness of the diffusion processes, the rate of acid generation in the device can exceed the rate of neutralization, particularly in the centers of the microparticles where the diffusion pathways are longest [168]. The autocatalytic effect is thus more important in larger devices because it takes longer for the bases to reach the center of larger microparticles to neutralize the acidic monomers [62]. The autocatalytic effect is represented in Figure 17.

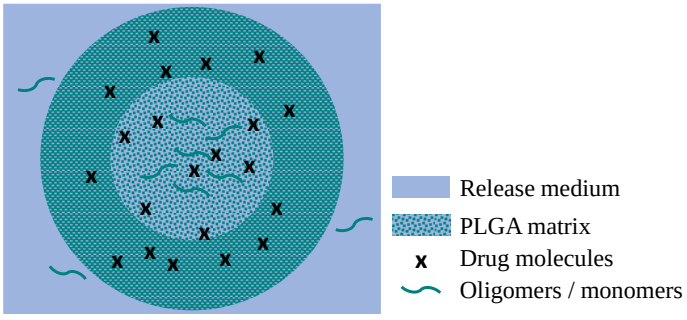


Figure 17. Schematic presentation of the autocatalysis drug release mechanism

VI.1.8. Formation of pores or cracks

Over the course of degradation, the hydrolysis of PLGA leads to the creation of pores within the polymer matrix: Pore formation is then governed by water absorption and erosion. These parameters are in turn influenced by the pH of the release medium, the salt concentration, the osmotic pressure, the polymer type, the presence of a plasticizer agent, etc [61], [82], [165], [169]. These pores serve as pathways for water penetration, triggering further polymer hydrolysis. Porogen agents can be added to the formulation to enhance the system's porosity [170], [171]. Concurrently, the degradation and erosion processes (especially bulk-erosion) can induce stress within the polymer, resulting in the formation of cracks on the surface or within the matrix, leading this latter to collapse [172]. Cracks can also appear when the osmotic pressure in the system becomes too high. These cracks can accelerate the ingress of water, potentially causing burst release, and accelerating polymer degradation. Both pore formation and cracks influence the drug release profile by altering the pathways for water penetration and drug diffusion. The pore and crack formation drug release mechanisms are represented in Figure 18.

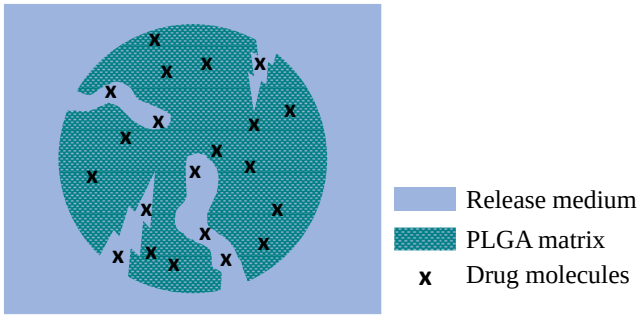


Figure 18. Schematic presentation of the pore and cracks formation drug release mechanisms

In conclusion, the release mechanisms governing drug delivery from PLGA DDS are multiple and tightly linked. Frequently, several of the above-mentioned release mechanisms are simultaneously involved in the drug release. It is thus sometimes quite complex to understand exactly which mechanisms are governing the release, as they change depending on the type of drug encapsulated, the release medium, the type of PLGA, the type of device, etc. In fact, release profiles can be very different depending on these physicochemical characteristics. The main release profiles that can be obtained from PLGA DDS are presented in the following section.

VI.2. In vitro drug release profiles

PLGA microparticles can exhibit different drug release profiles. Even though there are an infinite number of possible release behaviors, the most commonly reported in the literature are the biphasic and triphasic profiles. Indeed, the latter is obtained for large DDS, which exhibit heterogeneous degradation leading to a slower second phase.

VI.2.1. The triphasic profile

As its name suggests, the triphasic profile is made up of three successive phases: A rapid release phase, a temporary reduction in the release rate, and finally a more important and sustained release phase.

The first phase is usually called the "burst release phase", this phenomenon is characterized by rapid drug release in the first hours or days. This is frequently attributed to the diffusion of drug molecules which were adsorbed at the microparticle's surface, located near the surface of the polymer matrix, or within pores that are readily accessible to the surrounding medium [50]. This release is commonly prior to any polymer-erosion/degradation, or swelling [58].

The second phase, generally called the "lag-phase", is a period of significantly reduced release rate, which can last from a few days to several weeks. The common reason for a lag-phase is a slow drug diffusion through a dense polymer matrix with low porosity, which seems to be controlled by the limited drug solubility [47], [50]. In this phase occurring after the burst release, all the drug with direct surface access has already been released, and the remaining drug is well-trapped in the matrix. Therefore, there exists a concentration gradient between the release medium (sink conditions) and the saturated drug solution within the microparticles. This concentration gradient will induce a constant drug diffusion from the microparticles to the outer medium [151], [173]. The slope of the release kinetics of this second phase is thus dependent on the permeability of the matrix by the drug. The water penetration into the microparticle will enable this latter to swell, and the local swelling of some regions will allow for a progressive drug release [163]. Furthermore, pore closure, drug/polymer or drug/drug interactions can also be the reason for the decrease in the release rate. In some cases, this lag-phase is even non-existent, because of the fast polymer degradation that leads to a rapid complete drug release.

Sometimes called the "second burst", the third phase is a sustained release phase leading to complete drug exhaust. The drug is released from the matrix at a relatively constant and

controlled rate over an extended period. This phase is generally attributed to the increased drug mobility due to the polymer erosion onset allowed thanks to rapid water absorption, but it can also be ascribed to pores and cracks formation, and swelling [50], [66], [163]. Sometimes, this second burst is attributed to a point where the porous network becomes fully continuous within the microparticles [174]. This water income is at least partially driven by the osmotic pressure, created by the PLGA byproducts, and by the increasing hydrophilicity of the system, due to the shortening of the PLGA chains. The limited diffusion of PLGA degradation products increased the autocatalytic effect, and this highly acidic environment can induce an entire degradation of the microparticles' core, leading to a complete drug exhaust [175]. The water penetration and accumulation of degradation products can lead to an increase in osmotic pressure, which can cause the rupture of the microparticles, and consequently, all the remaining drug is released [61], [155].

The triphasic profile can be useful for pathological situations where a delay in the drug action may be beneficial, before moving on to controlled long-term administration.

VI.2.2. The biphasic profile

The biphasic profile is more often encountered for small DDS, where the second phase is relatively rapid [152]. The biphasic release profile is characterized by an initial rapid burst release phase followed by a slower sustained release phase. This profile is advantageous for combining the benefits of immediate release to treat acute symptoms, with prolonged treatment to maintain the therapeutic effect over time.

VI.2.3. The monophasic profile

The monophasic profile, i.e. a zero-order release, also exists but is much less encountered. In a monophasic drug release profile, the drug is released at a relatively constant rate over time. This uniform and sustained release is often desired for maintaining steady therapeutic levels and minimizing fluctuations in drug concentration. This type of release is particularly valued in the treatment of chronic diseases.

Amongst these release profiles, variations exist, depending on each phase duration and on the eventual presence of a burst release phase. These different types of release profiles are presented in Figure 19.

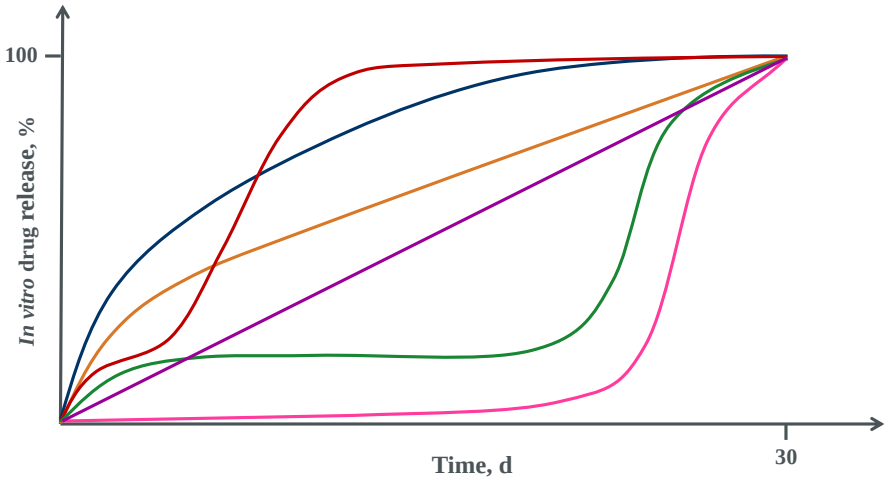


Figure 19. Main drug release profiles from PLGA-based DDS. Blue curve: Burst release followed by a rapid phase (biphasic); Orange curve: Burst release followed by a zero-order phase (biphasic); Red curve: Triphasic release with a short phase II; Purple curve: Monophasic profile; Green curve: Triphasic profile; Pink curve: Biphasic profile. Adapted from Fredenberg et al. (2011) [47]

VI.3. Factors affecting the drug release

Many factors can have an impact on the release mechanisms of microparticles, leading to very different release profiles. These factors may be related to experimental conditions or to the formulation parameters of the microparticles. Amongst all the microparticles' characteristics that can have an impact on the drug release, the ones that will have the greatest effects are listed below.

VI.3.1. Experimental conditions

A consensus has not yet been reached on establishing a regulatory standard for conducting *in vitro* release studies. However, it has been proven that the experimental conditions can play a major role in the resulting release behaviors and involved mechanisms.

- ***Medium pH and composition:*** For one application of microparticles or another, the physiological environments, and therefore the pH values associated with these environments, may vary. The pH can have a significant impact on the release mechanisms. It has been shown that at very low pH levels, PLGA degradation products are much less soluble, crystallize, and cannot be released into the medium, leading to microparticle breakage. Drug release is therefore much faster once the microparticle is broken [78]. In a study by Faisant *et al.* (2006), higher release rates were observed for higher pHs, due to a lower PLGA T_g for the high pH [176]. This study also highlights

the fact that drug solubility can be part of the explanation of the different release profiles according to the medium pH. Indeed, lowering the pH of the release medium can lead to greater drug solubility, increasing the drug release rate [177], [178]. It has been shown that the addition of any compound able to increase the drug solubility (usually a surfactant) significantly affects the resulting release kinetics [179], [180]. Usually, it is stipulated that sink or even perfect sink conditions must be maintained throughout the *in vitro* study, so that the release medium is not saturated with the drug. However, maintaining these conditions, particularly for very poorly soluble APIs, can lead to significant changes in the release setup, which may have an impact on the release mechanisms [173]. Osmolarity also has quite a significant effect on drug release: The higher the osmolarity, the slower the release, due to the decrease in osmotic pressure [176], [178]. However, this is dependent on the drug, and more importantly, on the polymer-drug interactions. Shameem *et al.* (1999) showed that increasing the buffer concentration increased the ionic strength of the release media, thus reducing the polymer-drug interaction and resulting in a faster release [181]. This time, the ionic strength, not the osmotic pressure, was the driving force of release.

- ***Incubation temperature:*** Sometimes, elevated temperatures of the release medium are used, in order to create "stress tests" during product development and production. This usually leads to accelerated drug release with increasing temperatures, which can be due to greater PLGA degradation and mobility, and a higher drug diffusivity [82], [176], [182]. The latter is indeed allowed by the medium temperature (37° C) closer to the T_g of PLGA (44-47° C). During the two first phases (burst and lag-phase), the increase in temperature induces a higher mobility in the polymer chains, leading to faster diffusion. During the third phase, the release is accelerated by the enhanced hydration and polymer erosion induced by elevated temperatures [178]. On the opposite, an increase in temperature can also lead to surface pore closure, thus decreasing the release rate [53]. Otte *et al.* (2021) [183] showed that the *in vitro - in vivo* release correlation was better for PLGA microparticles incubated at 36°C instead of 37°C.

- ***Agitation:*** The bulk fluid agitation limits the microparticle aggregation which offers for more surface to be in contact with the release medium, and hence, to fasten the release [184]. Moreover, the movement induced by the setup agitation creates mechanical stress on the device which will lead to a faster degradation and release [185]. An unstirred

medium will also cause a drug-saturated layer around the microparticles, which will decrease the release rate [186].

VI.3.2. Formulation parameters

- ***The microparticle size and geometry:*** Small microparticles generally release more rapidly than larger ones, and are therefore more likely to have a burst effect. Indeed, the diffusion pathways are longer in bigger microparticles, leading to a more progressive and triphasic release. Moreover, the surface-to-volume ratio is higher in smaller microparticles, and in certain microparticles' geometries, the water uptake is then more rapid and can lead to a burst effect [152], [187]. However, the autocatalytic effect is more pronounced in larger microparticles, resulting in faster release rates [46], [83], [188], [189]. Drug loading is also generally more important for larger microparticles, so a high heterogeneity in terms of particle sizes will thus lead to different release profiles within the same batch [190].

- ***The microparticle porosity:*** Porous microparticles have larger surface areas and shorter diffusion pathways, leading to a more pronounced burst release [46], [191]. More generally, due to a fast water-penetration, the release will occur faster in porous microparticles and the lag-phase will be shorter. The third phase will show a reduced release rate, as smaller amounts of drug remained in the microparticles [192].

- ***The polymer characteristics:*** It has been demonstrated several times that the lower the initial PLGA molecular weight, the faster the release [1], [82], [193], [194]. Low-molecular-weight polymers will form a less dense matrix because the chains will be less entangled as they both are shorter, and present more important levels of hydrophilic acid end groups. This will make it easier for water to diffuse through the matrix and speed up the release, which may cause a burst biphasic drug release profile. However, in some cases, a lower PLGA molecular weight will lead to a facilitated pore closure due to the higher chain mobility compared to high molecular weight PLGA [53], [203], thus increasing the release rate. In contrast, high-molecular-weight polymers will present a triphasic profile with a lower burst release [194], [195]. The polymer chain end group can play a role in the second drug release phase as a carboxylic group might be able to create ionic interactions with an encapsulated drug, and as a consequence, slow down the release [83], [196]. PLGA can be chemically modified to improve its compatibility

with the drug (PEGylation, terminal end group, etc.) [201]. The PLGA degradation will be more important for a high lactide-to-glycolide ratio, as lactic acid is more hydrophilic. As a consequence, the duration of the lag-phase will increase with a high molecular weight and a low level of glycolic acid [49], [197]. Furthermore, the monomer sequence distribution of PLGA can impact drug release: A sequenced alternative copolymers will exhibit a slower hydrolysis rate and thus, a lower burst release than random copolymers [204], [205]. More generally, all the parameters that will affect the degradation rate of PLGA will impact the intensity and duration of the phases. These parameters were already described in *Section II.2.1*.

- ***The type and amount of encapsulated drug:*** The encapsulation efficiency being less good for hydrophilic APIs [198], the latter adsorb onto the microparticles' surface, and this partly causes a burst effect as it can be rapidly released [145]. In addition, the water solubility is higher for hydrophilic drugs, which improves and accelerates the release of drug near the surface in the surrounding medium. Drug-polymer compatibility will also be a parameter to consider. Indeed, a drug with low solubility in PLGA will be dispersed in the matrix, and the drug clusters close to the surface will be released by creating a burst effect [58] (some co-solvents can be added to increase the solubility of the drug in the polymer solvent [213], [214]). Moreover, the acidic or basic nature of the drug can impact its release: Acidic APIs will be more likely dispersed in the matrix due to low interactions with PLGA, whereas basic APIs will be dissolved, so they will take longer to release. Neutral APIs will release even more slowly than acidic and basic APIs [82]. Furthermore, the higher the initial drug loading, the more likely it is that the drug will be in a crystalline state, and the more likely it is that crystal clusters on the surface of the microparticles will be released rapidly into the medium, creating a burst effect [50], [199]. The second release phase can be substantially dependent on the drug loading, but also on the type of drug and the other involved release mechanisms. Gasmi *et al.* (2016) showed that the second phase release rate of low-loaded dexamethasone microparticles was higher than that of higher-loaded ones, up to a critical load rate that induces the change from a triphasic to a biphasic release profile presenting a burst [50]. The release rate was thus slow for low and high drug loadings, and high for intermediate drug loadings. For prilocaine- and ketoprofen-loaded microparticles, the higher the initial drug loading, the faster the release (from a triphasic to a biphasic release profile as well)

[65], [66]. It has been noticed that the third phase can be delayed at high initial drug loading [66].

- ***The type and temperature of the post-processing treatment:*** Some treatments can be added to wash the microparticles' surfaces with different solutions, and limit the burst effect [145]. Moreover, the temperature of these solutions can be varied in order to obtain triphasic (low temperatures) or biphasic profiles (high temperatures) [200]. Heating the microparticles' surface above the PLGA T_g allows for surface pore closure, also reducing the burst effect [53]. The PLGA present on the surface of the microparticles can be heated, cross-linked, or coated with a drug-free layer of PLGA or another material. As a consequence, the burst release will be decreased [56], [202]. As already mentioned in *Section V.11*, Park *et al*. showed that the microparticle drying method could affect the burst release phenomenon. The freeze-drying process made microparticles more likely to undergo burst release than air- or vacuum-dried microparticles [133], [146].

Customizing the drug release profile, intensity, and duration of drug exposure is achievable through the variation of all these influencing factors, and thus more generally, by the manufacturing process [114]. The selection of PLGA physicochemical characteristics, such as molecular weight and the lactide-to-glycolide ratio, but also by combining different microparticles' sizes, for example (large and small), in order to have an intermediate bi- or triphasic profile, can be devised [187].

VII. Objective of this work

Poly(lactic-co-glycolic) acid (PLGA) microparticles serve as versatile carriers for sustained and controlled drug delivery, offering the potential for fine-tune therapeutic treatments. Its biocompatibility and biodegradability make it a material of choice for its safety of administration. Despite its high potential, its drug release mechanisms are not yet fully understood, thus preventing the development of new products on the market. As our understanding of PLGA release mechanisms deepens, insights are gained into optimizing formulations for enhanced efficacy, minimized side effects, and improved patient outcomes.

Usually, *in vitro* release studies are conducted with ensembles of microparticles. However, in ensembles, microparticles with various characteristics (sizes, porosities, drug loadings, etc.) coexist within the same batch. Nevertheless, it is widely recognized that the system's features can significantly impact the underlying physicochemical mechanisms. In order to overcome these group effects, the release kinetics will be carried out on single microparticles (of similar sizes), so that a physicochemical characteristic can be better correlated with release behavior. By studying microparticles individually, insights into the intricate interplay between the polymer matrix, encapsulated drug, and surrounding environment will be gained.

The aim of this work will be to better understand the *in vitro* drug release mechanisms of PLGA-based microparticles. By evaluating the impact of the physical state of the drug within the microparticles, the preparation method, the type of drug encapsulated, and the type of release setup on the release kinetics, we will be able to deepen our knowledge in terms of drug release.

In more detail, this manuscript will discuss:

- The release impact of the physical state of ibuprofen within the microparticles: Amorphous *versus* crystalline
- The release impact of the preparation method: A conventional *versus* a microfluidics technique
- The release impact of the type of encapsulated drug: Ibuprofen *versus* lidocaine
- The release impact of the *in vitro* drug release setup: A bulk fluid *versus* an agarose gel setup

VII. Objectifs de recherche

Les microparticules d'acide poly(lactique-co-glycolique) (PLGA) sont des vecteurs polyvalents pour l'administration prolongée et contrôlée de substances actives, offrant la possibilité d'adapter les traitements thérapeutiques. Sa biocompatibilité et sa biodégradabilité en font un matériau de choix pour sa sécurité d'administration. Malgré son fort potentiel, ses mécanismes de libération de substances actives ne sont pas encore totalement élucidés, ce qui empêche le développement de nouveaux produits sur le marché. Au fur et à mesure que nous approfondissons notre compréhension des mécanismes de libération du PLGA, nous acquérons des perspectives d'optimisation des formulations pour une meilleure efficacité, une minimisation des effets secondaires et une amélioration des résultats pour les patients.

Habituellement, les études de libération *in vitro* sont menées sur des ensembles de microparticules. Cependant, dans les ensembles, des microparticules aux caractéristiques diverses (tailles, porosités, taux de charge en substance active, etc) coexistent au sein d'un même lot. Néanmoins, il est largement reconnu que les caractéristiques du système peuvent avoir un impact significatif sur les mécanismes physicochimiques sous-jacents. Afin de surmonter ces effets de groupe, les cinétiques de libération seront effectuées sur des microparticules individuelles (de tailles similaires), de sorte qu'une caractéristique physicochimique puisse être mieux corrélée au comportement de libération. L'étude de microparticules individuelles permettra de mieux comprendre l'interaction complexe entre la matrice polymère, la substance active encapsulée et le milieu environnant.

L'objectif de ce travail sera de mieux comprendre les mécanismes de libération de substances actives *in vitro* des microparticules à base de PLGA. En évaluant l'impact de l'état physique de la substance active dans les microparticules, de la méthode de préparation, du type de substance active encapsulée et du type de dispositif de libération sur la cinétique de libération, nous allons pouvoir approfondir nos connaissances en matière de libération de substances actives.

De manière plus détaillée, ce manuscrit abordera :

- L'impact sur la libération de l'état physique de l'ibuprofène dans les microparticules : amorphe *versus* cristallin
- L'impact de la méthode de préparation sur la libération : technique conventionnelle *versus* technique microfluidique

- L'impact sur la libération du type de substance active encapsulée : ibuprofène *versus* lidocaïne
- L'impact sur la libération du setup de libération *in vitro* : un fluide en vrac *versus* un gel d'agarose

CHAPTER II - MATERIALS AND METHODS

I. Materials

Poly (D,L lactic-co-glycolic acid) (PLGA, 50:50 lactic acid:glycolic acid; Resomer RG 503H; Evonik, Darmstadt; Germany); ibuprofen (BASF, Ludwigshafen, Germany); lidocaine (Sigma–Aldrich Chemie GmbH, Steinheim, Germany); polyvinyl alcohol (Mowiol 4-88; Sigma-Aldrich, Steinheim, Germany); agarose (genetic analysis grade); potassium dihydrogen orthophosphate, disodium phosphate, potassium chloride, sodium chloride and sodium hydroxide (Acros Organics, Geel, Belgium); formic acid (Honeywell Reidel de Haën, Seelke, Germany); acetonitrile, ethanol, and dichloromethane (VWR, Fontenay-sous-Bois, France).

II. Methods

II.1. Microparticle preparation

Ibuprofen- and lidocaine-loaded PLGA microparticles were prepared using an oil-in-water (O/W) solvent extraction/evaporation technique: Either in a "classical beaker setup" or with a microfluidics device.

II.1.1. Preparation via the beaker method

Ibuprofen-loaded microparticles were prepared by dissolving PLGA 503H and ibuprofen in 4 mL of dichloromethane (DCM). This organic phase was emulsified with 2.5 L of an aqueous solution of poly(vinyl) alcohol (PVA) 0.25% w/w under stirring (900 rpm, Eurostar power-b; Ika, Staufen, Germany) for 30 min. Upon DCM partitioning into the outer aqueous phase, the PLGA precipitated, and microparticles formed. The latter were hardened by adding another 2.5 L of the same outer aqueous phase and further stirring at 700 rpm (Eurostar power-b) for 4 h. The microparticles were separated by filtration (Nylon filter, 0.45 µm, 13 mm; GE Healthcare Life Sciences Whatman, Kent, UK), washed with demineralized water, and freeze-dried for 3 days (Christ Alpha 2-4 LSC+; Martin Christ, Osterode, Germany) under the following conditions: Freezing at -50 °C for 2.5, primary drying at 0 °C and 1 mbar for 34 h, and secondary drying at 20 °C and 0.1 mbar for 34 h. Drug-free microparticles were prepared accordingly, without drug.

Lidocaine-loaded PLGA microparticles were prepared by dissolving PLGA 503H and lidocaine in 5 or 6 mL of DCM depending on the DL. This organic phase was emulsified with 2.5 L of an aqueous solution of PVA 0.25% w/w under stirring (900 to 1200 rpm in function of

the DL) for 30 min. Upon DCM partitioning into the outer aqueous phase, the PLGA precipitated, and microparticles formed. The latter were hardened by adding another 2.5 L of the same outer aqueous phase and further stirring at 700 rpm (Eurostar power-b) for 4 h. The microparticles were separated by filtration (Nylon filter, 0.45 µm, 13 mm; GE Healthcare Life Sciences Whatman, Kent, UK), washed with demineralized water, and freeze-dried for 3 days (Christ Alpha 2-4 LSC+; Martin Christ, Osterode, Germany) under the same conditions as for ibuprofen microparticles, described above.

The preparation of drug-loaded microparticles by the classical beaker method is represented in Figure 20.

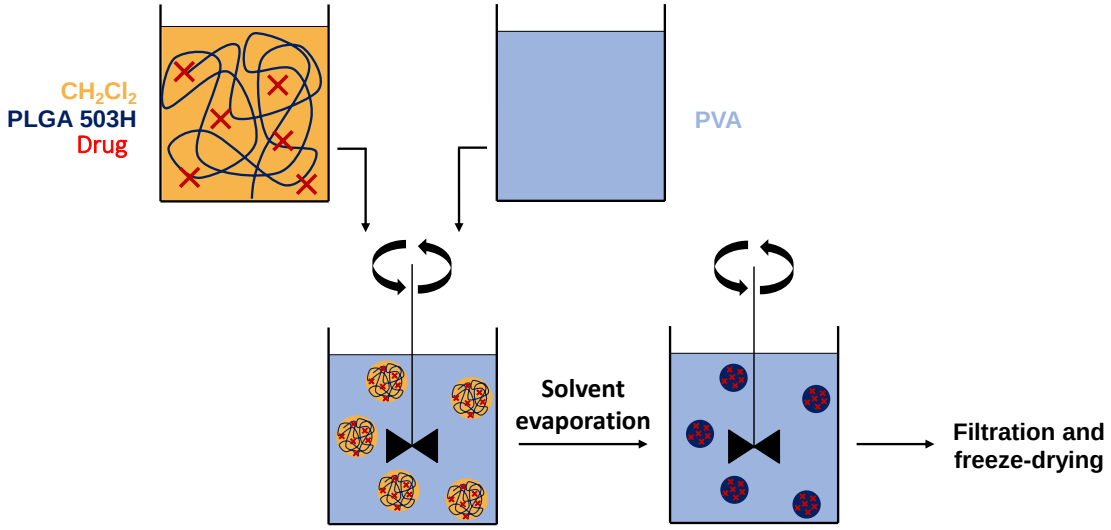


Figure 20. Preparation of microparticles via the beaker method

II.1.2. Preparation via a microfluidics device

Drug-loaded microparticles were prepared by dissolving PLGA 503H and ibuprofen or lidocaine in 40 mL of DCM. This organic phase was mixed with an aqueous PVA 1% w/w solution, using a "T-junction", at a flow monitored by the microfluidics device (OB1 MK3+; Elveflow, Paris, France) of 20 µL/min for the aqueous phase and 10 µL/min for the organic phase. Emulsion droplets were recovered in 2 L of an aqueous PVA 0.25% w/w solution, followed by stirring for 2 h to harden the microparticles. Microparticles were separated by filtration, washed with demineralized water, and freeze-dried for 3 d (as indicated in the beaker method). The software used is the Elveflow Smart Interface (ESI – 3.05.02).

The preparation of drug-loaded microparticles with a microfluidics device is represented in Figure 21.

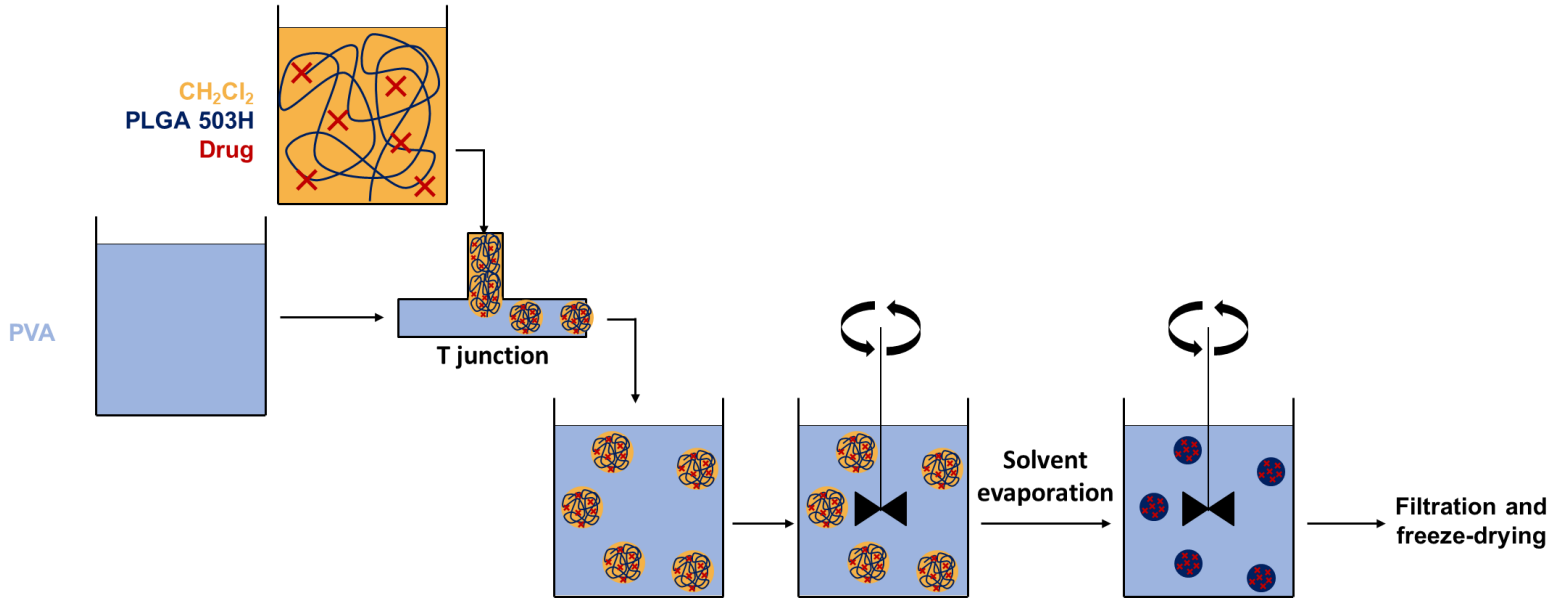


Figure 21. Preparation of microparticles via a microfluidics device

II.2. Agarose gel preparation

0.5% w/w agarose gel was prepared by dissolving the polysaccharide in phosphate buffer pH 7.4 (USP 42) heated at 135°C under stirring (400 rpm, IKA RCT standard, IKA-Werke, Staufen im Breigsau, Germany) for approximately 1 h until complete dissolution. The solution was poured into a well of a microplate, followed by cooling to room temperature (causing sol-to-gel-transition).

II.3. Microparticle characterization

II.3.1. Microparticle morphology and size

Microparticles' sizes were determined by optical microscopy: Pictures of MPs after production and freeze-drying were taken using a Nikon SMZ-U (Nikon, Tokyo, Japan) equipped with an AxioCam ICc1 camera, and pictures of MPs during release studies were taken using an Axiovision Zeiss Scope-A1 microscope, equipped with an AxioCam 305 color camera. The Axiovision Zeiss Software (Carl Zeiss, Jena, Germany) was used to treat the data. For each batch of microparticles, 200 diameter measurements are made, the standard deviation from the mean diameter is then used to calculate the polydispersity index (PdI) of the microparticle population within the batch as follows:

$$PdI\ (\%) = \frac{Standard\ deviation}{Average\ MPs\ diameter} \times 100$$

This PdI parameter represents the size distribution of the MPs, and will therefore give indication about the size's homogeneity of the batch. The lower this index is, the more homogeneous the population will be in terms of size, and conversely, the higher it is, the more heterogeneous the sizes will be within the batch.

II.3.2. Practical drug loading

The practical drug loading and encapsulation efficiency of ibuprofen microparticles were determined by dissolving approximately 5 mg microparticles in 5 mL acetonitrile, followed by filtration (PVDF syringe filters, 0.22 µm; GE Healthcare, Kent, UK). The drug content was determined by HPLC-UV analysis [Thermo Fisher Scientific Ultimate 3000 Series HPLC, equipped with an LPG 3400 SD/RS pump, an autosampler (WPS-3000 SL) and a UV-Vis detector (VWD-3400RS); Thermo Fisher Scientific, Waltham, USA]. A C18 reversed-phase column (Gemini 5µm; 110 Å; 150 x 4.6 mm; Phenomenex, Le Pecq, France) was used.

The mobile phase was a mixture of phosphate buffer (50 mM KH_2PO_4 with 22.5 mM NaOH, pH 6,8):acetonitrile (67:33, v:v). The detection wavelength was 225 nm, and the flow rate was 1 mL/min. Ten microliter samples were injected. The experiments were conducted in triplicate. Mean values +/- standard deviations are reported.

The practical drug loading and encapsulation efficiency of lidocaine microparticles were determined by dissolving approximately 1 mg microparticles in 3 mL acetonitrile, followed by filtration (PVDF syringe filters, 0.22 µm). The drug content was determined by HPLC-UV analysis. A C18 reversed-phase column (Gemini 5 µm; 110 Å; 150 x 4.6 mm) was used. The mobile phase was a 70:30 (v:v) mixture of acetonitrile:water, acidified at pH 3-4 with formic acid. The detection wavelength was 222 nm, and the flow rate was 1 mL/min. 10 µL samples were injected. The experiments were conducted in triplicate. Mean values +/- standard deviations are reported.

Formulas of drug loading (DL) and encapsulation efficiency (EE) are reported below:

$$DL\ (\%) = \frac{Mass\ of\ drug\ in\ MPs}{Mass\ of\ MPs} \times 100$$

$$\text{EE}\ (\%) = \frac{Mass\ of\ drug\ in\ MPs}{Initial\ mass\ of\ drug\ added} \times 100$$

The encapsulation efficiency can also be found to be the practical drug loading divided by the theoretical drug loading.

II.3.3. Drug release measurements from single microparticles in bulk fluid

Ibuprofen or lidocaine release into phosphate buffer pH 7.4 (or phosphate buffer saline pH 7.4 in one of the cases, specified later; USP 42) were measured from *single* microparticles in bulk fluid in 96- well standard microplates (Tissue culture plate 96 well; Carl Roth, Karlsruhe, Germany) as follows: One microparticle was introduced into a well filled with 100 µL of phosphate buffer pH 7.4 (50 mM KH_2PO_4 with 39 mM NaOH) and sealed with an aluminum foil (Sarstedt AG & Co, Germany). The microplates were horizontally shaken at 80 rpm and kept at 37°C (GFL 3033; Gesellschaft für Labortechnik, Burgwedel, Germany). At predetermined time points, a Hamilton syringe (Microlite #710, 100 µL; Hamilton, Bonaduz, Switzerland) was used to replace the entire bulk fluid with fresh release medium carefully. The withdrawn samples were analyzed for their drug contents by HPLC-UV. The mobile phase used for the dosage of ibuprofen samples was a 67:33 (v:v) mixture of phosphate buffer

pH 6.8:acetonitrile. The detection wavelength was 225 nm, and the flow rate was 1 mL/min. Twenty µL samples were injected. The mobile phase used for the dosage of lidocaine samples was a 70:30 (v:v) mixture of acetonitrile:phosphate buffer pH 6.8. The detection wavelength was 222 nm, and the flow rate was 1 mL/min. Twenty µL samples were injected. A schematic presentation of this experimental setup is represented in Figure 22. The experiments were conducted 8 times. Perfect sink conditions were provided throughout the observation periods. Mean values +/- standard deviations are reported.

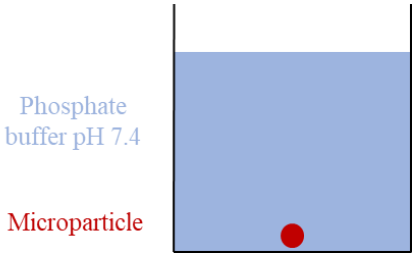


Figure 22. Schematic presentation of the experimental bulk fluid setup

II.3.4. Drug release measurements from single microparticles in agarose gel

Ibuprofen or lidocaine release were measured from *single* microparticles entrapped in an agarose gel (0.5%) prepared with a phosphate buffer pH 7.4 (or a phosphate buffer saline at pH 7.4, in one of the cases, specified later) in 96- well standard microplates, as follows: 50 µL solution of agarose (prepared as described in *section II.2*) were poured into a well followed by cooling to room temperature. A *single* microparticle was placed in the middle of the surface of the gel. Another 50 µL of agarose solution was poured into the well (at a temperature close to gel formation), then cooled to room temperature. One hundred µL phosphate buffer pH 7.4 were added on the top of the gel. The well was sealed with aluminum foil and horizontally shaken at 80 rpm and 37°C (GFL 3033). At predetermined time points, a Hamilton syringe was used to replace the entire bulk fluid with fresh release medium carefully. The withdrawn samples were analyzed for their drug contents by HPLC-UV analysis, as described in *section II.3.3*. A schematic presentation of this experimental setup is represented in Figure 23. The experiments were conducted 8 times. Perfect sink conditions were provided throughout the observation periods. Mean values +/- standard deviations are reported.

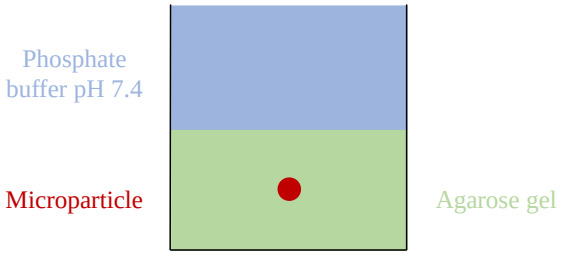


Figure 23. Schematic presentation of the experimental agarose gel setup

II.3.5. Microparticle swelling

Microparticles were treated using the method described for the *in vitro* drug release studies from *single* microparticles (see *Section II.3.3.* and *II.3.4.*). At predetermined time points, pictures of the microparticles were taken using an Axiovision Zeiss Scope-A1 microscope. The diameters of the microparticles were determined with the Axiovision Zeiss Software (Carl Zeiss).

The increase in microparticles' diameter in function of the time was calculated using the following formula:

$$Increase\ in\ diameter\ (\%)(t) = \frac{diameter\ (t)}{diameter\ (t=0)} \times 100$$

Where "diameter (t = 0)" represents the initial microparticle diameter before release and "diameter (t)" the microparticle diameter at a certain timepoint t.

Furthermore, dynamic changes in the microparticles' wet mass were determined as follows: At predetermined time points, samples were carefully withdrawn and excess water removed using Kimtech precision wipes (Kimberly-Clark, Rouen, France). The microparticles' wet mass was measured using an ultra-microbalance (XPR6U; Mettler-Toledo, Greifensee, Switzerland).

II.3.6. Thermogravimetric analysis (TGA)

TGA thermograms of raw materials (as received: Ibuprofen, lidocaine, PLGA 503H) and of microparticles were recorded using the Q500 equipment (TA instruments, France), and the results were analyzed using the Universal analysis software (TA instruments, France). 5 to 8 mg samples were heated in sealed aluminum pans from 0 to 550°C at a rate of 5°C/min.

II.3.7. Differential scanning calorimetry (DSC)

DSC thermograms of raw material (as received: Ibuprofen, lidocaine, PLGA 503H), physical mixtures of PLGA 503H and ibuprofen or lidocaine, and of microparticles were recorded with a DSC3 Star System (Mettler Toledo, Greifensee, Switzerland). The ibuprofen MPs analyzed after exposure to the release medium (PB pH 7.4) were previously freeze-dried (as described in *Section II.1.1*). For the physical mixtures, PLGA 503H was previously milled with a blender and lidocaine was milled manually with a mortar and pestle for few minutes. Approximately 5mg samples were heated in sealed aluminum pans (caps perforated) from -70°C to 120°C, cooled to -70°C and reheated to 120°C, all at a rate of 10°C/min. The reported glass temperatures (T_g) were determined from the 1st heating cycles in the case of the microparticles and ibuprofen, and from the 2nd heating cycles in the case of PLGA 503H. The experiments were conducted in triplicates.

II.3.8. Scanning Electron Microscopy (SEM)

The microparticles' internal and external morphology obefore and after exposure to the release medium was studied using a JEOL Field Emission Scanning Electron Microscope (JSM-7800F, Tokyo, Japan). Samples were fixed with a ribbon carbon double-sided adhesive tape and sputter coated with a thin chrome layer. Before analysis the *samples were freeze-dried after exposure to the release medium*, as described in *Section II.1.1*. Cross-sections of the microparticles were obtained upon inclusion into water-based glue (UHU, Bolton Group, Buehl, Germany), drying for 48 h, and slicing with a razor blade or using a cryostat (Leica CM3050 S, Wetzlar, Germany), after inclusion into the same glue and fixation with OCT (Embedding medium for frozen tissue specimen to ensure Optimal Cutting Temperature, VWR BDH, Chemicals, United Kingdom).

II.3.9. Energy dispersive X-ray analysis (EDX)

The repartition of lidocaine inside the microparticles before exposure to the release medium was assessed using a JEOL Field Emission Scanning Electron Microscope (JSM-7800F, Tokyo, Japan) equipped with an EDS microanalysis system (X-Mas SDD detector, Aztec 3.3 software; Oxford Instruments Oxfordshire, England). Cross-sections of the microparticles were obtained as described in the *Section II.3.8*. Samples were fixed with a ribbon carbon double-sided adhesive tape and sputter coated with a thin carbon layer.

II.3.10. X-ray powder diffraction

X-ray powder diffraction analyses were performed with a Panalytical X'Pert Pro diffractometer equipped with a Cu X-ray tube (λ = 1.54 Å) and Hilgenberg glass capillaries (diameter 0.7 mm) in transmission mode with the X'Celerator detector. The diffractograms were recorded from 4 to 60° (2θ, 0.0167° steps).

II.3.11. X-ray microcomputed tomography (XμCT)

X-ray micro computed tomography analysis was performed with a SkyScan 1172 micro CT scanner (Bruker, Kontich, Belgium) to characterize the inner structure of the microparticles (before and after exposure to the release medium) in a non-invasive manner. Samples were placed on a polystyrene support. A camera array with size 4000x2672 was used, resulting in a resolution of 2.26 µm. The rotation step was 0.25 degrees.

II.4. Drug release measurements from loaded agarose gels

One hundred µL agarose gels were prepared as described in *Section II.2.* and exposed to 25 or 100 µL of a solution of ibuprofen or lidocaine in phosphate buffer pH 7.4 (120 µg/mL) in microplate wells for 2 d at 37 °C under horizontal agitation (80 rpm, GFL 3033). This allowed us to load the agarose gels with 1.5 or 6 µg ibuprofen or lidocaine, respectively. The drug was dissolved in the gel, schematically illustrated in Figure 24 (crosses represent individual drug molecules/ions). The bulk fluid was replaced by 100 µL fresh phosphate buffer pH 7.4, and drug release was measured as described in *Section II.3.4*. Perfect sink conditions were provided in the bulk fluid throughout the observation periods. The experiments were conducted in triplicate, and mean values +/- standard deviations are reported. The release kinetics obtained were normalized to 100%, corresponding to the plateau value. A schematic presentation of this experimental setup is represented in Figure 24.

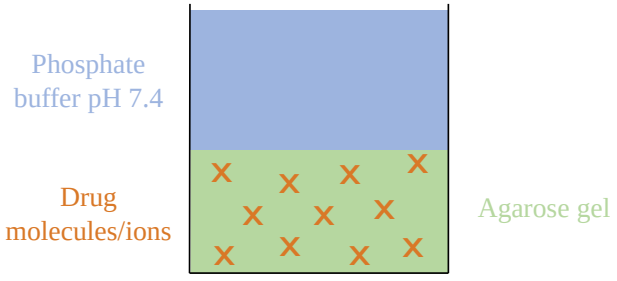


Figure 24. Schematic presentation of the loaded agarose gel setup

II.5. Drug extraction from agarose gel

One hundred µL of a 70:30 (v:v) mixture of phosphate buffer pH 7.4 and ethanol is brought into contact with the 100 µL agarose gel after removal of the microparticle, and is triturated. The whole is left in contact for 2 hours at 37°C, 80 rpm, and the phosphate buffer is sampled for drug content analysis in HPLC-UV, and replaced with fresh one. This procedure is repeated 3 times to extract the total amount of ibuprofen remaining in the gel. This protocol was conducted in n = 8.

II.6. Drug solubility measurements

Solubility tests were conducted for lidocaine in phosphate buffer and phosphate buffer saline pH 7.4. Excess amounts of lidocaine were exposed to 30 mL of one or other of these media, under stirring at 37°C. Bottles were horizontally shaken (80 rpm, 37°C) for 21 d. At pre-determined timepoints, 500 µL were withdrawn, filtered (PVDF syringe filters, 0.22 µm) and diluted before analysis in HPLC-UV, as described in *Section II.3.3*.

CHAPTER III – IMPACT OF THE PHYSICAL STATE OF IBUPROFEN ON THE SINGLE RELEASE KINETICS OF PLGA MICROPARTICLES

I. Objectives of the work

When microparticles are prepared according to an emulsion-solvent evaporation technique in a conventional way (described in Chapter II, *Section II.1.1*), their characterization under the optical microscope showed differences in their appearance. Some microparticles appeared transparent, while others were opaque. Interestingly, it was noticed that for the same batch, both types of microparticles could be found.

The hypothesis for this difference in appearance would be that the drug is present in a different physical state in these two types of microparticles. The active ingredient may indeed be dissolved in the PLGA matrix and thus be in an amorphous form, which would potentially make the microparticle transparent. On the other hand, if the active ingredient is dispersed in the matrix, it will remain in crystalline form, and may thus give an opaque appearance to the microparticle. It can therefore be assumed that if the API content in the MP exceeds the API solubility in the polymer, the API is likely to be found in both physical states. MPs with a higher API content should therefore appear more opaque than MPs where all the API is solubilized in the PLGA matrix in an amorphous form.

The purpose of this study is to determine the potential reason for this difference in appearance, within the same batch or not, and whether this difference could affect the drug release. To this end, the microparticles will be studied individually. In some cases, the opaque microparticles will be separated from the transparent ones, in order to correlate their appearance to a certain physicochemical characteristic, or a certain release behavior. In addition, the change in appearance of MPs over time will be monitored to evaluate their stability, and its possible impact on ibuprofen release will be studied.

II. Results and discussion

II.1. General characteristics of the investigated microparticles

The characteristics of the investigated microparticles in this study are listed in Table 2. The sample name, the preparation method, the average size of the MPs in the corresponding batch, the polydispersity index (PdI), the average drug loading and encapsulation efficiency, as well as the initial average diameter of the MPs investigated in the release studies for each setup, are reported.

Table 2. Characteristics of the investigated microparticles in this chapter

Sample name	Preparation method	Average diameter (µm)	PdI (%)	DL_{exp}± SD (%)	EE (%)	Initial average diameter of investigated MPs during release (µm)
LAL-020	Standard	344	37	8±0.46	80	Opaque: 336 Transparent: 243
LAL-030	Standard	362	44	14±0.05	95	/
LAL-031	Standard	269	41	20±0.71	98.5	2021.04: 264 µm 2023.04: 259 µm
LAL-046	Standard	203	30	47.5±0.4	95	Opaque: 270 Transparent: 255
LAL-047	Microfluidics	206	25	38±1.32	84	/
LAL-048	Microfluidics	233	33	47±4.9	95	/

II.2. Characterization and release studies of single opaque and transparent MPs

Before better understanding the mechanisms by which active ingredients are released from PLGA MPs, it is important to carry out an in-depth characterization of these MPs, in order to determine which parameters might influence release. The first characterization consists of an observation under an optical microscope. Indeed, this provides an overview of particle size, morphology, appearance, and size distribution. It was therefore noticed that, within the same batch, some MPs were fully transparent, while others were more opaque or even completely white. Interestingly, there was no direct apparent link between the drug loading and the fact that opaque and transparent MPs are found in the same batch. Indeed, in Figure 25 a, a batch loaded with only 8% of ibuprofen (LAL-20) contains transparent but also opaque MPs, as does batch b, loaded with 47.5% of ibuprofen (LAL-046). Nevertheless, some of the MPs in Figure 25 b appear slightly more opaque than the transparent MPs of batch LAL-020 (the quality of the images taken with the microscope can be responsible for the different appearance of the MPs).

a) b)

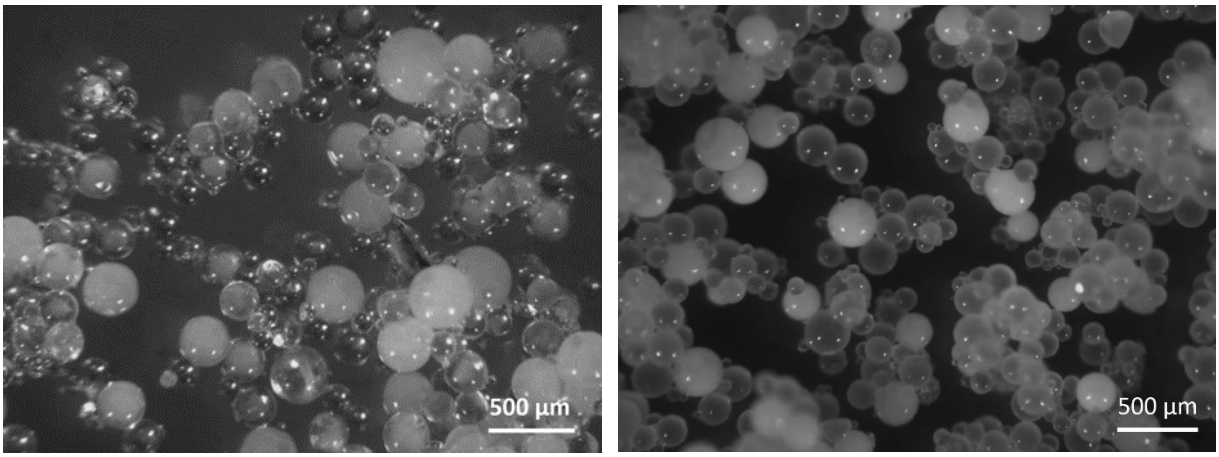


Figure 25. Optical microscopy pictures of the investigated microparticles a) LAL-020 (8% ibuprofen-loaded, and b) LAL-046 (47.5% ibuprofen-loaded), before exposure to release medium

X-ray diffraction analysis was used to determine whether the MPs present Bragg peaks, characteristics of crystalline structures, or a large diffusion halo, characteristic of an amorphous form. Indeed, this technique is based on a beam of X-rays, diffracted or not by the material it passes through. The direction of the diffracted X-rays is characteristic of a specific organization of the crystalline network.

Figure 26 shows the X-ray diffraction patterns of the two different batches represented on the optical microscopy pictures (Figure 25), and the patterns of drug-free microparticles as well as raw materials (mixtures of opaque and transparent microparticles, if applicable). It can be seen that pure ibuprofen exhibits Bragg peaks, characteristics of crystals, while pure PLGA exhibits an amorphous halo. Fully transparent drug-free microparticles (first picture in Figure 42) exhibit the same amorphous halo as pure PLGA. Interestingly, the same Bragg peaks are visible in batch LAL-046, while they are barely noticeable in batch LAL-020, which suggests that more crystalline ibuprofen is present in batch LAL-046.

These results are consistent with the DL of the MPs: A more loaded batch (LAL-046) has a greater chance of containing ibuprofen crystals than a less loaded batch (LAL-020). Even though only small diffraction patterns were detected for the batch loaded with 8% ibuprofen, it can be assumed that a small part of crystalline ibuprofen is enough to make MPs opaque and generate peaks on the diffractogram.

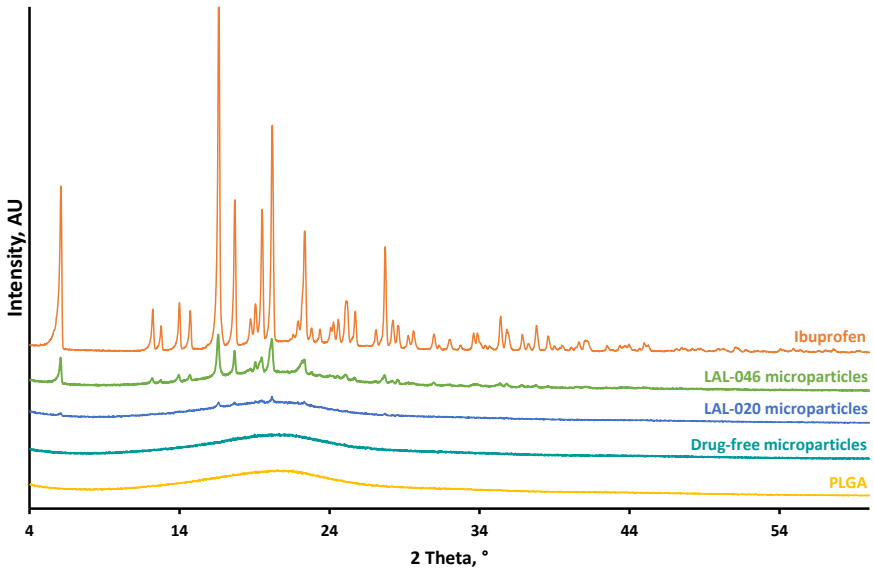


Figure 26. X-ray diffraction patterns of the drug-free, LAL-020 and LAL-046 microparticles, and raw materials (PLGA & ibuprofen)

However, it is difficult to carry out a quantitative study using X-rays with the sample preparation method used here, because the signal intensity depends, in particular, on the quantity introduced into the capillary. This led us to further investigate the physical state of ibuprofen in the MPs using differential scanning calorimetry (DSC). This thermal analysis measures the difference in heat exchange (endothermic or exothermic transition) between a sample to be analyzed and a reference, and can provide information about the melting temperature of a product, its glass transition temperature (T_g), its recrystallization temperature

if applicable, and enthalpy reaction values. The obtained thermograms are shown in Figure 27 (these results were obtained by analyzing a mixture of opaque and transparent MPs, if applicable). Arrows are pointing out the T_g.

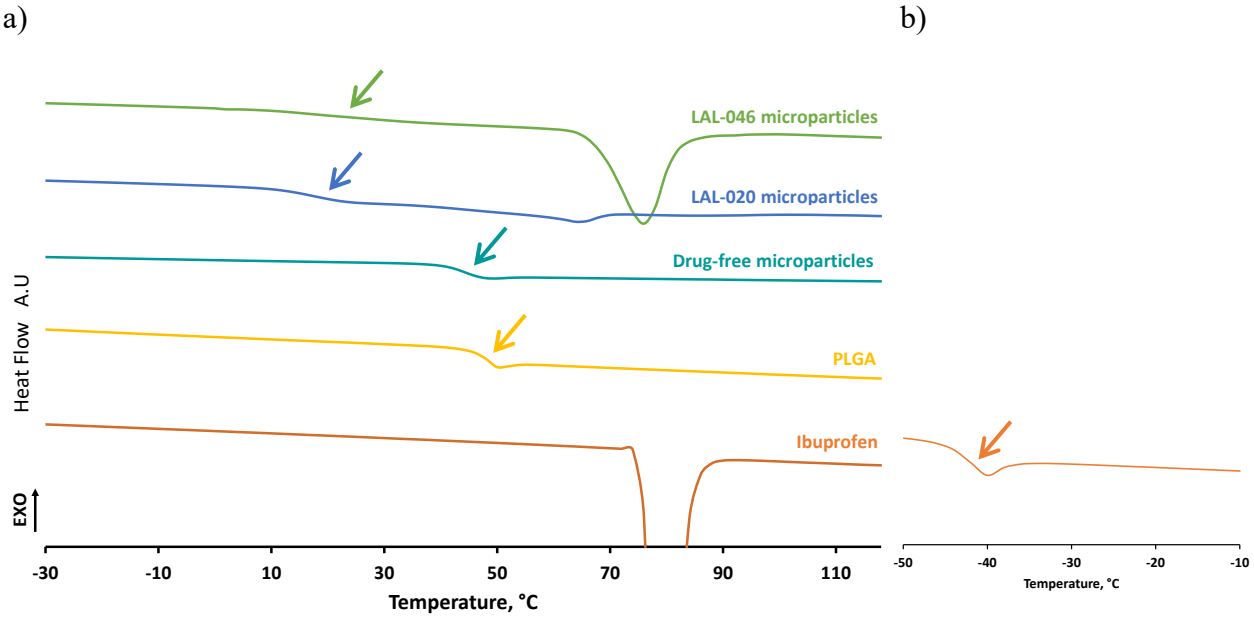


Figure 27. a) DSC thermograms of the: Drug-free and investigated LAL-020 and LAL-046 microparticles (before exposure to the release medium, first heating cycle) and raw materials (PLGA, second heating cycle & ibuprofen, first heating cycle); b) DSC thermogram of ibuprofen (before exposure to release medium, second heating cycle)

Pure ibuprofen exhibits a melting peak starting around 76 °C, during the first heating cycle (orange thermogram in Figure 27 a). The quench cooling followed by subsequent heating of ibuprofen has allowed to highlight a T_g of ibuprofen around -44 °C (Figure 27 b). The T_g of pure PLGA 503 H is around 47 °C (yellow thermogram). Surprisingly, the T_g of drug-free MPs (44 °C) was slightly lower than the T_g of pure PLGA, probably due to the presence of a small amount of water in the MPs, which presents a plasticizing effect. The thermogram of LAL-020 showed a C_p jump, characteristic of a glass transition at T_g = 16 °C, followed by a small endotherm, attributed to the dissolution of crystalline ibuprofen remaining in the polymer, appearing before the ibuprofen melting endotherm. Concerning batch LAL-046, its T_g was around 20 °C, and the dissolution peak was much more pronounced than in batch LAL-020, so there was indeed more crystalline ibuprofen in the most highly loaded MPs (LAL-046). The observed T_{gs} are lower in the case of the drug-loaded MPs, compared to the T_g of raw PLGA. This is explained by the plasticizing effect of ibuprofen. According to the Gordon-Taylor equation, the T_g value of PLGA will be shifted to lower temperatures in the presence of ibuprofen because of its lower T_g. The fact that the T_g in batch LAL-020 is a bit lower than that in batch LAL-046 could be explained by the fact that more ibuprofen was solubilized in PLGA

in the case of batch LAL-020, or that a few more water molecules were present in the LAL-020 MPs.

These analyses showed that in batches of MPs that appeared quite similar at first sight, the crystallinity could be more or less pronounced. However, as mixtures of opaque and transparent MPs were studied, no clear evidence indicated that the opaque MPs contained ibuprofen in crystalline form, and the transparent MPs contained ibuprofen in amorphous form. Before further investigating the characterization and release behavior of opaque and transparent MPs from the same batch separately, the focus will be on batches which were more homogeneous in terms of appearance. Two examples are shown in Figure 28: A 14% ibuprofen-loaded batch composed of fully transparent MPs (Figure 28 a) and a 38% ibuprofen-loaded batch made of a majority of opaque MPs (Figure 28 b).

a) b)

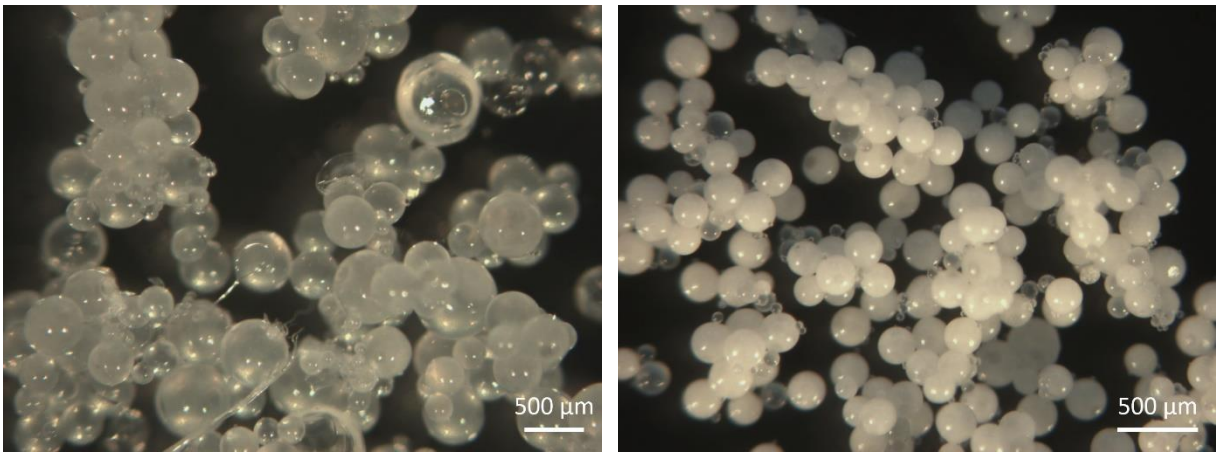


Figure 28. Optical microscopy pictures of a) 14% ibuprofen-loaded LAL-030 microparticles and b)38% ibuprofen-loaded LAL-047 microparticles, before exposure to release medium

With batch LAL-030 being lightly loaded (14% ibuprofen), there was an important probability that all the ibuprofen would have solubilized in amorphous form in the PLGA, giving it a transparent appearance. On the contrary, in batch LAL-047, which contained more drug (38% ibuprofen), the matrix was probably saturated with amorphous drug, causing the creation of crystalline ibuprofen clusters, rendering the MPs opaque. This was indeed confirmed by the X-ray analysis of these 2 batches, represented in Figure 29. In addition, a solubility study of ibuprofen in PLGA was conducted to determine when saturation of PLGA with ibuprofen is reached, the results will be presented later in this manuscript (*Chapter V, II.2.,* Figure 57).

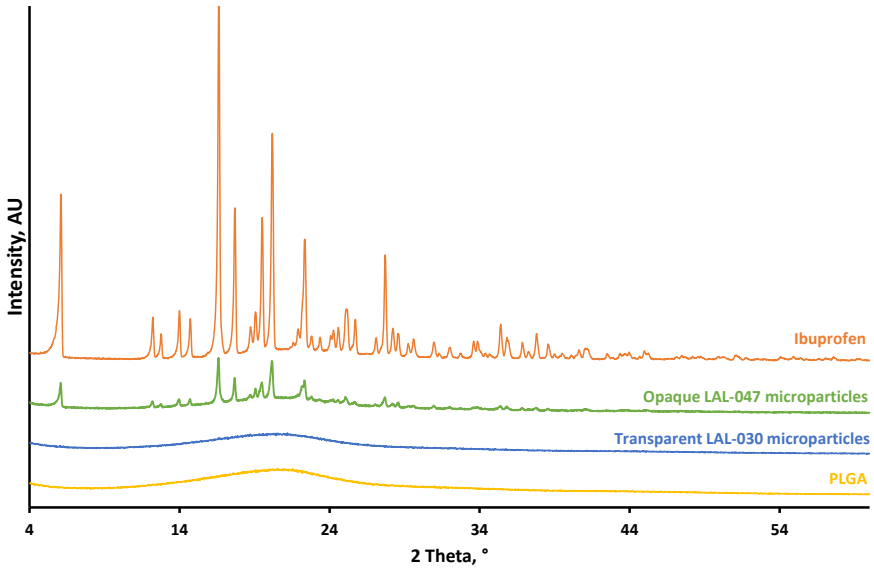


Figure 29. X-ray diffraction patterns of the transparent 14% ibuprofen-loaded LAL-030 and the opaque 38% ibuprofen-loaded LAL-047 microparticles, and raw materials (PLGA & ibuprofen)

Transparent MPs from batch LAL-030 showed a large diffusion halo characteristic of an amorphous form, without any characteristic Bragg peak characteristic of crystalline ibuprofen, the totality of ibuprofen is thus dissolved in the PLGA matrix. On the other hand, opaque MPs of batch LAL-047 showed ibuprofen characteristic diffraction patterns, indicating the presence of crystalline ibuprofen in these MPs, probably due to the saturation of the matrix by a large amount of ibuprofen. The relationship between an amorphous or crystalline drug and its transparent or opaque appearance has been demonstrated.

Therefore, the difference in appearance between these two batches is due to the physical state of ibuprofen within the MPs, which appears to be linked to their different drug loadings. However, it has to be confirmed for MPs within the same batch, which is theoretically loaded with the same amounts of ibuprofen in opaque and transparent MPs. The opaque and transparent MPs of batch LAL-020 have been separated for deeper investigation concerning their physical states. To this end, DSC thermograms of opaque and transparent MPs are shown in Figure 30.

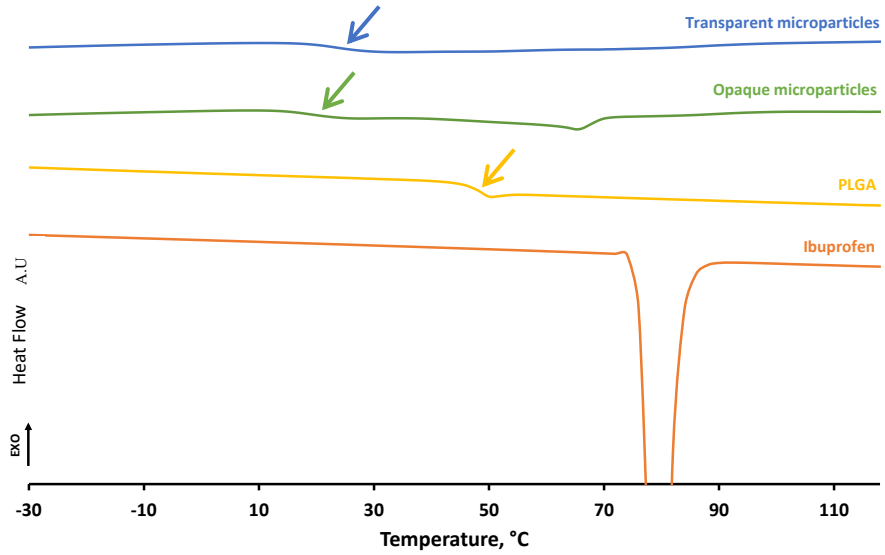


Figure 30. DSC thermograms of the: Investigated LAL-020 opaque or transparent microparticles (before exposure to the release medium, first heating cycle) and raw materials (PLGA, second heating cycle & ibuprofen, first heating cycle)

It can be seen that the transparent MPs showed only a T_g, which is decreased compared to the one of pure PLGA (until around 23 °C), due to the plasticizing effect of ibuprofen, but no evidence of crystalline ibuprofen. On the other hand, opaque MPs showed a T_g around 19 °C, which is slightly lower than the T_g of transparent MPs. This could be explained by the fact that the co-amorphous mixture is more enriched in ibuprofen in opaque MPs than in transparent MPs, which plasticizes more PLGA. In that case, the matrix was potentially saturated with dissolved ibuprofen, and the remaining drug organized into crystal clusters, inducing an endothermic peak around 70 °C, characteristic of the dissolution of ibuprofen. This kind of peak is characteristic of crystalline drug, which dissolves in the matrix when the temperature increases gradually, before reaching the melting temperature.

First of all, the surface appearance was further investigated to assess the potential relationship between the physical appearance, whether opaque or transparent, and a certain surface characteristic. This surface aspect was thus precisely studied thanks to the scanning electron microscope (SEM). This analysis consists of an electronic beam scanning the surface of a sample to be analyzed, previously covered with a thin metal layer. The electrons re-emitted by the surface are then collected by detectors, which analyze the signal received and reconstruct a three-dimensional image of the surface. The SEM pictures obtained for opaque and transparent MPs for batch LAL-020 are shown in Figure 31 a and b respectively.

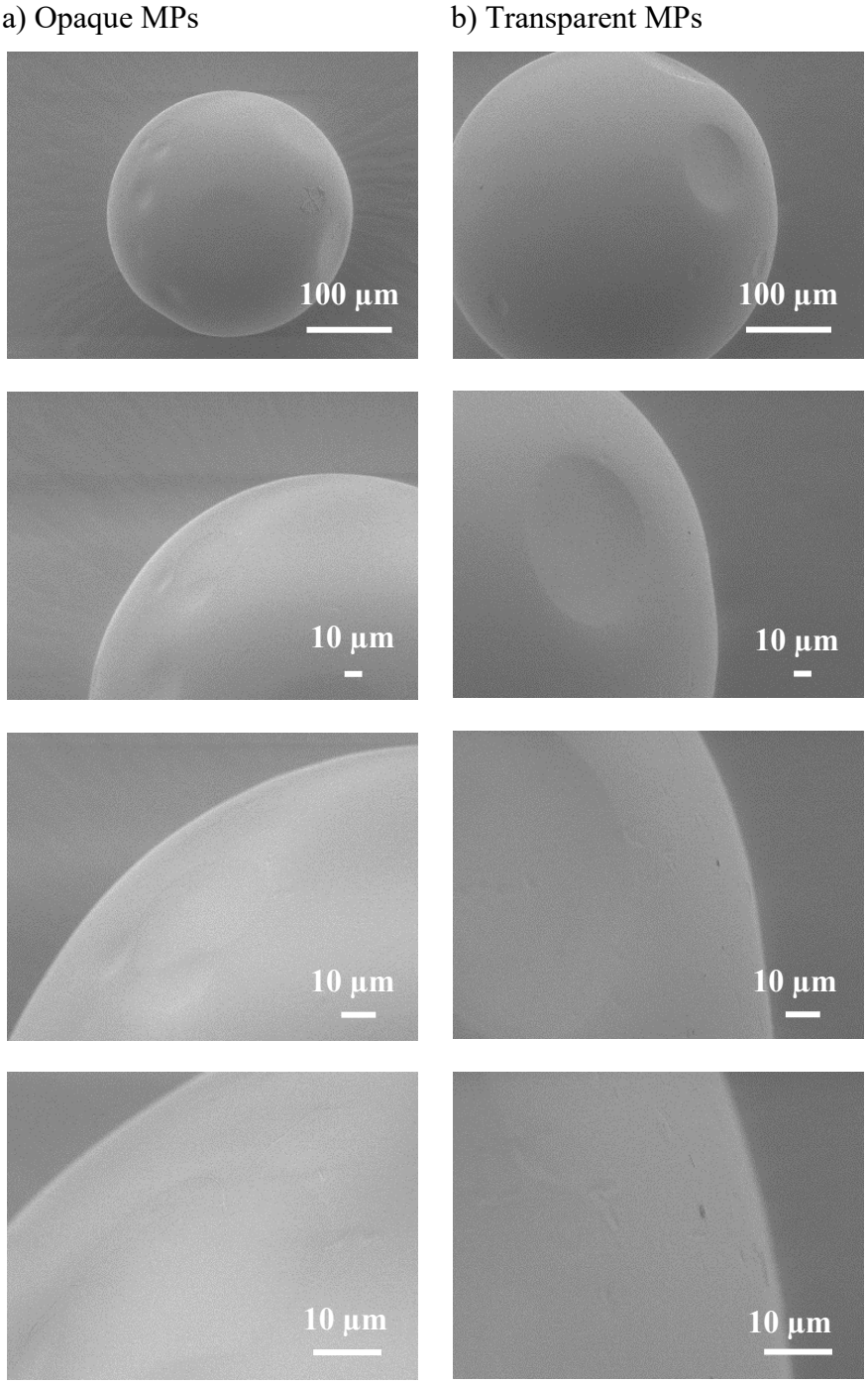


Figure 31. SEM pictures of surfaces of the investigated a) opaque or b) transparent LAL-020 microparticles, before exposure to release medium

As it can be seen in these pictures, there is no difference in surface aspect for opaque or transparent MPs: Surfaces are smooth and non-porous (this was confirmed for several MPs), so the physical state difference between opaque and transparent MPs will not affect the external characteristics of the MPs. However, even if the difference in crystallinity of ibuprofen within the MP does not seem to impact its external structure, it could potentially have an influence on its release behavior. The release and swelling kinetics of opaque and transparent MPs are given

in Figure 32 a and b respectively. It should be pointed out that during release, the microparticles, which were initially spheres, flatten. The swelling of the microparticles was therefore monitored by the increase in their diameter, instead of their volume. The initial diameters of the investigated MPs are reported on the right-hand side of each graph. Please note that each curve represents the release or swelling kinetic of a single MP, and to facilitate graph analysis, the marks assigned to MPs for release kinetics are the same as those assigned for swelling kinetics.

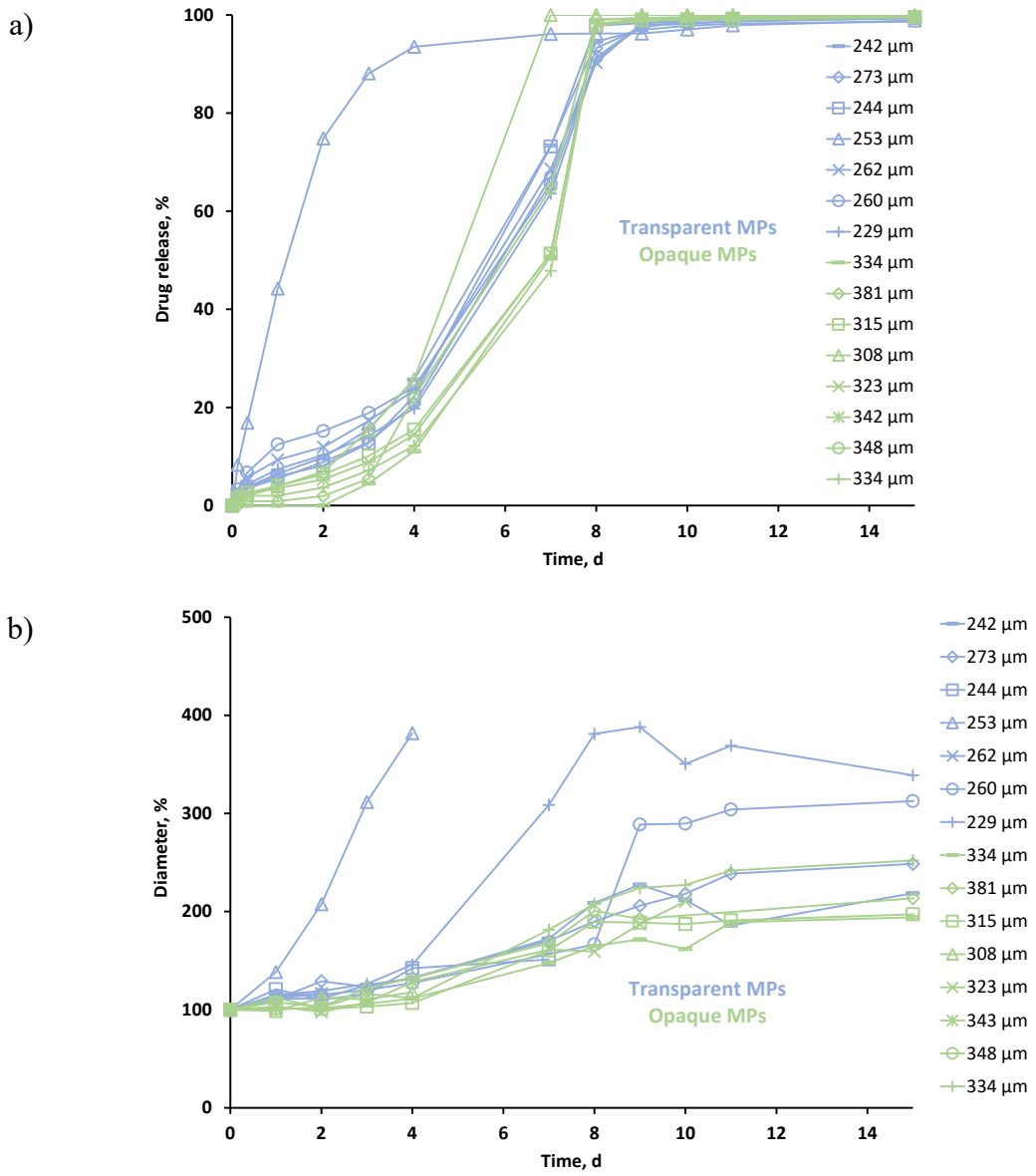
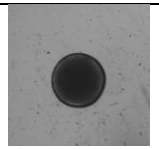
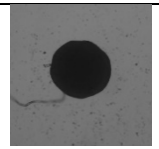
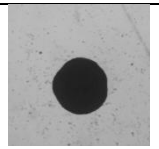
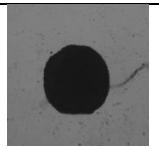
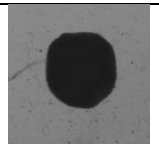
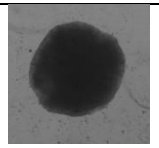
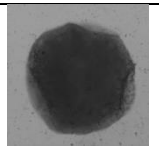
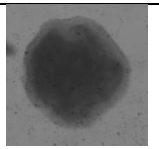
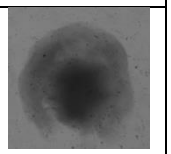


Figure 32. Ibuprofen single a) release and b) swelling behavior of the LAL-020 transparent or opaque microparticles

As it can be seen in Figure 32 a, the release kinetics profiles of both opaque and transparent MPs are all similar and biphasic, without showing any burst, except for one MP for which the release started prior to the others and for which the release was faster (biphasic profile with burst). If the kinetics for each batch are detailed, it can be seen that the release of opaque MPs seems to start a bit later than the release of transparent MPs, even if the duration of the release is the same (8 days). This can be correlated with the swelling kinetics (Figure 32 b). Indeed, the substantial swelling started around day 4 for opaque MPs, whereas the onset of swelling for transparent MPs started from the first days of exposure to the release medium. This slightly more rapid swelling of transparent MPs could be due to the fact that they were smaller than the opaque ones (average of 243 µm for transparent and 336 µm for opaque), leading to faster water penetration in the entire MP. Nevertheless, this swelling difference is slight and induces a slight release difference as well. However, the release behavior of the MP that differs from the others has to be investigated in more depth. Figure 33 shows optical microscopy pictures for some MPs during the release studies of opaque (Figure 33 a) and transparent MPs (Figure 33 b).

a) Opaque MP

	5 min	1 d	2 d	3 d	4 d	7 d	8 d	9 d	15 d
381 µm									

b) Transparent MPs

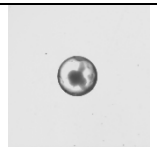
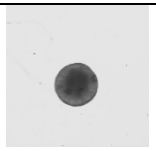
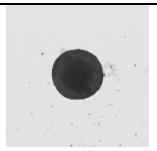
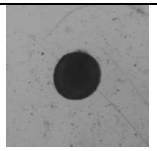
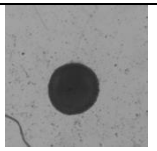
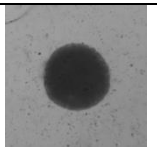
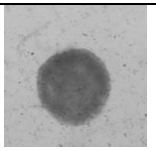
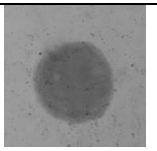
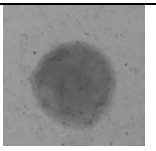
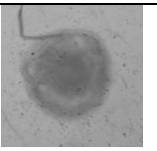
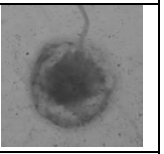
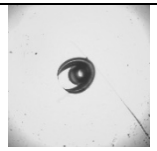
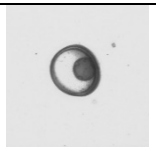
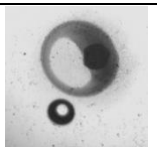
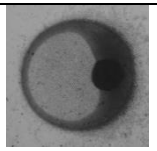
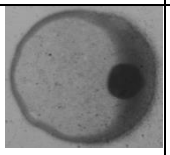
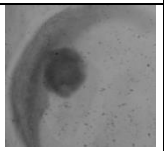
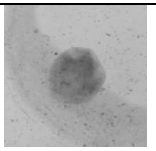
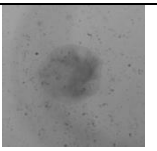
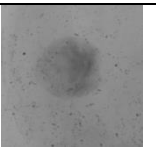
	5 min	1 d	2 d	3 d	4 d	7 d	8 d	9 d	10 d	11 d	15 d
273 µm											
253 µm											

Figure 33. Optical microscopy pictures of the investigated a) opaque and b) transparent LAL-020 microparticles upon exposure to PB pH 7.4. The exposure times are indicated at the top, the initial microparticle diameter on the left-hand side

Interestingly, Figure 33 shows that on the starting day of the release (after 5 minutes of exposure to the release medium), opaque MPs appeared black while transparent ones did not (the microscope's light beam comes from below). However, after one day of exposure, initially transparent MPs became opaque and appeared black (example in Figure 33 b, initial diameter of 273 µm), and opaque MPs remained opaque. It can thus be assumed that ibuprofen contained in transparent MPs, initially amorphous, became crystalline after exposure to the release medium.

Surprisingly, one of the transparent MPs remained transparent even after contact with the release medium, as can be seen in Figure 33 b (initial diameter of 253 µm), and this MP was the one releasing differently from the others in Figure 32 a. This release profile could be explained by a MP with ibuprofen which stays in an amorphous form all along the release, which corresponds to what was observed on optical microscope pictures with this MP staying transparent. Indeed, amorphous drugs are more rapidly solubilized in a medium than crystalline drugs, which explains why the release of this MP is over after 4 days of exposure to the release medium, compared to 8 for all the others. Furthermore, this MP seems to be composed of one MP encapsulated in a bigger one, probably a hollow one. This could happen during the formation process of MPs produced with a classical beaker setup, but this has never been observed again. This special organization of PLGA and ibuprofen within this MP could maybe explain why it remained amorphous all throughout the release. Another reason could be that the ibuprofen loading rate is low in this MP: A very low amount of amorphous ibuprofen solubilized in the PLGA matrix would remain amorphous due to favorable surrounding conditions. This hypothesis was then verified by analyzing the total amount of ibuprofen released for each MP. These results are presented in Figure 34.

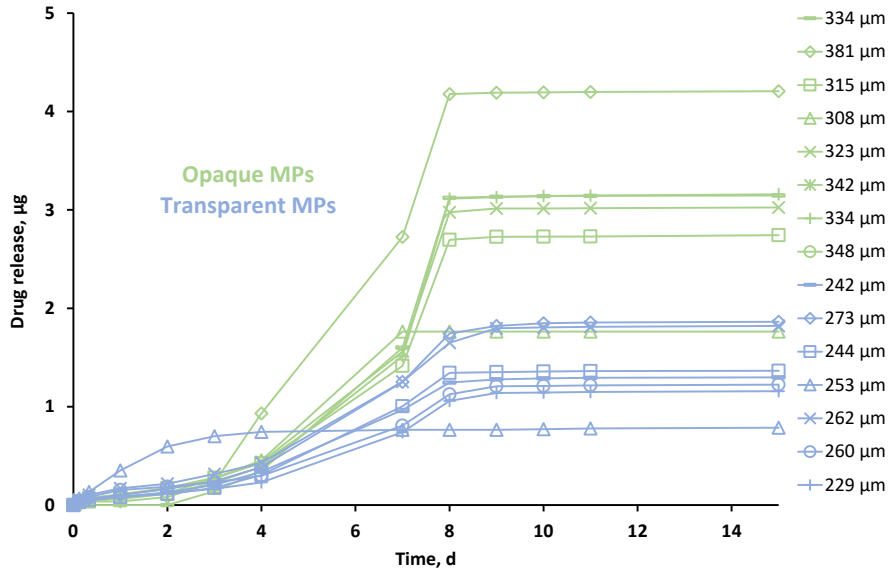


Figure 34. Amounts of ibuprofen released from single LAL-020 opaque or transparent microparticles

As expected, the MP differing from the others was less loaded with ibuprofen (a bit less than 1 µg). It can be clearly seen that opaque MPs are initially more loaded than transparent ones, but they are also bigger than them (initial diameter on the right-hand side of Figure 34). Logically, the larger the MP, the more API it can encapsulate. In this batch, the selection of similar-sized MPs was not really possible because transparent MPs were almost systematically smaller than opaque ones. (as it can be seen in Figure 25 a).

In order to confirm the hypothesis that initially transparent MPs become opaque from the first day exposure to the release medium, a DSC analysis was carried out before and after exposure to PB, on a batch (LAL-031, 20% ibuprofen) whose MPs were initially all transparent. The thermograms obtained are shown in Figure 35.

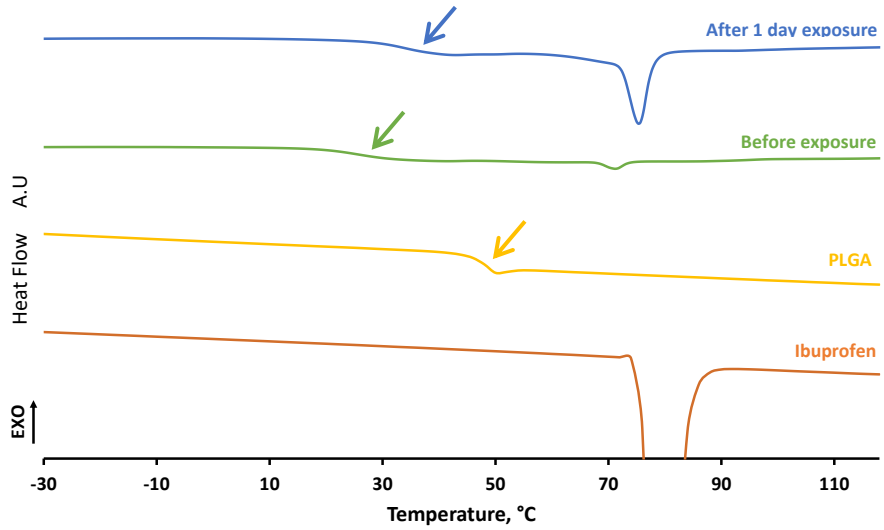


Figure 35. DSC thermograms of the: Investigated LAL-031 microparticles before and after 1-day exposure to the release medium (first heating cycle) and raw materials (PLGA, second heating cycle & ibuprofen, first heating cycle)

DSC analysis of these MPs prior to the release medium exposure showed a very small dissolution profile, while the majority of ibuprofen dissolved in the matrix and was therefore present in amorphous form. If the theory is confirmed, a more important melting peak should be observed after one day in the medium. Nevertheless, these results should be viewed with care, because the MPs exposed to the medium for one day were freeze-dried before DSC analysis, and the freeze-drying process can create artifacts during the drying phase by crystallizing ibuprofen. This phenomenon was not reported for MPs after their post-production freeze-drying, even though they were in contact with an aqueous medium during manufacturing, so it can be presumed that the results are reliable.

As expected, MPs exposed to PB showed a more important dissolution/melting peak than before exposure, confirming that initially amorphous ibuprofen crystallizes very rapidly in contact with the release medium. The crystallinity was ten times more important than before exposure to PB. Moreover, the T_g rose from 26 to 33 °C for MPs exposed to the medium, meaning that the initially amorphous ibuprofen that plasticized PLGA dissociated from the matrix as it crystallized. Nevertheless, as this T_g still does not correspond to the one of pure PLGA (T_g = 47 °C), it means that a fraction of ibuprofen did not recrystallize and thus remained amorphous. However, this amorphous fraction might crystallize later during the release.

To determine whether a higher MP loading rate will lead to the same conclusion (no release difference between opaque and transparent MPs), batch LAL-046, loaded with an average of 47.5% ibuprofen and presenting the same opaque and transparent MPs was studied (Figure 25 b). In this case, MPs' sizes were similar between opaque and transparent MPs. The obtained single release kinetics are shown in Figure 36.

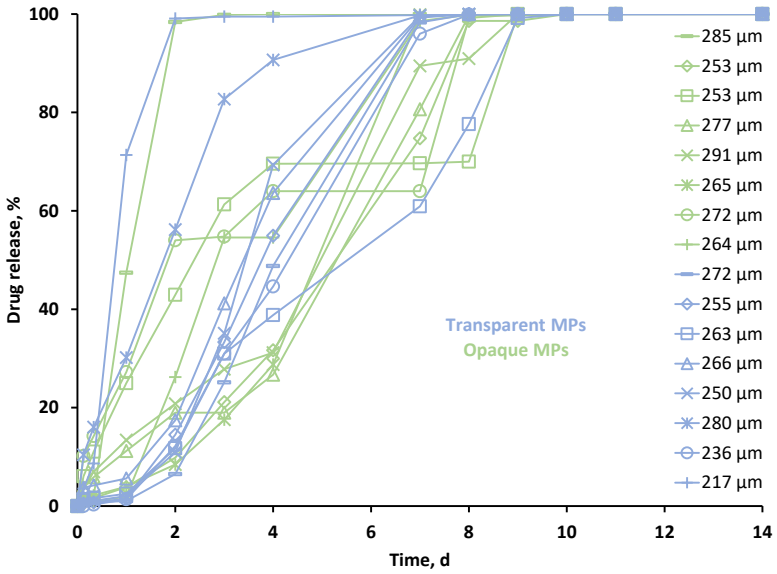


Figure 36. Ibuprofen single release of the LAL-046 opaque or transparent microparticles

At this high loading rate, it is expected for release kinetics to be more heterogeneous between them. In fact, in this case, all the transparent MPs became opaque the first day, meaning that at least a fraction of ibuprofen recrystallized. As a consequence, each time a crystal breaks, ibuprofen is released in the medium, and if the crystals are close to each other, it will create ways for the release medium to penetrate and break other crystals, inducing a fast release. On the other hand, MPs with less connected drug crystals will release slowly. This could explain why the release can last between 2 and 9 days for this batch. At the end, the same conclusion can be drawn: There is no difference in the release of opaque and transparent MPs, as the drug becomes rapidly crystalline after a few hours' exposure to the release medium.

In order to determine if the individual loading rate was different between opaque and transparent MPs for this batch, as it was for LAL-020, the total amount of ibuprofen released is given in Figure 37. Please note that in this case, the selected MPs had more comparable sizes between opaque and transparent, and the physical appearance did not seem to vary according to the size of the MPs.

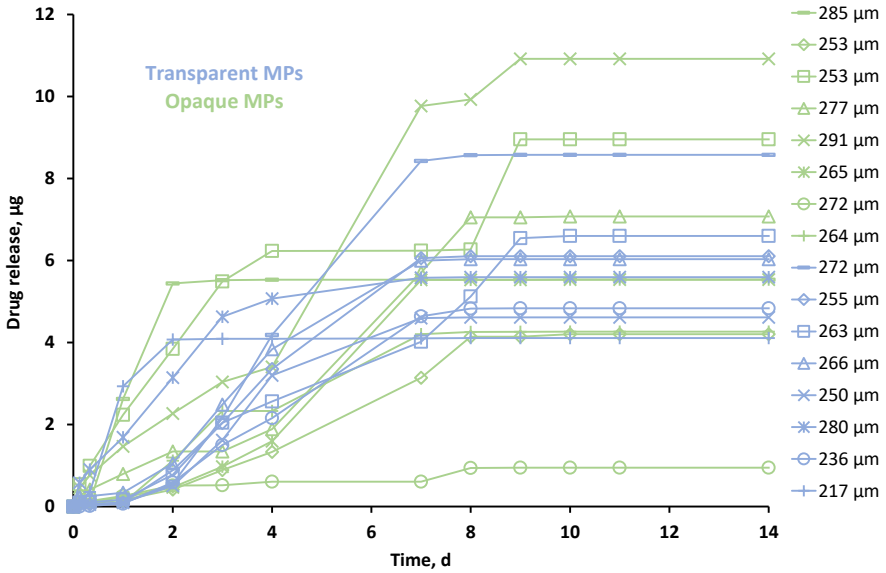


Figure 37. Amounts of ibuprofen released from single LAL-046 opaque or transparent microparticles

Contrary to batch LAL-020, as MPs have a similar diameter, opaque MPs are not more loaded than transparent MPs. The physical appearance seems thus to be independent from the loading of each MP: A more highly-loaded MP will not necessarily be more crystalline than a less loaded one. However, there is an important variability in loading between the MPs, which could explain the heterogeneous kinetics observed in Figure 36. The production process is probably responsible for this non-uniform distribution of the drug and the difference in appearance. Indeed, the precipitation speed of the droplet in the aqueous phase can vary, and possibly create differences in drug encapsulation. It can be assumed that a fast precipitation will induce a trapping of the drug that will stay in an amorphous form, whereas if the precipitation is slower, the drug will have more time to organize into a more stable state, leading to the apparition of crystals. This could explain why for the batch LAL-020 presented before, there was a correlation between the large size of the MPs and their opacity, and vice versa for the transparent ones. Indeed, for large MPs, as it takes longer for the solvent to evaporate, ibuprofen has more time to crystallize. This would also explain why the transparent MP with an initial diameter of 272 µm was loaded with 8 µg of ibuprofen for a total MP's mass of about 13 µg. Normally, at this high DL, a part of ibuprofen, which could not be solubilized in the saturated PLGA matrix, should have crystallized (this will be further investigated in Chapter V). This is potentially due to an oversaturation of the polymer with an amorphous drug, which occurs during the production process. This theory will be further investigated later in this manuscript.

Previously, it has been shown, thanks to the SEM images, that there was no difference in surface characteristics between the opaque and transparent MPs in the LAL-020 batch.

However, the internal structure has not been investigated yet. Therefore, X-ray micro computed tomography (XµCT) images were recorded to determine the internal porosity of opaque and transparent MPs. This analysis allows us to obtain virtual cross-sections of samples in a non-destructive manner. The principle of this technique is to scan the sample with a penetrating X-ray beam, and the recovered X-rays, similar to the initial ones or attenuated by the material it went through, are collected on a detector. Stacks of the two-dimensional images obtained are constructed to recreate a three-dimensional image of the sample. This analysis was carried out on initially porous MPs loaded with 47% ibuprofen (LAL-048), as the batch LAL-046, but produced *via* microfluidics. The objective was just to assess whether the porosity could be influenced or not by the physical state of the drug within the MPs of the same batch. The XµCT images obtained for opaque and transparent MPs are shown in Figure 38 a and b, respectively.

a) Opaque MPs b) Transparent MPs

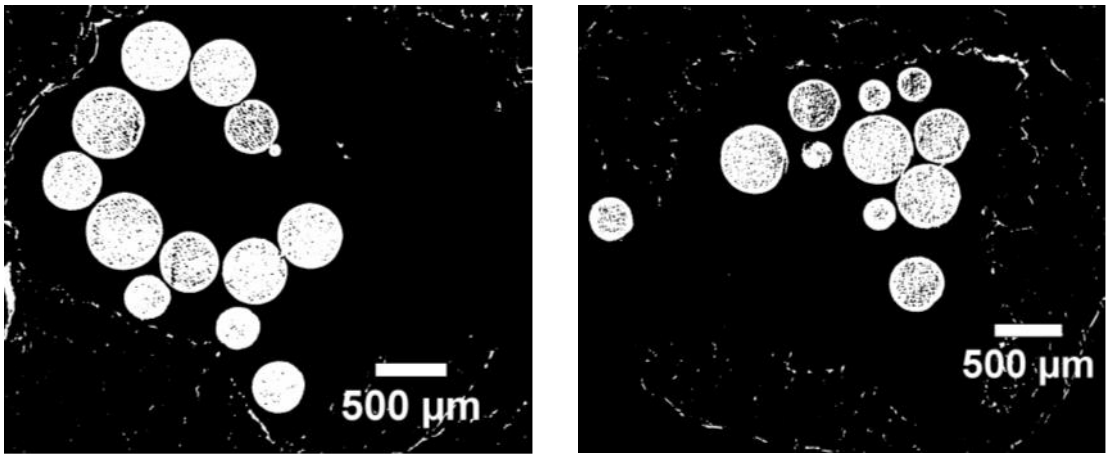


Figure 38. XµCT images obtained for a) opaque or b) transparent LAL-048 47% ibuprofen-loaded microparticles

These images were obtained after color binarization to better see the porosity: MPs are the white spheres, and porosity appears as small black dots inside. It can be seen that some MPs are a bit more porous than others in both cases, but there is no significant difference in porosity between opaque and transparent MPs for this batch.

This study on the impact of the opaque or transparent appearance of MPs was essential to ensuring that future release studies would be carried out on MPs that are comparable. It has been shown that transparent MPs, containing amorphous ibuprofen, crystallized in contact with the release medium and then became opaque. This initial difference in appearance has thus no impact on release, as it disappears within the first few hours of the release study. These results are in accordance with the conclusions drawn from the study of Vay *et al.* (2011) [135], who

compared the release of a fully amorphized drug after solubilization in one solvent, with that of a less well solubilized drug in another solvent. This difference in solubility made it possible to obtain microparticles in which the drug was totally amorphous in the first case, while in the second case the drug was in crystalline form. In both cases, the release kinetics were the same, as the initially amorphous drug crystallized on contact with the release medium.

For future release studies, if the investigated MPs also present this type of difference within the batch, opaque or transparent MPs can be chosen indifferently to be studied without this having any impact on the release behavior. It will therefore not be specified whether the selected MPs were initially opaque or transparent.

II.3. Physical aging and stability studies of MPs

It is well known that an amorphous material is in a non-stable state due to a thermodynamic imbalance. The MPs containing amorphous ibuprofen can thus present the same instability, leading to a rearrangement into crystals over time. PLGA chains in the glassy state can also undergo slow relaxation processes as they attempt to attain a certain thermodynamic equilibrium. MPs were followed for 2 years, in order to monitor the evolution of transparent MPs, for some differently loaded ibuprofen standard batches. Optical microscopic pictures were taken after 2 years of storage in the fridge in hermetical vials, and the physical appearance was compared to those observed after the production of the batch in Figure 39. Unfortunately, the microscope picture quality is quite poor and varied over time in function of the applied settings.

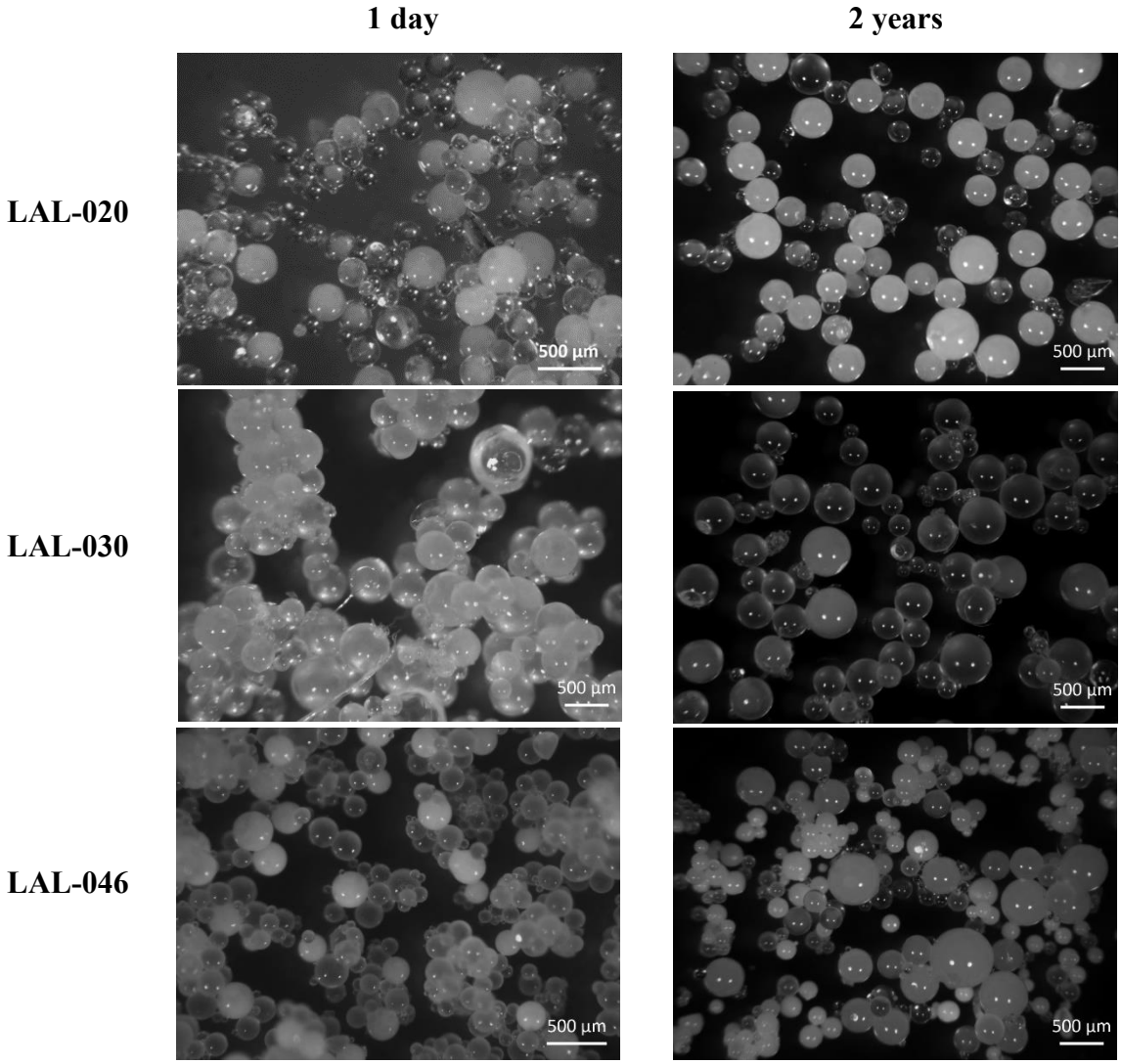


Figure 39. Optical microscopy pictures of the LAL-020, LAL-030 and LAL-046 microparticles taken at t = 1 d after freeze-drying and t = 2 y, before exposure to release medium

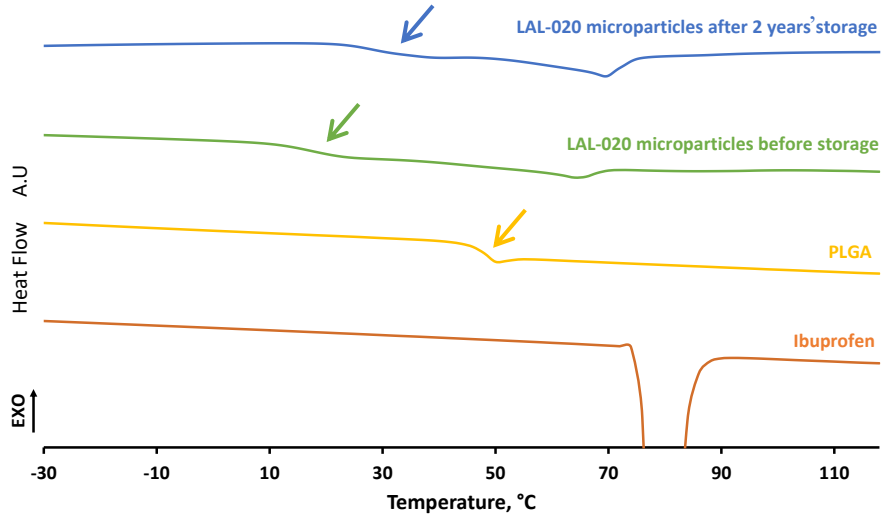
After production, as already mentioned, the most opaque MPs seem to be the largest of the batch. This could be due to the preparation method itself: The standard method used does not guarantee the homogeneity of the emulsion droplets formed. It can therefore be presumed that the larger the droplets, the longer it will take for the DCM to diffuse into the aqueous phase, giving the ibuprofen molecules more time to arrange themselves into crystals rather than remaining in amorphous form if precipitation is rapid (which would be the case for transparent MPs).

After 2 years of storage, the ratio of opaque to transparent MPs appears to have increased for all batches monitored. It seems a little more obvious in the LAL-020 and LAL-046 batches than in the LAL-030. Interestingly, in the case of LAL-020 and LAL-030, the MPs that were

opaque after production, as well as those that became opaque after 2 years, were the biggest of the batch. This is probably indicative of greater thermodynamic instability in the largest MPs. For example, if there is a defect in the MP or something which could be a nucleation point, the amorphous ibuprofen can start to crystallize.

In addition to the visual observation of MPs, DSC analyses were performed on batches after production and after 2 years of storage. Thermograms of LAL-020 and LAL-030 are compared in Figure 40 a and b, respectively.

a)



b)

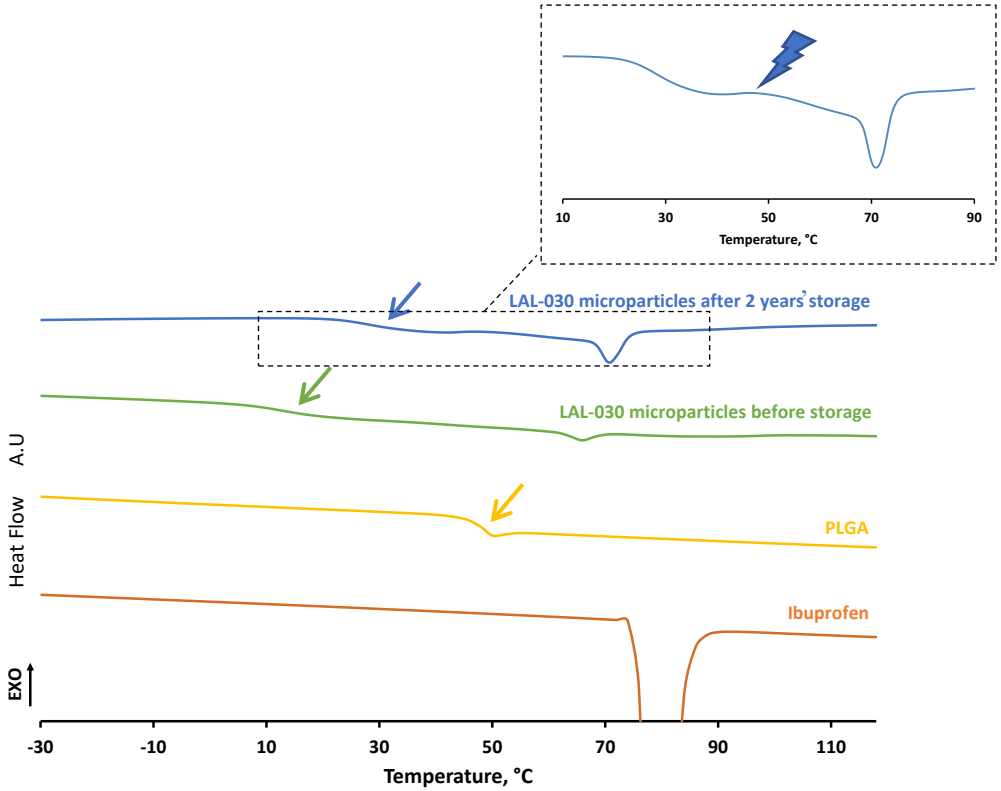


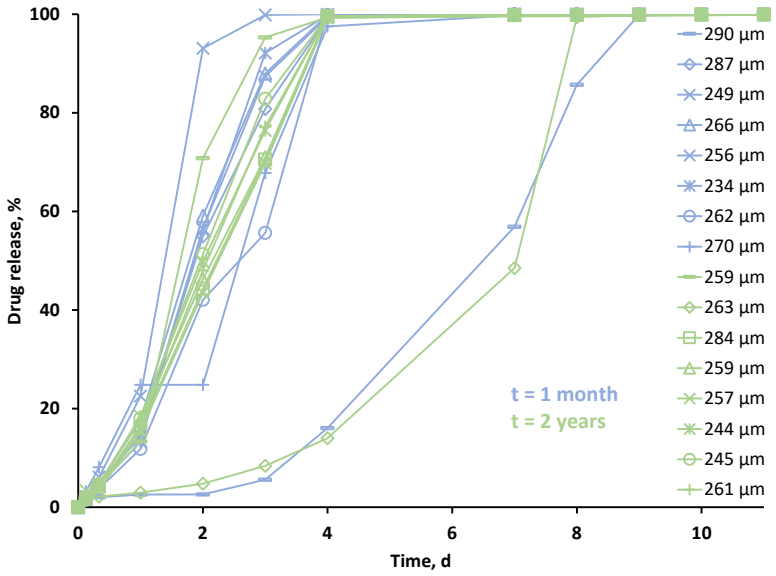
Figure 40. DSC thermograms of the: Investigated a) LAL-020 and b) LAL-030 microparticles (before exposure to the release medium, first heating cycle) obtained before storage and after 2 y storage, and raw materials (PLGA, second heating cycle & ibuprofen, first heating cycle)

After production, both batches exhibited a T_g around 16 °C for LAL-020 and 13 °C for LAL-030, and a slight dissolution profile around 65 °C. After 2 years of storage, T_g increased (29.5 °C for LAL-020 and 29 °C for LAL-030), and the dissolution profile intensified for both batches. These analyses reveal that amorphous ibuprofen has indeed been converted to the crystalline state during those 2 years. As the dissolution peak is more important and the T_g has increased after 2 years, this proves that less ibuprofen is solubilized in the amorphous state in the matrix. Another phenomenon, which is highlighted by the zoom and pointed out with the blue lightning sign on Figure 40, is the crystallization of the amorphous ibuprofen upon heating. The amorphous ibuprofen is in an unstable state after 2 years of storage and therefore recrystallizes more easily than before storage, for which no crystallization reaction was observed.

In order to ensure that this change in the physical state of ibuprofen within the MP had definitely no impact on the release behaviors, 2 release studies were carried out 2 years apart on batch LAL-031, 20% ibuprofen-loaded. This batch was similar to batch LAL-030 in terms

of physical appearance, all the MPs were transparent (fifth picture in Figure 42). The obtained single and average of single release kinetics are shown in Figure 41 a and b, respectively.

a)



b)

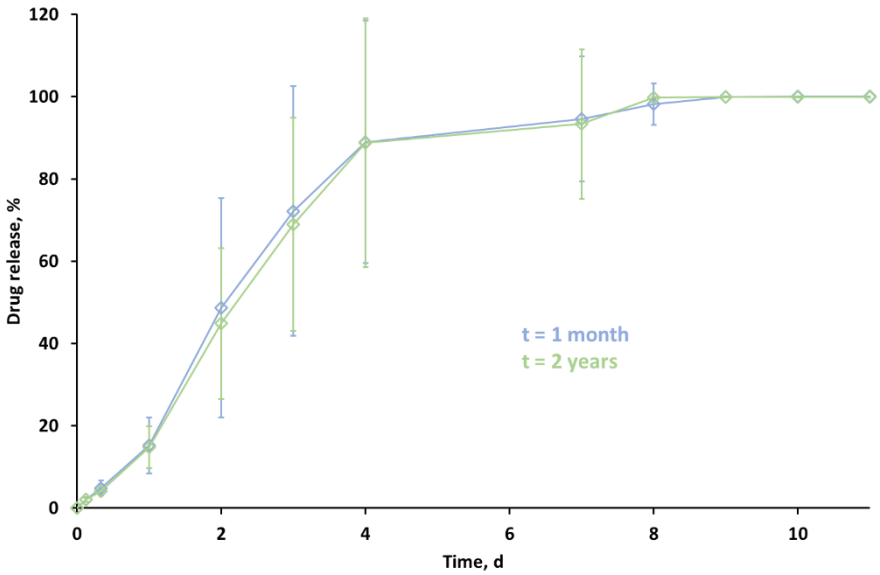


Figure 41. a) Single and b) average of single release kinetics of the LAL-031 MPs, 1 month after production and after 2 y storage

The single release kinetics of the same batch two years apart showed no significant difference. For most of the MPs, the release is biphasic, and over after 4 days of exposure to the release medium. Both times, one MP exhibited a longer release (8-9 d) (Figure 41 b). This is explained by the fact that these MPs started to swell later than the others, which provoked a delay in the release (the correlation between swelling and release will be further investigated in the following chapters).

III. Conclusion

Studying single MPs instead of groups allowed us to better monitor the correlation between a specific characteristic of the MP and its release behavior. Thanks to this specific way of investigation, we were able to assess the impact of the physical appearance of the MPs on the release of ibuprofen. It has been proven that the fact that some MPs appear opaque and others transparent has no incidence on release kinetics. As a result, the fact that initially transparent MPs tend to become opaque over time has no consequence on release, leading us to believe that MPs are stable, for at least several years when stored in the fridge, away from humidity.

III. Conclusion

L'étude de MPs individuelles plutôt que de groupes a permis de mieux évaluer la corrélation entre une caractéristique spécifique de la MP et son comportement de libération. Grâce à cette méthode d'investigation spécifique, nous avons pu évaluer l'impact de l'aspect physique des MPs sur la libération de l'ibuprofène. Il a été prouvé que le fait que certaines MP apparaissent opaques et d'autres transparentes n'a pas d'incidence sur la cinétique de libération. Par conséquent, le fait que des MPs initialement transparentes tendent à devenir opaques au fil du temps n'a aucune conséquence sur la libération, ce qui nous amène à penser que les MPs sont stables, au moins pendant plusieurs années, lorsqu'elles sont conservées au réfrigérateur, à l’abri de l’humidité.

CHAPTER IV – IMPACT OF THE PREPARATION METHOD ON IBUPROFEN RELEASE FROM SINGLE PLGA MICROPARTICLES

I. Objectives of the work

The growing popularity of microfluidics as a new method for microparticle production is of interest to industry because of its repeatability and reproducibility, and has facilitated the steps towards scale-up compared to more conventional production methods, as mentioned above. Moreover, this production technique uses less solvent (amongst others) than a conventional beaker method, which reduces the quantity of waste, making it a more economical method that is also more respectful of the environment. However, this preparation process is responsible for microparticle specific characteristics, potentially different from microparticles prepared following other methods. These intrinsic microparticle characteristics could potentially affect their behavior in *in vitro* release studies.

The objective of this work was to compare two different microparticle preparation processes: A conventional beaker setup method, *versus* a method involving a microfluidics device. These two preparation methods, already described in Chapter II, *Sections II.1.1* and *II.1.2*, Figure 20 and Figure 21, respectively, present similarities as well as differences. These differences in the preparation process will potentially have a strong impact on the microparticles' characteristics. This study will enable us to assess which microparticle characteristics each preparation method leads, and whether these characteristics have a significant impact on microparticle release behaviors.

II. Results and discussion

II.1. General characteristics of the investigated microparticles

The characteristics of the investigated microparticles in this study are listed in Table 3. The sample name, the preparation method, the average size of the microparticles in the corresponding batch, the polydispersity index, the average drug loading and encapsulation efficiency, as well as the initial average diameter of the MPs investigated in the release studies for each setup, are reported. In order to reduce bias as much as possible in the comparison of the preparation method, the investigated MPs always have a similar diameter, to within a few tens of micrometers, and a similar drug loading.

Table 3. Characteristics of the investigated microparticles in this chapter

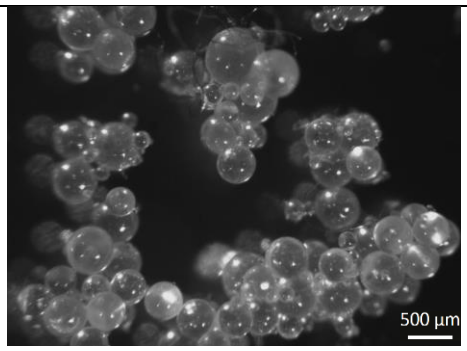
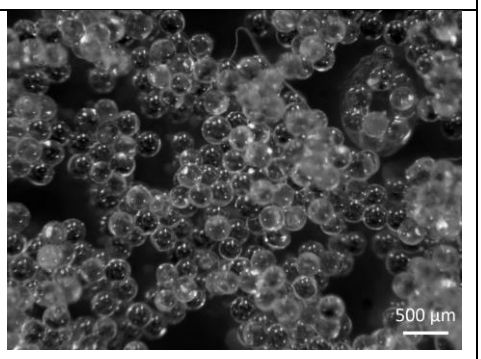
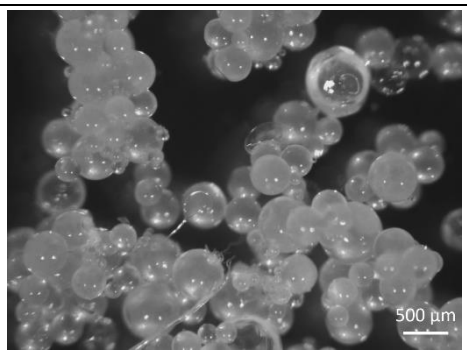
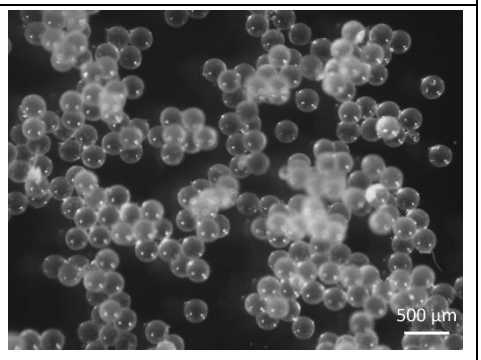
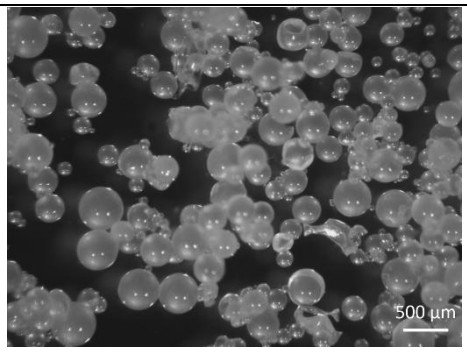
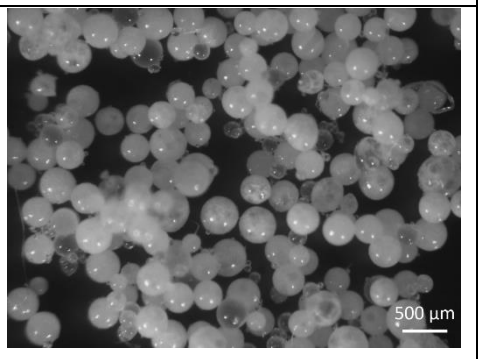
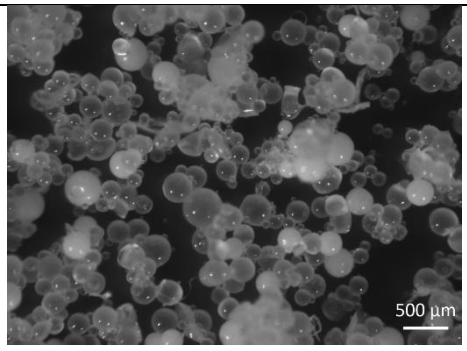
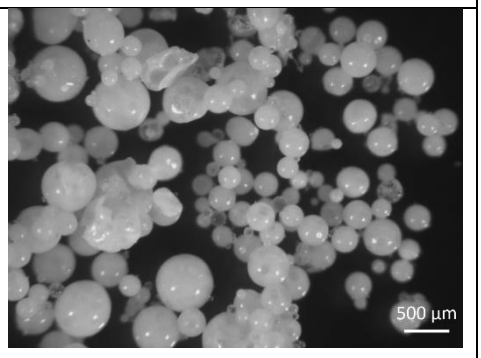
Sample name	Preparation method	Average diameter (µm)	PdI (%)	DL_{exp}± SD (%)	EE (%)	Initial average diameter of investigated MPs during release (µm)
LAL-030	Standard	362	44	14±0.05	95	286
LAL-031	Standard	269	41	20±0.71	98.5	259
LAL-038-1	Microfluidics	262	5	15±0.22	75	239
LAL-041	Standard	242	32	36±1.92	91	270
LAL-042	Microfluidics	326	20	23.5±0.64	78	267
LAL-044	Microfluidics	319	44	36±0.58	96	297
LAL-046	Standard	203	30	47.5±0.4	95	237
LAL-048	Microfluidics	233	33	47±4.9	95	286

First of all, it can be noted that the preparation method does not impact the size of the MPs because, independently of the method, the average sizes are comprised between 200 and 360 µm. Sometimes, for a comparable DL, standard MPs are bigger than the microfluidics' MPs, sometimes it is the opposite. However, the homogeneity in terms of size distribution differs according to the method used. The polydispersity index (PdI), which characterizes this size homogeneity, is lower for MPs produced in microfluidics. This means that when they are produced in microfluidics, the size of MPs is more homogeneous than that of MPs produced with the beaker method. This could be explained by the fact that microfluidics is a fully automated device, which controls the flow of the two phases, and allows for a regular mixing. The droplet size being similar, the resulting MPs after hardening have a similar size. This method is then reproducible by anyone applying the same parameters (flow rate, phase ratio, phase concentration, etc.).

On the other hand, the beaker method involves a manipulator to push the organic phase through the syringe into the aqueous phase: The droplets formed will differ according to the speed and force applied to the syringe, but also to the height and shape of the blade, the width of the beaker, or the height of the syringe at the injection time, since the shearing of the droplets will differ according to all these parameters. This suggests that the drug release will probably be more homogeneous for MPs produced using a microfluidics device than with the classical beaker method. Indeed, in the case of a subcutaneous injection, for example, if MPs are differently sized, this will result in a poorly controlled release, as smaller MPs will quickly release the drug, while larger ones will take longer to release. Obviously, this has to be seen in the context of MPs of a few microns. Furthermore, if a parallel is drawn with the previous Chapter, it was noted that the MPs produced in microfluidics have a similar appearance within the same batch (either all opaque or all transparent), whereas in the standard method, some batches presented opaque and transparent MPs for the same batch. Consequently, the comparative release study of opaque and transparent MPs from the same batch produced in microfluidics was not carried out.

II.2.1. Preliminary characterization of MPs produced by the beaker or microfluidics method

Before detailing the release kinetics for MPs obtained with each of the preparation methods, it is important to determine whether the production method can have an impact on the physical characteristics of the MPs. The optical microscopic pictures of the investigated batches of MPs are shown in Figure 42.

Practical DL (%) Beaker method – Microfluidics method	**Beaker method**	**Microfluidics method**
0 – 0		
14 – 15		
20 – 23.5		
36 – 36		

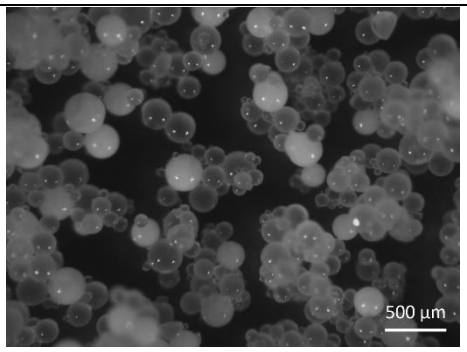
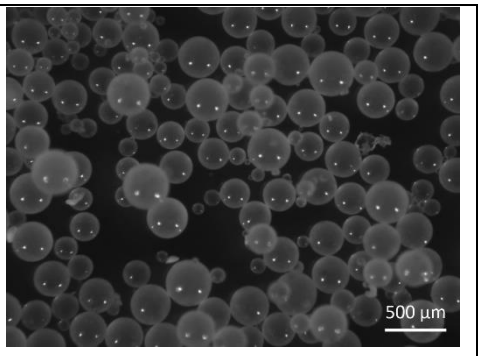
47.5 – 47		

Figure 42. Optical microscopy pictures of the drug-free & the investigated ibuprofen-loaded microparticles, prepared by the beaker or the microfluidics method, in function of the drug loading, before exposure to release medium

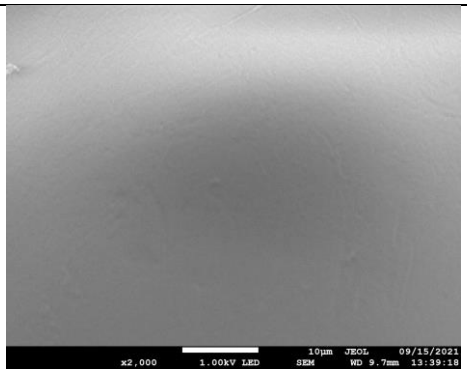
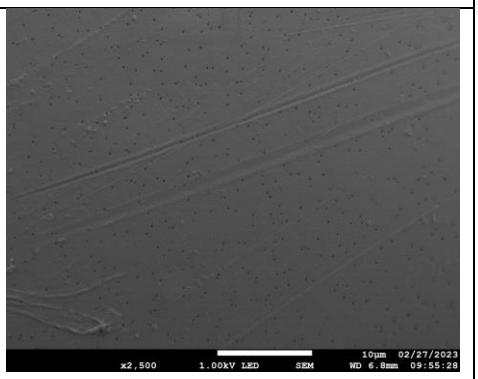
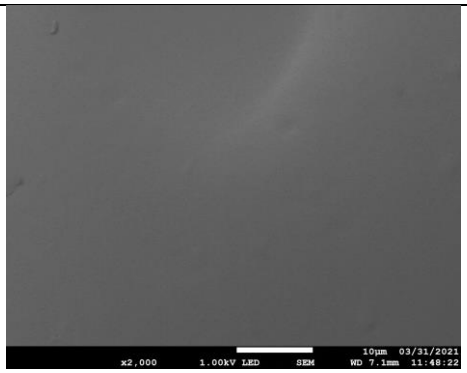
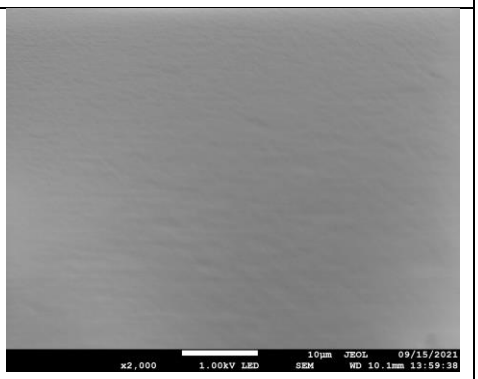
The dispersity of the MP populations obtained, previously described with the PdI, is also visible in the optical microscopy pictures. In most cases, for the same DL, MPs produced using microfluidics are more homogeneous in terms of size than those produced using the beaker method. Nevertheless, depending on the batch, some microfluidics MP populations seem more polydisperse than others (for example, in Figure 42, the one loaded with 36% ibuprofen is much more polydisperse than the one loaded with 15%). This may be explained by the fact that the device did not always have a completely stable flow rate throughout production, due to problems with the device itself. This resulted in different droplet sizes within the same batch. However, with a more precise device, these differences in size should be avoided.

In Table 3, the encapsulation efficiencies (EE) of the two methods are also reported. It has to be pointed out that, generally, the EE of microfluidic MPs is lower than that of MPs produced using the beaker method. This could be explained by the fact that in the beaker method, droplets are quickly hardened, thanks to the fast stirring and the high aqueous phase volume, whereas in the microfluidics method, droplets are produced first, and agitation takes place only after all droplets have been produced, and not during the process. Moreover, the stirring speed is less important, in order to maintain the droplet size and not apply shearing that could modify them. As a consequence, droplets stayed longer in the aqueous phase before being hardened. This results in a diffusion of the drug into the aqueous phase, so the resulting MP will be less loaded than expected, and the encapsulation is then lowered. This hypothesis has been confirmed by measuring the amount of drug recovered in the aqueous phases.

Despite this lower EE (which can however be overcome by a weak agitation system concomitant with droplet production), microfluidics has the advantage of being more easily scalable than the standard method, because the same parameters could be applied to many

microfluidics devices, instead of transposing the classical beaker method to industrial-sized tanks and blades, and readjusting the parameters to have the same shearing of the droplets, and then the same MP sizes.

As for release studies, selected MPs have sizes close to within a few tens of microns, and similar DLs based on the average DL of each batch. Sizes and DLs will therefore have no impact on the MPs release behaviors. However, the differences between the two methods previously highlighted may have other impacts on the characteristics of the MPs, particularly from a structural point of view. This is what will be attempted to be determined through SEM imaging. Surfaces and cross-sections of the investigated MPs are given in Figure 43 and Figure 44, respectively.

Practical DL (%) Beaker method – Microfluidics method	**Beaker method**	**Microfluidics method**
0 – 0		
14 – 15		

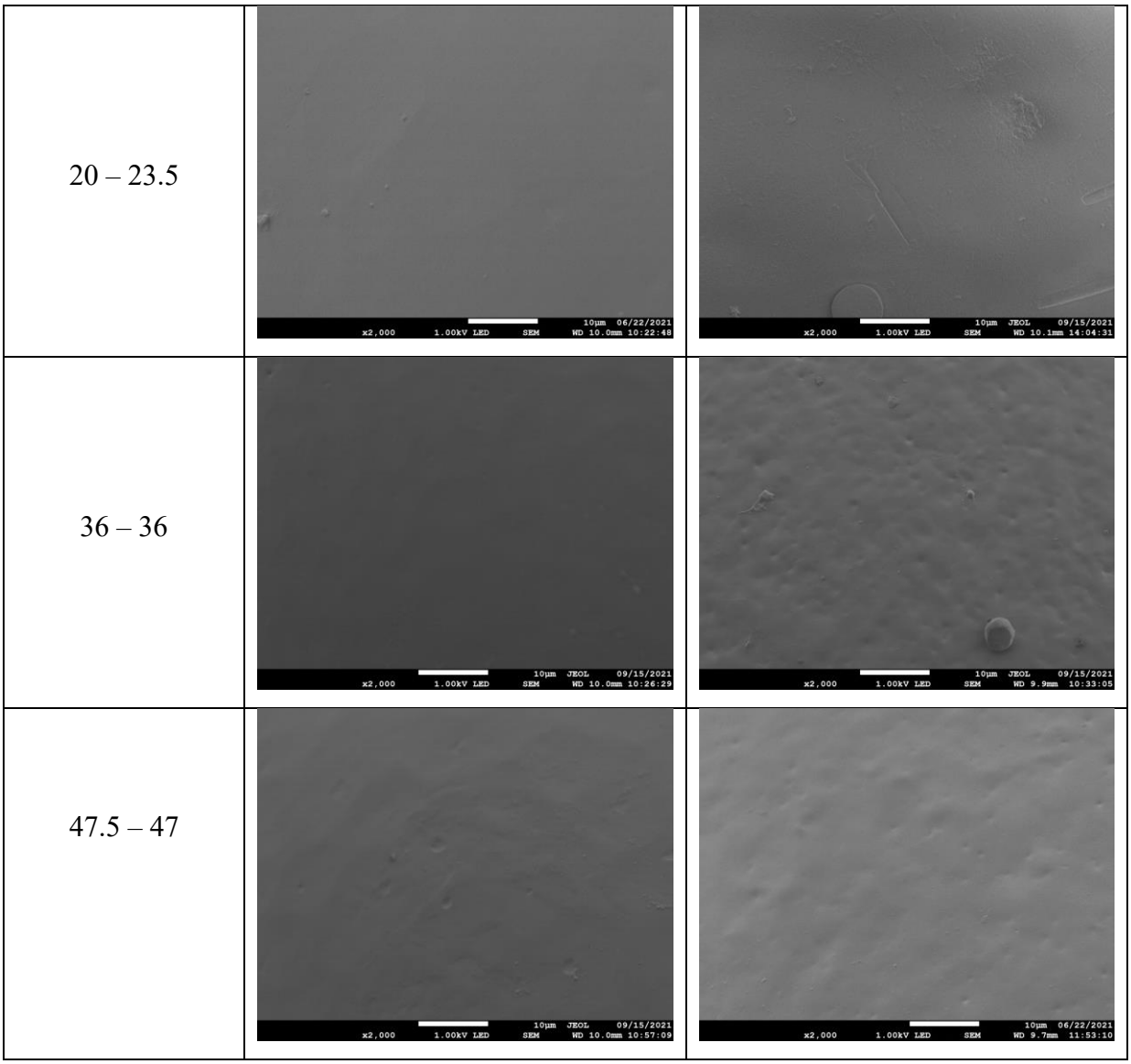
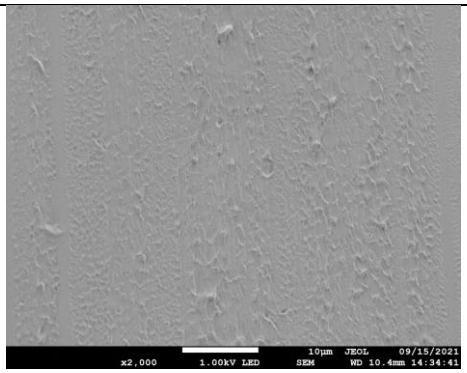
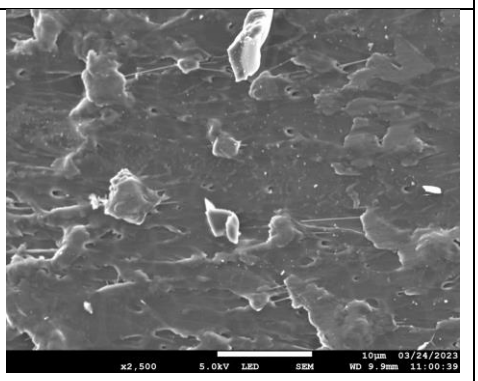
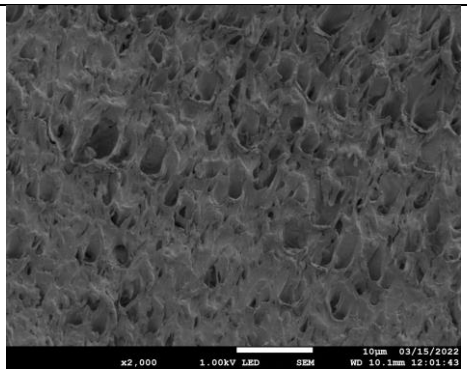
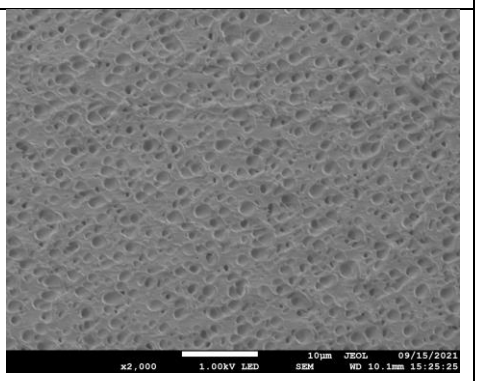
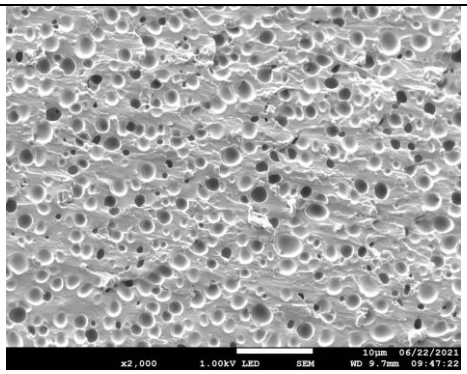
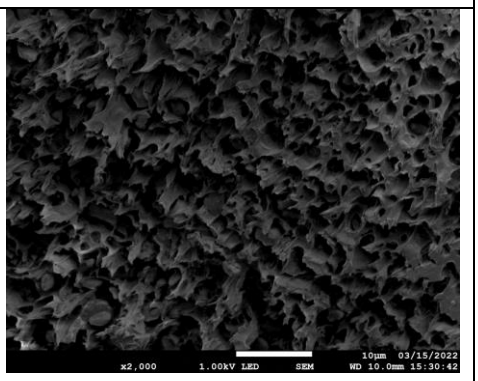


Figure 43. SEM surface pictures of the drug-free & the investigated ibuprofen-loaded microparticles, prepared by the beaker or the microfluidics method, in function of the drug loading, before exposure to release medium

In general, MPs' surfaces are smooth and non-porous. However, with a closer look, nanopores can be seen on the surface of non-loaded MPs produced in microfluidics. Underneath the more irregular surface of microfluidic MPs, a significant pore network can be made out for MPs loaded with 36% or more ibuprofen, which is less obvious for MPs produced in the standard way. This may have consequences for the release behavior of MPs, because if the pore network of MPs produced in microfluidics is rapidly accessible, water will easily penetrate the entire MP, and drug release will be fast. This difference could potentially be due to the slower hardening process for the microfluidic MPs. Indeed, as DCM takes longer to leave the MP, the surface has more time to organize around the pores, which makes the surface topography less

smooth in that case. The porosity has been further investigated by performing MP cross-sections. The internal structures of the MPs are then shown in Figure 44.

Practical DL (%) Beaker method – Microfluidics method	**Beaker method**	**Microfluidics method**
0 – 0		
14 – 15		
20 – 23.5		

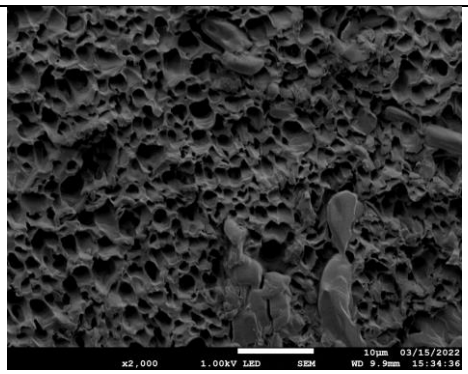
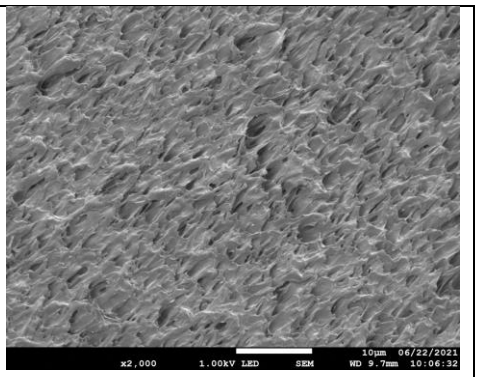
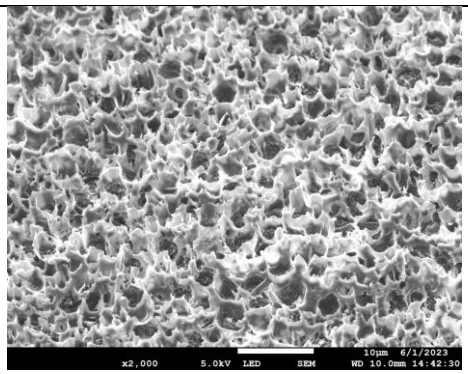
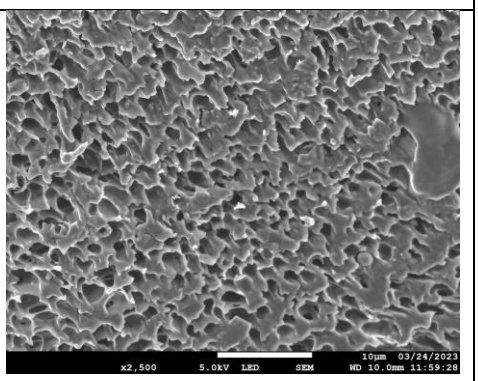
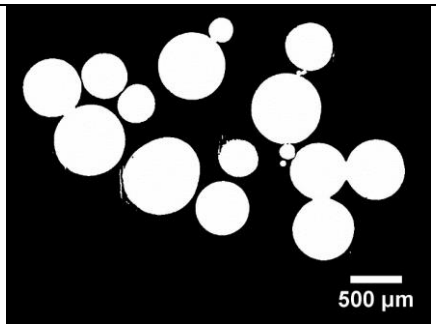
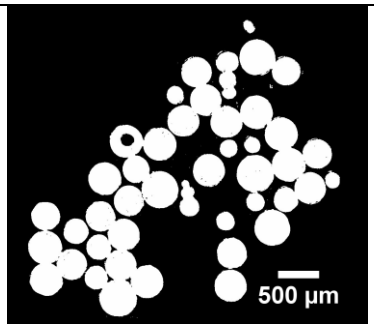
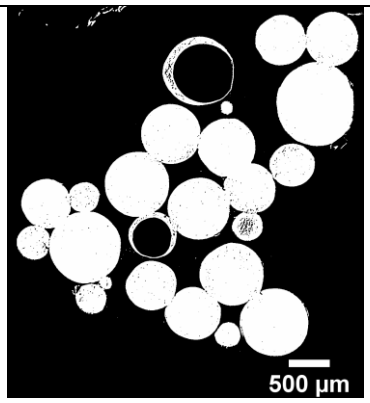
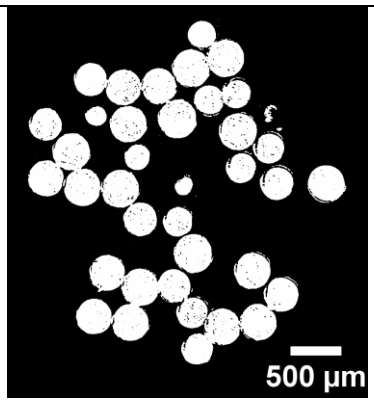
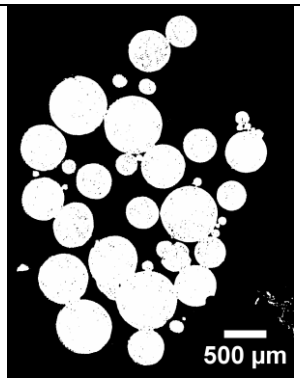
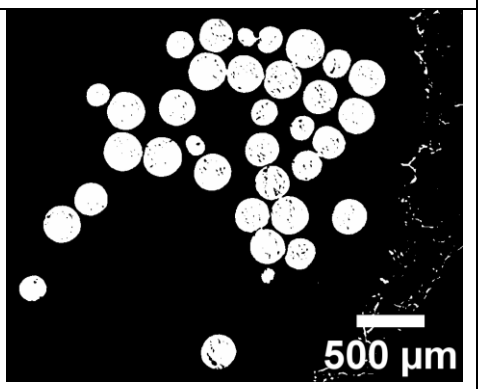
36 – 36		
47.5 – 47		

Figure 44. SEM cross-section pictures of the drug-free & the investigated ibuprofen-loaded microparticles, prepared by the beaker or the microfluidics method, in function of the drug loading, before exposure to release medium

The drug free MPs exhibited a non-porous internal structure, whether prepared using the beaker method or microfluidics. The small imperfections that can be seen are artifacts of the cutting process: The movement of the blade can induce the formation of small PLGA particles that detach from the matrix. When MPs are loaded with ibuprofen, regardless of the quantity they contain or the method of preparation, they become porous. Although all loaded MPs have an important porous network, they differ from one another. For example, the pores of MPs loaded with 20% ibuprofen from the conventional method and those of MPs loaded with 15% from the microfluidics method appear less interconnected than in the other batches; Each pore appears individualized but also shallower. This kind of porous structure could result in weaker water penetration into the MP, leading to a slower drug release than would be the case if all the pores were well-connected to each other. Thus, the preparation method does not seem to affect the structure of the pore network, as different networks are observed with the two methods.

It is sometimes difficult to determine from these SEM images whether the densities of the porous networks are different depending on the DL and the preparation method. X-ray microcomputed tomography (XμCT) analyses were then performed on these MPs to try to make

a clearer difference in porosity appear. Nevertheless, it has to be noted that this method of analysis does not provide a quantitative analysis of porosity. The results are presented in Figure 45.

Practical DL (%) Beaker method – Microfluidics method	**Beaker method**	**Microfluidics method**
0 – 0		
14 – 15		
20 – 22		

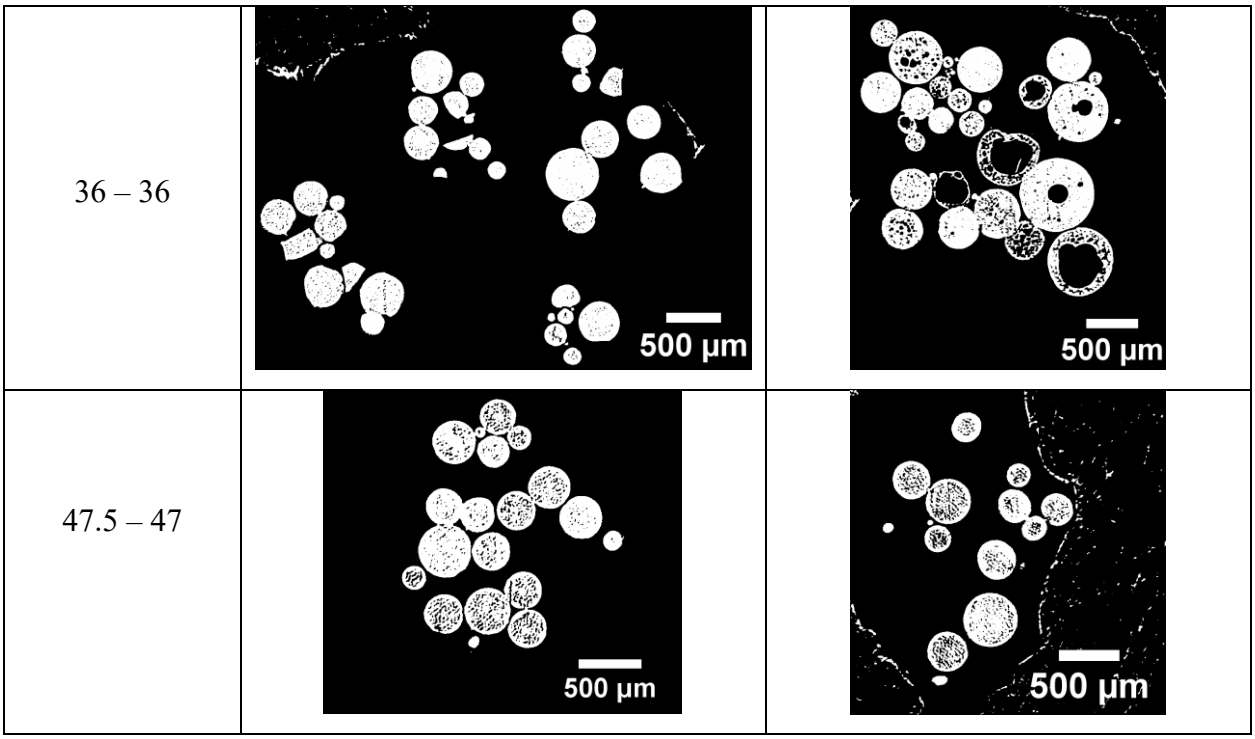


Figure 45. X-ray µCT virtual cross-sections of the drug-free and the investigated ibuprofen-loaded microparticles, prepared by the beaker or the microfluidics method, in function of the drug loading, before exposure to release medium

Thanks to this new analysis technique, it was possible to determine a little more precisely whether there was an impact of the preparation method on the porosity of the resulting MPs. For all MPs, whatever the DL, those produced in microfluidics are more porous than those produced conventionally (this is slightly less obvious for MPs loaded with 47% ibuprofen). Then, contrary to what had been observed previously, the porosity of the MPs is impacted by the preparation method. Indeed, this is explained by the fact that during the production process of microfluidic MPs, in order not to clog the fine tubes of the microfluidics device, the concentration of the organic phase had to be divided by 10 compared to that used in the beaker method. This results in the formation of droplets less concentrated in PLGA, which makes, after DCM evaporation, the matrix less dense and therefore more porous.

Moreover, it has to be noted that some MPs exhibit big holes, and this is particularly visible for microfluidic MPs loaded with 36% ibuprofen and, to a lesser extent, for 14%-loaded MPs produced with the beaker method. This type of structure is possibly due to air bubbles which are entrapped during the precipitation process (sometimes well visible for microfluidic MPs, potentially due to a non-fully hermetical circuit), but this type of issue can easily be overcome. It becomes obvious that these very porous structures will have an influence on drug release. In fact, the MPs exhibiting big holes will probably be less drug-loaded than the others,

so the release profiles within the same batch will be heterogeneous. In addition, the presence of this hole, potentially filled with air, will make the MP float above the release medium, the MP will not be fully immerged in it, and this will impact their release behavior. This has been identified in some batches, especially for microfluidic MPs. These MPs presenting cavities can be identified by weighing them, and seeing if they present a lower weight than the other MPs of the batch, but the difference in weight is slight (a few micrograms), so it is difficult to identify them before the release with a non-destructive analysis.

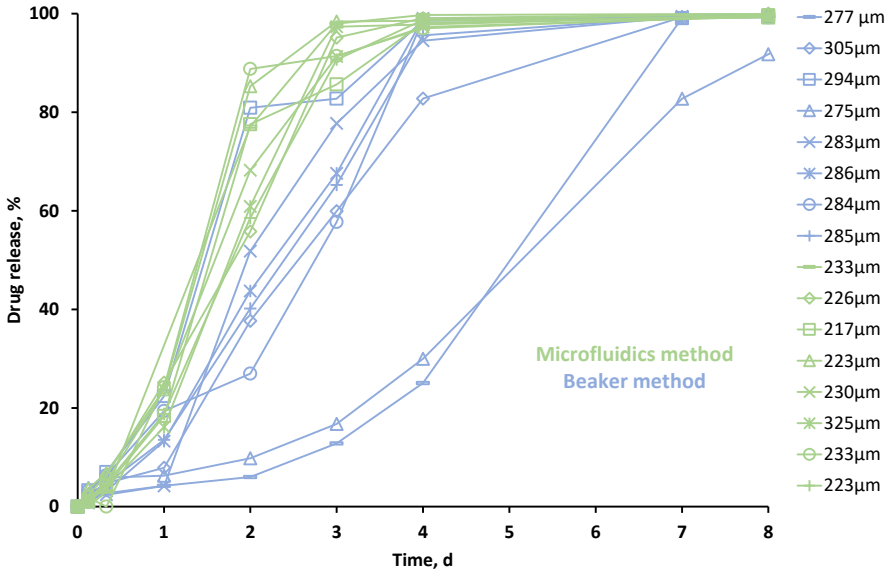
This analysis also allowed us to investigate the impact of drug loading on MP porosity. As expected, the higher the drug content, the greater the porosity. Indeed, with increasing the amount of ibuprofen, the amount of PLGA is lowered; The formed matrix is then less dense because of the PLGA amounts that are not sufficient to form a non-porous structure around the drug.

According to this, it can be assumed that the resulting release profile of MPs produced either *via* the beaker method or *via* a microfluidics device will be different. This will be determined thanks to the following release studies.

II.2.2. Comparative release studies of beaker-produced and microfluidic MPs

The release and swelling kinetics of the batches LAL-030 and LAL-038-1, loaded with 14-15% ibuprofen and obtained with the beaker or the microfluidics method, are presented in Figure 46 a (release kinetics) and b (swelling kinetics).

a)



b)

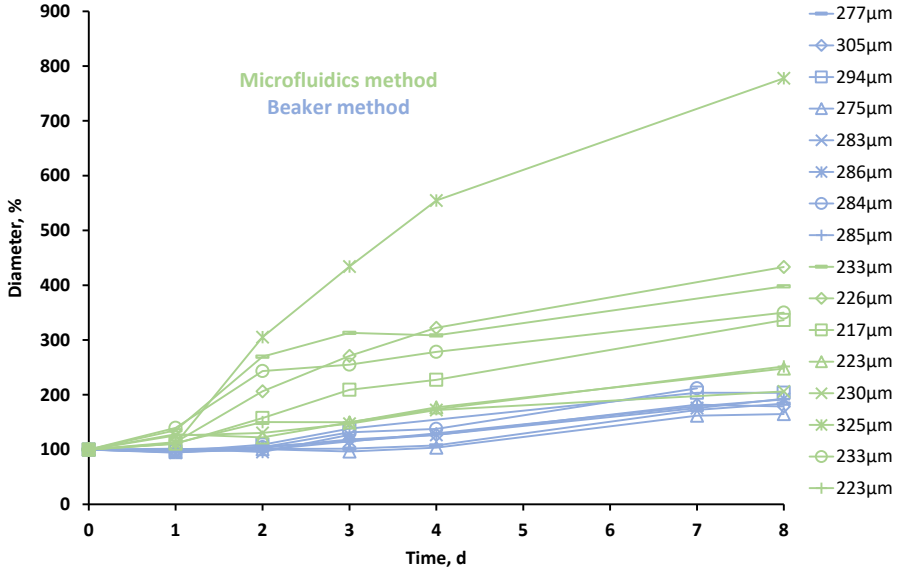
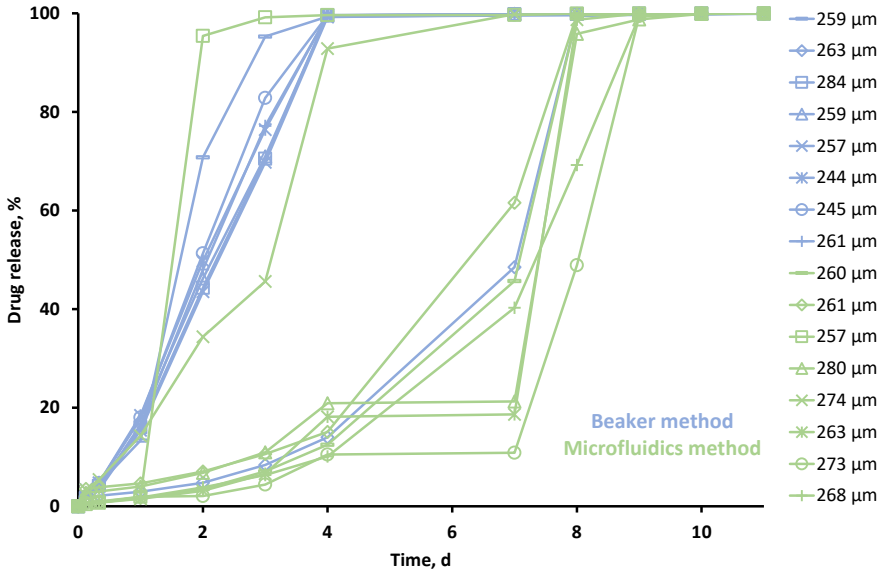


Figure 46. Ibuprofen single a) release and b) swelling kinetics of LAL-030 (beaker method) and LAL-038-1(microfluidics method) microparticles, upon exposure to phosphate buffer pH 7.4

As it was expected, according to the porosity results, MPs produced in microfluidics being more porous, they released ibuprofen a bit faster than the MPs produced with the beaker method. Due to the facilitated water penetration through the pores, the swelling was improved for microfluidic MPs, and the drug diffusion was faster. The release kinetics are more homogeneous for microfluidic MPs, which is in accordance with the fact that the production process is more controlled in microfluidics, leading to more similar MPs in terms of structure and then release behavior. MPs produced with the beaker method had a less important swelling, due to their lower porosity, resulting in slower release kinetics. Interestingly, by looking closer at the swelling kinetics, it can be seen that two MPs (initial diameters of 277 and 275 µm) almost didn't swell all along the exposure. These two MPs are also the ones that release later than the others in Figure 46 a. The swelling seems thus to be correlated to the release of ibuprofen. In fact, the substantial swelling of microfluidic MPs started after 1-day exposure to the release medium, which corresponds to the day where there is the highest slope of the release kinetics. Contrary to what was explained with the SEM images of MPs' cross-sections, the apparently less-connected pores didn't slow down the release rate of ibuprofen from microfluidic MPs. The fact that the pore density was higher in these MPs (as determined with XµCT measurements) was probably sufficient to provide good water penetration within the MPs.

This hypothesis, that MPs produced in microfluidics will release faster than the beaker-produced ones due to their denser porous network, amongst others, will now be investigated for more loaded ibuprofen MPs. The release and swelling kinetics of MPs produced either by the beaker method, or by the microfluidics method, and loaded with 20-23.5% ibuprofen, are shown in Figure 47 a and b respectively. Note that the fastest-releasing microfluidic MP (initial diameter of 257 µm) was broken on the 1st day of release, which is why it released all the ibuprofen very quickly afterwards, so this MP should not be taken into account.

a)



b)

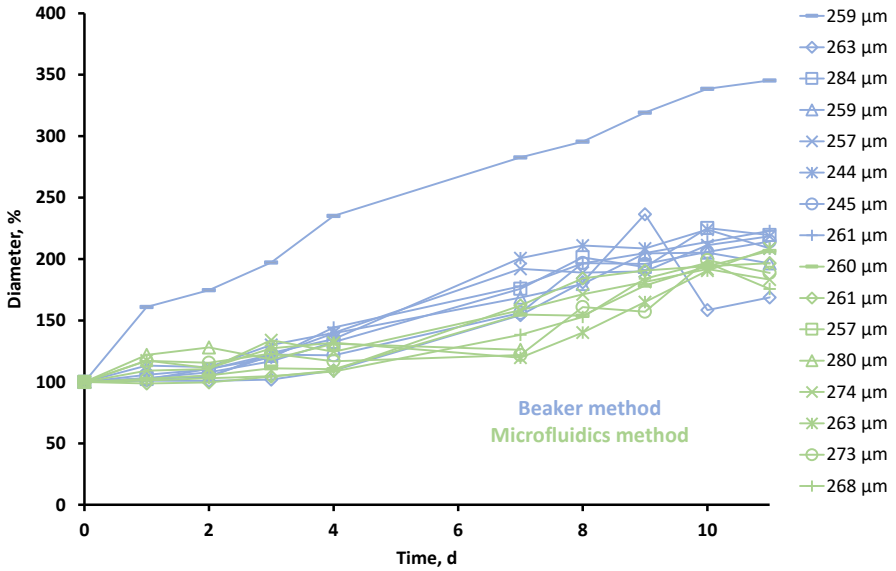
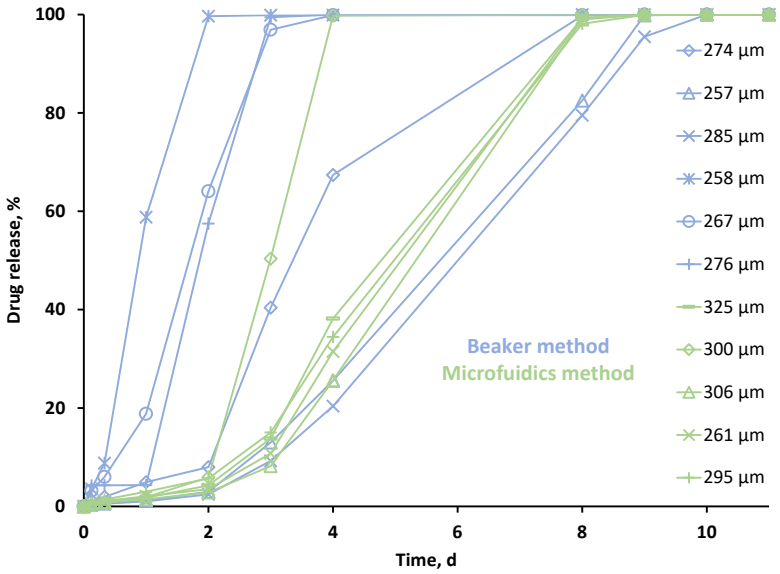


Figure 47. Ibuprofen single a) release and b) swelling kinetics of LAL-031 (beaker method) and LAL-042 (microfluidics method) microparticles, upon exposure to phosphate buffer pH 7.4

Surprisingly, in that case, microfluidics release kinetics were much more heterogeneous and slower than the standard ones for almost all of them. Swelling kinetics are homogeneous and similar for the two preparation methods, so swelling does not seem to be the only reason for the release of ibuprofen in that study. Indeed, if a focus is made on MPs produced by the beaker method, the substantial swelling started after two days of exposure to the release medium, but the release started in the first hours of exposition. The principal reason for the release is probably due to the fast water penetration through the pores, allowing for the diffusion of ibuprofen molecules, before PLGA had the time to swell. Nevertheless, it can be noticed that the MP that swells faster (after 1 d) and the most importantly (initial diameter of 259 µm) is also the MP that releases the most quickly, with the highest slope between the first and second days of exposure. In addition, the release of the MP which took 4 days to swell (initial diameter of 263 µm) started to be significantly important after 4 days as well. The swelling seems to play an important role amongst the release mechanisms. However, it was previously said that MPs presenting a higher porosity and pores better interconnected would be more likely to facilitate the water income within the MP and thus accelerate the drug release. In this case, by considering the SEM and XµCT analysis, MPs produced *via* microfluidics should have released faster, as they showed greater porosity in XµCT and better-connected pores according to SEM images. In this case, porosity was not the main responsible for water penetration into the MPs. DSC analysis of these MPs was carried out to determine whether the PLGA T_g of MPs produced using the beaker method was lower than that of MPs produced *via* microfluidics. A lower T_g would have indicated a stronger plasticizing effect of ibuprofen on the polymer, i.e., even more disentangled polymer chains, allowing water to penetrate easily. Unfortunately, this analysis did not reveal any difference in this respect, as the T_g was 26 °C for both batches (data not shown). The hypothesis that can be suggested is that of an inhomogeneous distribution of ibuprofen in the MP. It is possible that for MPs prepared using the beaker method, the majority of ibuprofen is distributed close to the surface of the MPs, easily accessible, and rapidly releasable as soon as the MPs come into contact with the release medium. On the contrary, for microfluidic MPs the distribution would be more homogeneous throughout the MP, requiring more time before the water has penetrated the entire MP and the ibuprofen present in its core can be released. Finally, one hypothesis that should not be forgotten is the autocatalytic effect. Indeed, it was said in the first Chapter that the autocatalytic effect could be greater for less porous MPs, because the acid degradation products could not be eliminated *via* the pores, which increased the degradation of PLGA to a much greater extent than for porous MPs.

These assumptions could also be applied to the release study for the following batches. These batches were more loaded than the previous ones (36% ibuprofen). The release and swelling kinetics of LAL-041 (produced with the beaker method) and LAL-044 (produced with the microfluidics device) are presented in Figure 48 a and b respectively.

a)



b)

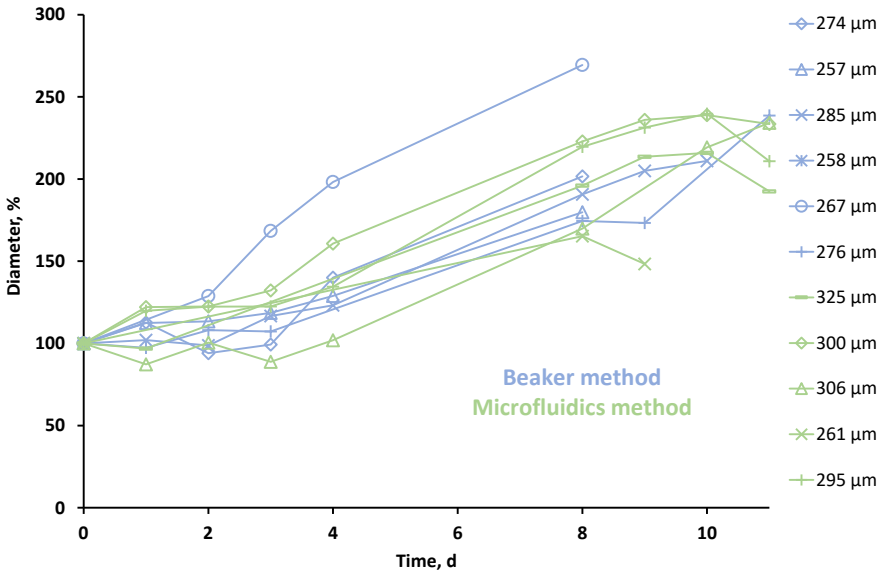


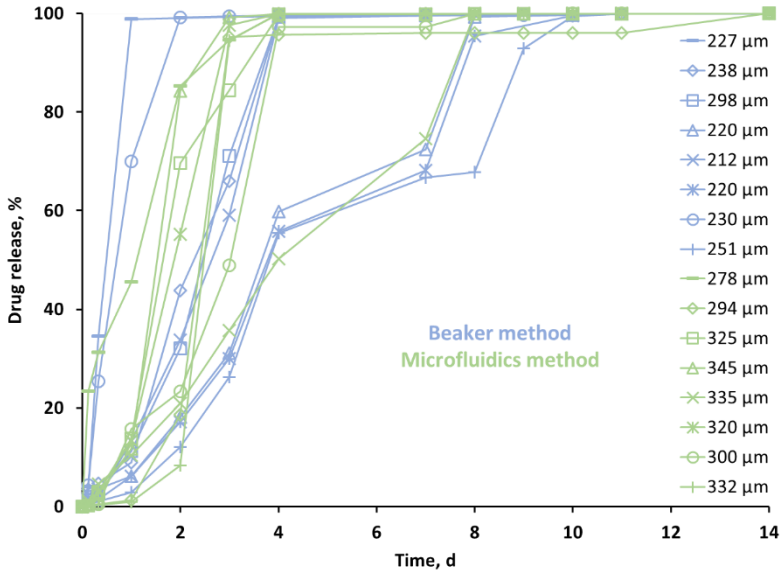
Figure 48. Ibuprofen single a) release and b) swelling kinetics of LAL-041 (beaker method) and LAL-044 (microfluidics method) microparticles, upon exposure to phosphate buffer pH 7.4

In this case, it is difficult to draw a clear trend from these kinetics. There are a few more standard MPs that release faster than microfluidic MPs, but this is not significant. Surprisingly, according to the XµCT images obtained (Figure 45), the porosity of the MPs produced in

microfluidics was much greater than for standard MPs, and as a result, the drug release rate should have been faster for these MPs, but this was not the case. At this important DL, variability between MPs is high, with some MPs releasing all their ibuprofen within 2 days, while others took over a week. Indeed, there is more chance for a significant amount of ibuprofen to be close to the MP surface. Once surface-located ibuprofen is rapidly released after contact with the release medium, the pores created in place of the drug, will allow for the penetration of important amounts of water into the system. This will be followed by drug dissolution and rapid diffusion through water-filled channels for molecules located further in the center of the MPs. Swelling therefore has less impact here, as ibuprofen is probably released before the polymer has had time to swell. This can be seen in Figure 48 b, with swelling generally limited to 2 times the initial diameter of the MP and appearing after a few days of release.

As might be expected, by further increasing the loading rate of ibuprofen in MPs, release kinetics become even more uncontrolled. This can be seen in Figure 49 a and b, which respectively represents the release and swelling kinetics of MP batches LAL-046 (beaker method) and LAL-048 (microfluidics), loaded with 46% ibuprofen.

a)



b)

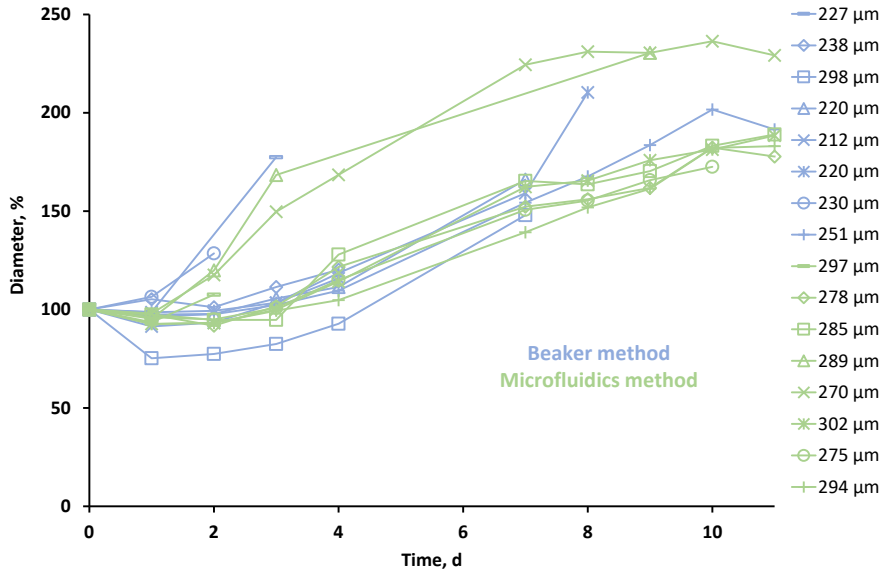


Figure 49. Ibuprofen single a) release and b) swelling kinetics of LAL-46 (beaker method) and LAL-048 (microfluidics method) microparticles, upon exposure to phosphate buffer pH 7.4

As in the previous case, release kinetics are very heterogeneous, ranging from 1 day to 10 days, so there is great variability between MPs, meaning that the resulting releases are uncontrolled. Between the two batches, there was apparently no significant difference in porosity between the standard and microfluidic methods (Figure 45), they were both highly porous. However, some differences can be seen in these XµCT images: Some MPs appeared more porous than others, especially for standard MPs. This could partially explain why some of them released much faster than others. The water penetration being enhanced in these porous MPs, the drug diffusion is improved and the total release of ibuprofen is faster.

Obviously, swelling doesn't play a major role in the release of highly loaded MPs, since it only appears after 4 day for most MPs, by which time their drug release has already reached 100% for the majority, and 60% for the others. For the latter, swelling is the mechanism that probably enables them to release the rest of the drug that has not found a way through the pores to diffuse, and it was only once the chains were disentangled thanks to swelling that the drug could be released.

III. Conclusion

The conclusion to be drawn from this study is that the preparation method of MPs definitely has an impact on their physical characteristics. The parameters specific to each method (organic phase concentration, droplet formation mechanism, precipitation process, etc.) have a significant impact on their porosity and homogeneity in terms of both size and distribution of the active ingredient, with the ensuing consequences for release kinetics. These disparities in characteristics linked to the production method have enabled us to better highlight the relationship between the specific characteristics of MPs and their release behavior. The distribution of the drug within MPs and the autocatalytic effect, although we have not been able to demonstrate them, seem to be preponderant parameters in the release of ibuprofen from PLGA MPs.

III. Conclusion

La conclusion que l'on peut tirer de cette étude est que la méthode de préparation des MPs a un impact certain sur leurs caractéristiques physiques. Les paramètres propres à chaque méthode (concentration de la phase organique, mécanisme de formation des gouttelettes, processus de précipitation, etc.) ont un impact significatif sur leur porosité et leur homogénéité, tant en termes de taille que de distribution de la substance active, avec les conséquences qui en découlent sur les cinétiques de libération. Ces disparités de caractéristiques liées à la méthode de production nous ont permis de mieux mettre en évidence la relation entre les caractéristiques spécifiques des MPs et leur comportement de libération. La distribution de la substance active au sein des MPs et l’effet autocatalytique, bien que nous n'ayons pas pu les démontrer, semblent être des paramètres prépondérants dans la libération de l'ibuprofène à partir des MPs de PLGA.

CHAPTER V – IMPACT OF THE TYPE OF DRUG ON SINGLE PLGA MICROPARTICLE BEHAVIOR

I. Objectives of the work

Amongst the different factors that can affect the behavior of microparticles in release, it has been shown that the preparation method can have an impact, but the type of encapsulated API should not be neglected. Indeed, it was observed in the *Section II.2.1.4.* of Chapter I that the behavior of PLGA, particularly its degradation, could vary depending on the acidic or basic nature of the drug and its plasticizing effect, potentially affecting the resulting release. In order to evaluate the impact of the acidic or basic nature of the drug, we chose to compare the release of two APIs: An acidic drug, ibuprofen, with a pKa of 4.9, versus a basic drug, lidocaine, with a pKa of 7.7. Ibuprofen and lidocaine molecules are represented in Figure 50 a and b respectively.

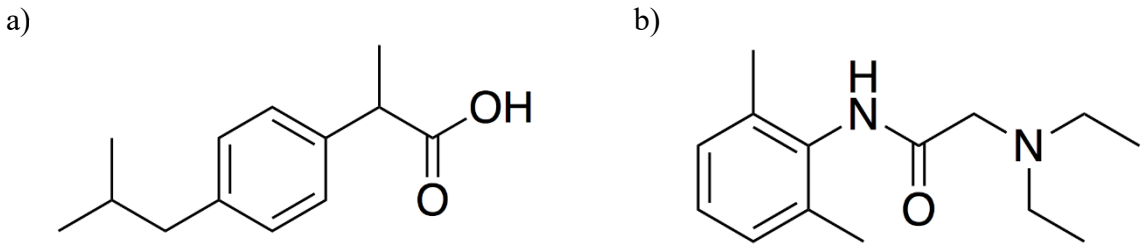
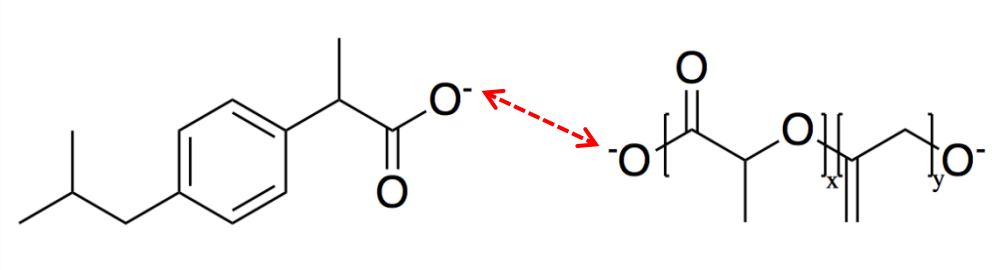


Figure 50. Chemical structures of a) ibuprofen and b) lidocaine

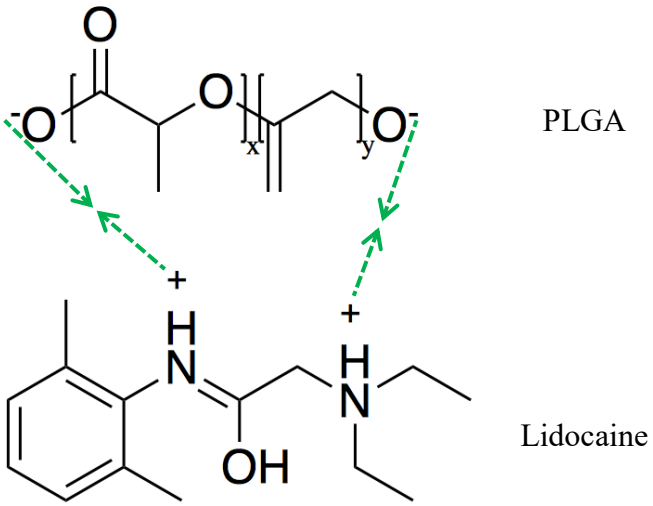
Due to their different pKa, these drugs will not have the same ionic charge at pH 7.4: Ibuprofen will be negatively charged, whereas lidocaine will be positively charged. At this physiological pH, PLGA is charged negatively, which means that it can engage in ionic interactions with compounds. According to the theory developed by D.Klose *et al*. (2007) [83], as ibuprofen and PLGA are both charged negatively, they will ionically repulse each other, and ibuprofen will be easily released from the PLGA matrix (Figure 51 a). Conversely, since lidocaine and PLGA have opposite charges, they will attract each other, and lidocaine will take longer to be released from the matrix (Figure 51 b).

a)



Ibuprofen PLGA

b)



PLGA

Lidocaine

Figure 51. Schematic representations of the ionic attraction existing between a) ibuprofen and PLGA or b) lidocaine and PLGA at pH 7.4, repulsive interactions are shown in red and attractive interactions in green

The aim of this study is to determine whether the type of API encapsulated, and in particular whether it is acidic or basic, will impact the release kinetics of PLGA MPs.

Moreover, the plasticizing effect of these drugs will also be investigated, because this could have an important influence on the PLGA behavior upon release.

II. Results and discussion

II.1. General characteristics of the investigated microparticles

The characteristics of the investigated microparticles in this study are listed in Table 4. The sample name, the encapsulated API, the preparation method, the average size of the microparticles in the corresponding batch, the polydispersity index, the average drug loading and encapsulation efficiency, as well as the initial average diameter of the MPs investigated in the release studies for each setup, are reported. In order to minimize bias in the comparison of ibuprofen and lidocaine release, the MPs selected are always of a similar diameter, to within a few tens of micrometers, and have a similar drug loading.

Table 4. Characteristics of the investigated microparticles in this chapter

Sample name	Drug	Preparation method	Average diameter (µm)	PdI (%)	DL_{exp} ± SD (%)	EE (%)	Initial average diameter of investigated MPs during release (µm)
LAL-020	Ibuprofen	Standard	344	37	8±0.46	80	333
LAL-030	Ibuprofen	Standard	362	44	14±0.05	95	286
LAL-031	Ibuprofen	Standard	269	41	20±0.71	98.5	259
LAL-086	Lidocaine	Standard	275	16	8.5±1.23	86	265
LAL-087	Lidocaine	Standard	241	27	14±1.88	96	277
LAL-094	Lidocaine	Standard	294	34	29.5±2.33	84	/
LAL-112	Lidocaine	Standard	179	26	35±1.24	88	/
LAL-115	Lidocaine	Standard	215	30	47.5±0.77	95	/
LAL-117	Lidocaine	Standard	298	39	21±0.75	84	290

In general, and at first sight, lidocaine MPs do not appear to have different characteristics from those obtained with ibuprofen. However, the preparation parameters had to be adapted in order to obtain spherical MPs of the desired sizes. Indeed, with the same parameters as for ibuprofen MPs (4 mL of dichloromethane and stirring speed at 900 rpm), non-spherical MPs were obtained. The optimal parameters determined were 6 mL and 1200 rpm for the batches LAL-086 and LAL-087, and 5 mL and 900 rpm for the batches LAL-115 and LAL-117. It may be noted that the encapsulation efficiency is equivalent to that of ibuprofen (between about 80 and 95%). The process of MPs' formation used here (oil-in-water emulsion) is perfectly adapted to encapsulate lipophilic drugs, whereas the water-in-oil-in-water emulsion is suitable for hydrophilic drugs. This similar EE could be due to their relatively close lipophilicity values ($LogP_{ibuprofen}$ = 3.97; $LogP_{lidocaine}$ = 2.44). As drugs all have more or less different physicochemical properties, the MP manufacturing process can be impacted and sometimes needs to be readapted. This can lead to different MPs' characteristics and thus release behaviors.

II.2. Characterization and release studies of ibuprofen- and lidocaine-loaded microparticles

II.2.1. Preliminary characterization of raw materials and ibuprofen & lidocaine MPs

Lidocaine MPs underwent the same characterization as ibuprofen MPs, starting with optical microscopy. Figure 52 shows the obtained lidocaine MPs for different DLs.

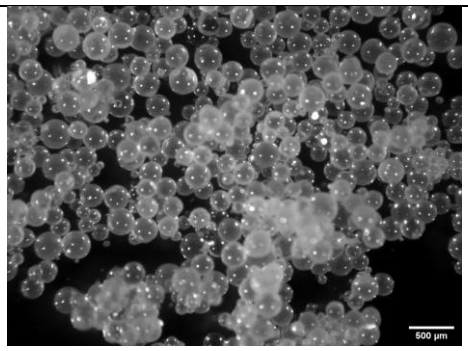
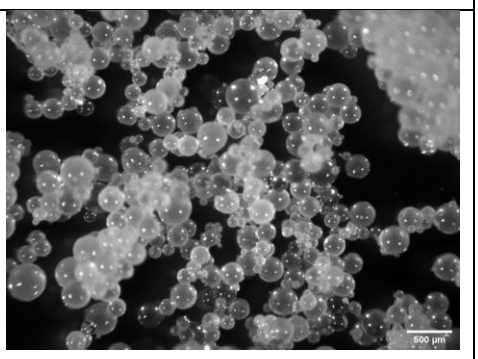
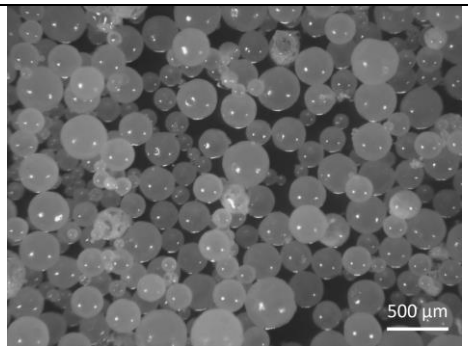
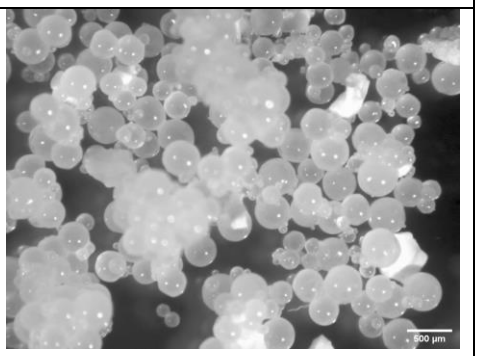
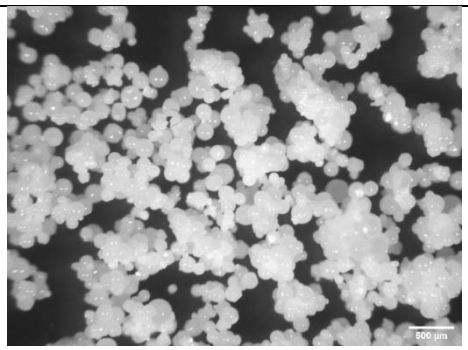
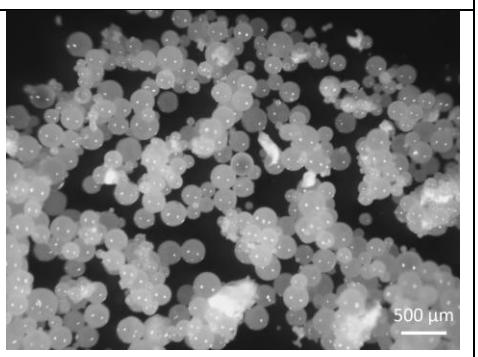
Practical DL (%)	8.5	14
		
Practical DL (%)	21	29.5
		
Practical DL (%)	35	47.5
		

Figure 52. Optical microscopy pictures of lidocaine-loaded microparticles, in function of the drug loading, before exposure to release medium

At first glance, the lidocaine MPs obtained are not different from ibuprofen MPs. However, it can be noted that this is not what was observed for ibuprofen MPs, i.e., the presence of opaque and transparent MPs within the same batch, as described in Chapter III. The fact that this phenomenon was not observed for lidocaine MPs may be due to the precipitation mechanism, which is modified by the presence of lidocaine in the organic phase, which changes its physicochemical properties. Moreover, this organic phase is less concentrated than for ibuprofen MPs. Properties such as viscosity, a better homogeneity within the droplet, or the chemical interactions that may exist between the active ingredient and the components of the organic phase, may influence the resulting precipitation of droplets and therefore the physical

state of the drug in the hardened MPs. It is evident from Figure 52 that the higher the DL, the less transparent the MPs, which wasn't always the case for ibuprofen MPs, as seen previously in Chapter III. However, there is a more pronounced white appearance for MPs loaded with 35% lidocaine than for those loaded with 47.5% lidocaine. This can be explained by the fact that the organic phase droplets precipitated less quickly in the case of MPs loaded with 35%, giving lidocaine time to crystallize. The physical state of raw materials and lidocaine within the transparent or opaque MPs was confirmed with X-ray diffraction analyses, presented in Figure 53. To this end, the totally transparent batch loaded with 8.5% lidocaine (LAL-086), the slightly less transparent but not fully opaque batch loaded with 21% lidocaine (LAL-117), and the totally opaque batch loaded with 35% lidocaine (LAL-112) were compared. In this way, it will be confirmed whether the same conclusions are observed as for ibuprofen MPs, i.e., if the lidocaine MPs are transparent, lidocaine is present in amorphous form in the MPs, whereas if the MPs are opaque, it is present in crystalline form.

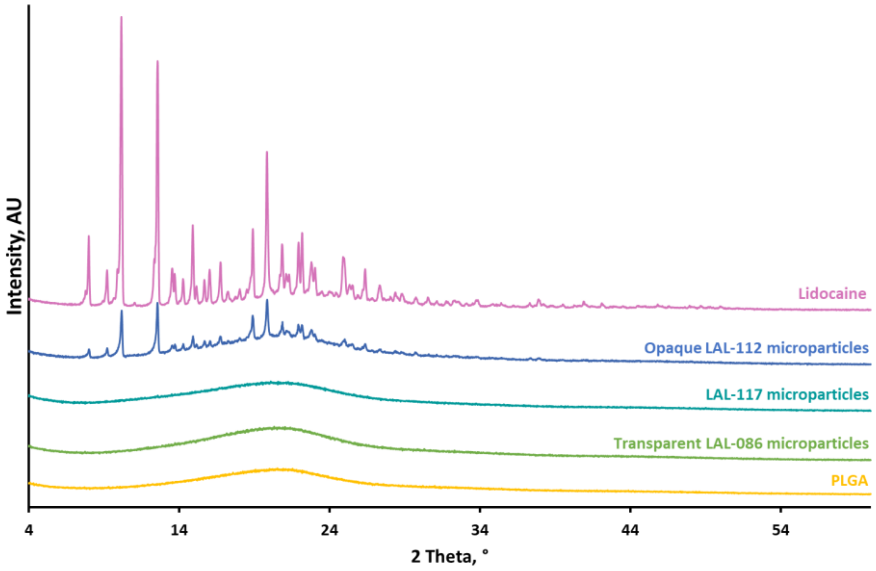


Figure 53. X-ray diffraction patterns of the transparent 8.5% lidocaine-loaded LAL-086, the transparent slightly opaque 21% lidocaine-loaded LAL-117 and the opaque 35% lidocaine-loaded LAL-112 microparticles, and raw materials (PLGA & lidocaine)

As expected, transparent lidocaine MPs showed a large diffusion halo, characteristic of amorphous lidocaine, while opaque MPs showed Bragg peaks, characteristic of crystalline lidocaine. For MPs that were rather transparent but with a slight opacity, it was finally discovered that the lidocaine they encapsulated was fully amorphous.

If the precipitation mechanism is slightly different in the case of lidocaine MPs in comparison to ibuprofen ones, as seen previously, the surface of the MPs could be different as well. For an equivalent DL, lidocaine MPs' surfaces are compared to ibuprofen MPs' surfaces thanks to SEM. The pictures are shown in Figure 54.

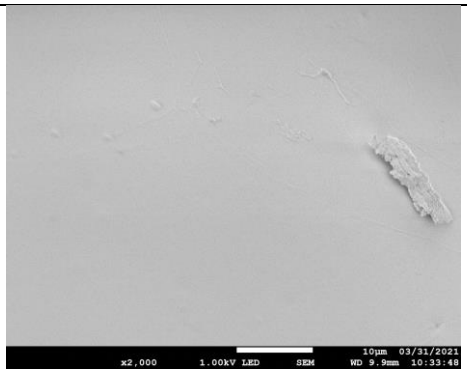
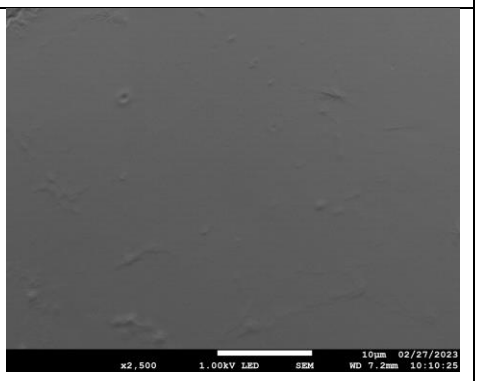
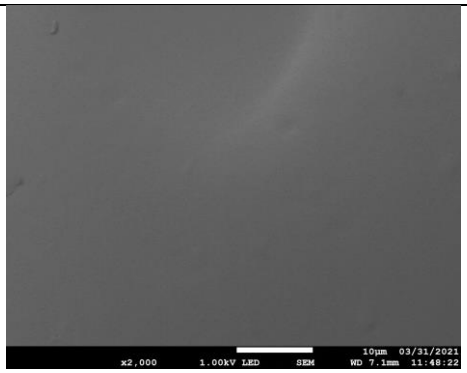
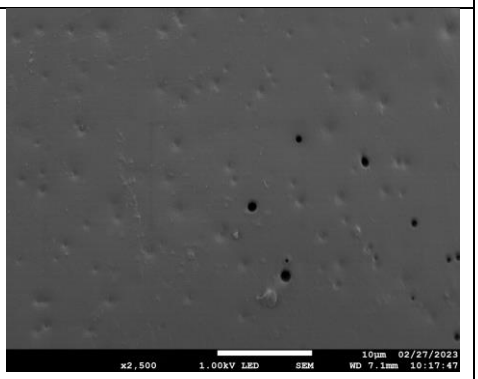
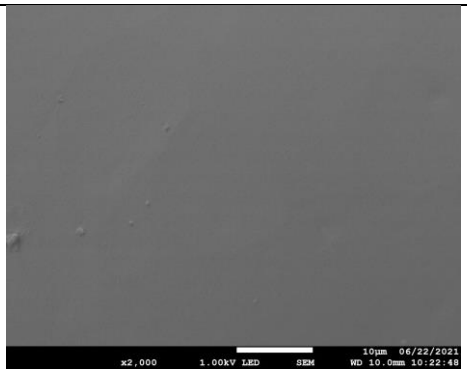
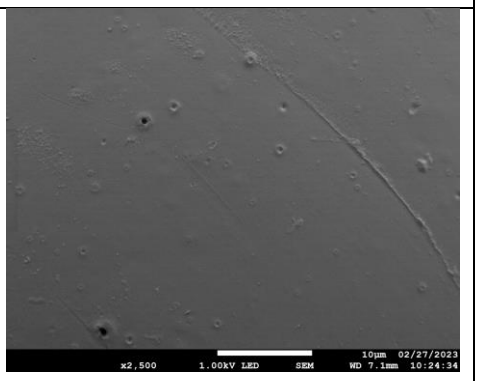
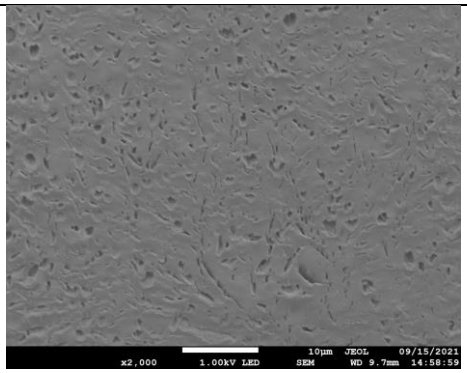
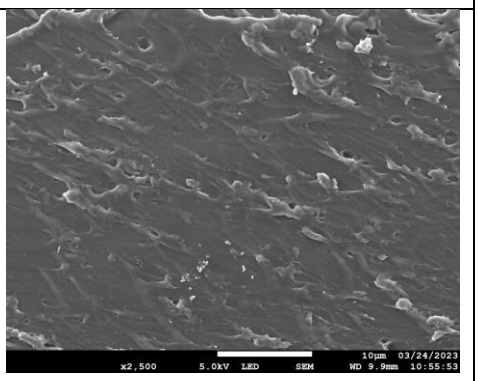
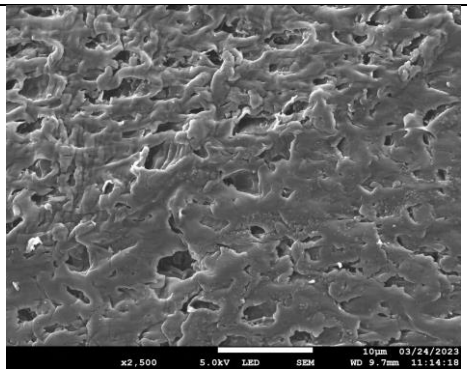
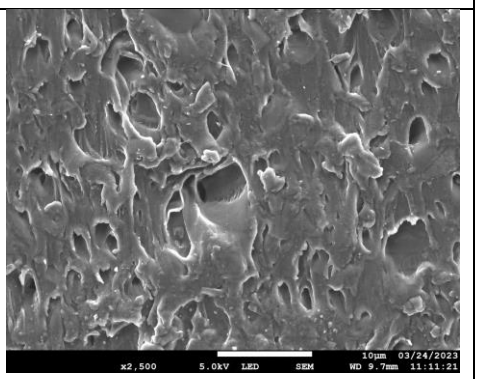
Practical DL (%) Ibuprofen – Lidocaine	**Ibuprofen MPs**	**Lidocaine MPs**
8 – 8.5		
14 – 14		
20 – 21		

Figure 54. SEM surface pictures of the investigated ibuprofen-and lidocaine-loaded microparticles, in function of the drug loading, before exposure to release medium

It was seen in Chapter II that all the standard ibuprofen MPs produced exposed a non-porous surface. Interestingly, this was not the case for all the standard lidocaine MPs. For 8.5% loading of lidocaine, the surface remained non-porous, but when the DL increased, pores

appeared. The porous external surface of these lidocaine MPs is probably due to the fact that the organic phase had a lower concentration than for ibuprofen MPs, leading to a less dense matrix, inducing pores at the surface. In addition, the more the DL increases, the lesser the amount of PLGA, and then the more porous the surface of the MPs. This external porosity will most likely generate faster and greater water penetration into the MPs, leading to faster release of lidocaine compared to ibuprofen MPs with similar DL, but this will be further investigated later. If the process parameters that were adapted for lidocaine MPs in comparison with ibuprofen MPs had an impact on the external porosity, a more significant internal porosity should also be observed. Cross-sections of lidocaine MPs and, for reason of comparison also similarly loaded ibuprofen MPs, were analyzed thanks to SEM, and shown in Figure 55.

Practical DL (%) Ibuprofen – Lidocaine	**Ibuprofen MPs**	**Lidocaine MPs**
8 – 8.5		
14 – 14		

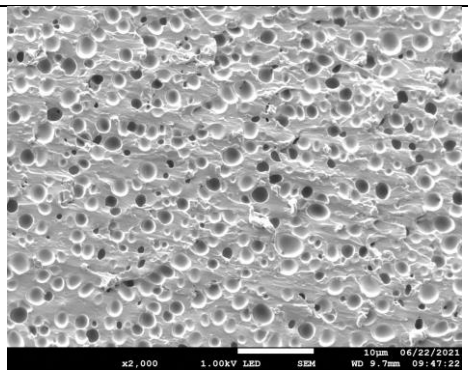
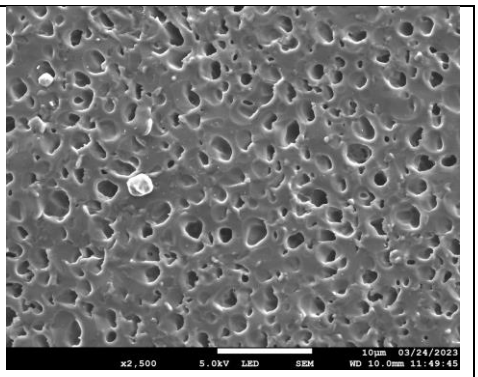
20 – 21		

Figure 55. SEM cross-section pictures of the investigated ibuprofen- and lidocaine-loaded microparticles, in function of the drug loading, before exposure to release medium

Surprisingly, the internal porosity was not more important, even less important for the less loaded lidocaine MPs (8.5%) (*please note the different scaling of the 8 and 20% ibuprofen MPs)*. The porous structure is quite different, and the connection between pores may probably vary between batches. These observations tend to suggest a slightly faster release for lightly loaded ibuprofen MPs (8% in our case), because although their surface is non-porous, their internal porosity means that once water has penetrated, the drug can diffuse and the polymer can swell more rapidly, thereby increasing the release rate.

So far, it has been shown that the API has an impact on the physical characteristics of MPs, as the preparation process had to be adapted with regard to the active ingredient, but it was not the physicochemical properties of the drug itself that were responsible for these characteristics. In order to better understand the impact of the drug on PLGA, calorimetric analyses were performed, on both MPs and raw materials. Differential scanning calorimetry and thermogravimetric analysis (TGA) were thus carried out. The latter involves measuring the weight loss of a sample as a function of temperature or time at a given temperature. Weight loss can be due to solvent evaporation, chemical degradation, or sublimation. The TGA results are presented in Figure 56.

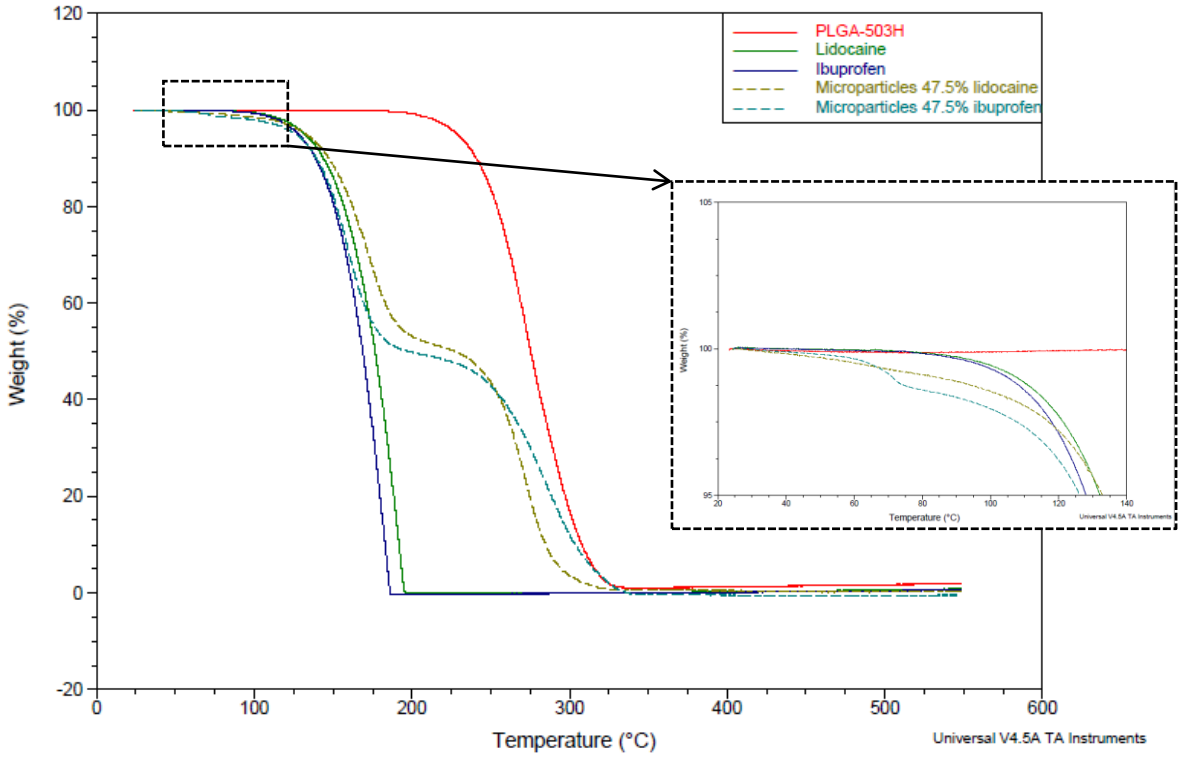


Figure 56. Thermogravimetric analysis of 47.5% ibuprofen- and lidocaine-loaded microparticles (dashed lines), and raw materials (solid lines)

TGA results showed PLGA degradation appearing at around 200 °C, while the loss mass for ibuprofen and lidocaine appeared already at 80 °C. This weight loss is more likely due to drug sublimation than degradation, the latter being total at about 190 °C for ibuprofen and 200 °C for lidocaine. For the MPs, it was first observed probable solvent/water evaporation starting around 40 °C (zoom on Figure 56), followed by the sublimation and degradation of the API (ibuprofen degradation started almost 20 °C earlier than lidocaine), and then polymer degradation.

Following these TGA analyses, DSC measurements were performed on physical mixtures of PLGA and ibuprofen or lidocaine. The maximum temperature of the cycle was kept at 120°C in order to ensure that the materials would not degrade between the two heating cycles. Indeed, at 120 °C, only 2% of the drug was sublimated (Figure 56), and PLGA was not degraded. The objective of performing DSC on physical mixtures of drug and polymer is to find out whether the drug has a plasticizing effect on the PLGA, whether it solubilizes in the polymer matrix, and if so, at which drug level the matrix becomes saturated. The results of these DSC analyses are presented in Figure 57.

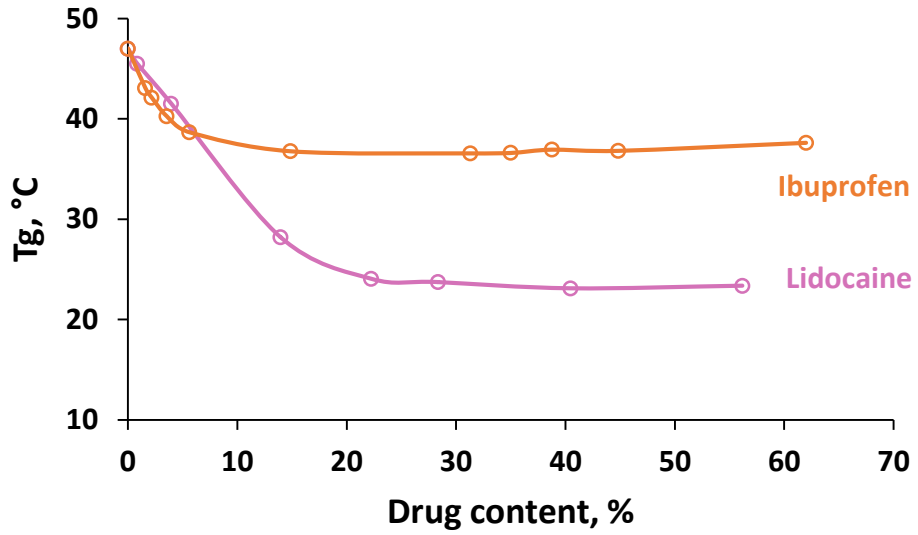


Figure 57. Dependence of the glass transition temperature of ibuprofen:PLGA powder blends (orange curve) or lidocaine:PLGA powder blends (pink curve) on the drug contents of the mixtures (measured by DSC, determined from the second heating cycle)

In both cases, the T_g decreases as soon as a drug is added, but not to the same extent for each active ingredient. The decrease in T_g is greater for physical mixtures with lidocaine, meaning that its plasticizing effect is stronger than that of ibuprofen. In fact, the T_g of pure PLGA 503H, initially around 47°C, can be lowered to about 23°C in the case of lidocaine, compared with only 37°C in the case of ibuprofen. Moreover, the amount of drug that is able to plasticize PLGA before its saturation is reached is greater for lidocaine. The plateau, i.e. the point at which the matrix can no longer accept amorphous drug, is reached at around 15% for ibuprofen, compared with just over 20% for lidocaine. These results are consistent with the X-ray analysis carried out on the batch loaded with 21% lidocaine (presented in Figure 53.), which shows that at this encapsulation rate, all the lidocaine remained in amorphous form, as the matrix was able to accept this quantity. The fact that lidocaine has a greater plasticizing effect than ibuprofen may have repercussions on drug release. Indeed, for the same DL, lidocaine-loaded MPs will have PLGA chains that require less energy to become mobile than in ibuprofen-loaded MPs. The polymer matrix will become more easily disorganized in the case of lidocaine MPs, leaving more freedom for lidocaine molecules to diffuse more easily and rapidly into the release medium, compared with ibuprofen-loaded MPs. It should be pointed out that the T_g of ibuprofen, determined on the 2nd heating cycle, happened at -44 °C. However, it was not possible to determine the T_g of lidocaine, as it undergoes strong recrystallization in the T_g zone upon heating, preventing its measurement. In 2022, Xu *et al.* [215] were able to

predict that the T_g of lidocaine is around -62 °C thanks to the formation of a co-amorphous system with prilocaine, using the Gordon Taylor equation.

The conclusion that can be drawn from the behaviors that should be obtained in drug release, is that the existing ionic interactions between the drug and PLGA were rather in favor of a faster release of ibuprofen. However, the external porosity of lidocaine MPs will favor its release compared to ibuprofen, and the internal porosity shown with SEM cross-sections seems similar for both drugs for most MPs. Nevertheless, highly-loaded ibuprofen MPs are a bit more porous than those of lidocaine, tending towards a slightly faster release for ibuprofen. Finally, since the plasticizing effect is greater with lidocaine, lidocaine MPs would be more prone to faster release. In summary, it is relatively difficult with these previous analyses to determine precisely which trend will be observed in the release kinetics.

To better understand and anticipate the MPs' release behavior, the internal structure of lidocaine MPs has been further investigated, thanks to EdX (energy dispersive X-ray analysis) measurements. This technique is based on the detection of the energy of photons emitted following an electronic excitation of the surface sample. Each energy value is characteristic of a chemical element, meaning that it is possible to identify which kind of atom is present in a specific area of the sample. It therefore makes sense that to differentiate between two components, they need to have different atoms in their structure.

The lidocaine molecule possesses two nitrogen atoms in its chemical structure (Figure 50 b); This differentiates it from a PLGA molecule (Figure 2), which contains only hydrogen, carbon, and oxygen atoms. Unfortunately, as the ibuprofen molecule does not have an atom that distinguishes it from PLGA, EdX analyses of ibuprofen MPs could not be carried out. As described in the method in *Section II.3.9*, this analysis is allowed thanks to the carbon covering of the MPs' surfaces. Consequently, the signal of carbon will be high and should not be considered during the analyses because it will not reflect the real amount of carbon present in the samples. On the other hand, oxygen and nitrogen signals will be helpful to monitor. Indeed, if in a determined zone of the MP, no nitrogen and only oxygen is detected, it means that only PLGA is present. On the contrary, if nitrogen is detected, this proves the presence of lidocaine molecules in that zone. In addition, if the signal of the nitrogen atom is as high as the oxygen signal, it means that only lidocaine is present in this specific area (probably in crystal form), because if there had been PLGA, which contains many oxygen atoms on its chain, the oxygen signal would have been much more important than the nitrogen one. Since the lidocaine molecule has one oxygen atom for every two nitrogen atoms, one would expect the oxygen

signal to be twice as high as the nitrogen signal, but it will be seen later that this is not the case, as the nitrogen signal is quite weak and difficult to detect, unlike oxygen. Because of this low nitrogen signal, the EdX results shown in Figure 58 were obtained with MPs highly loaded with lidocaine (47.5%).

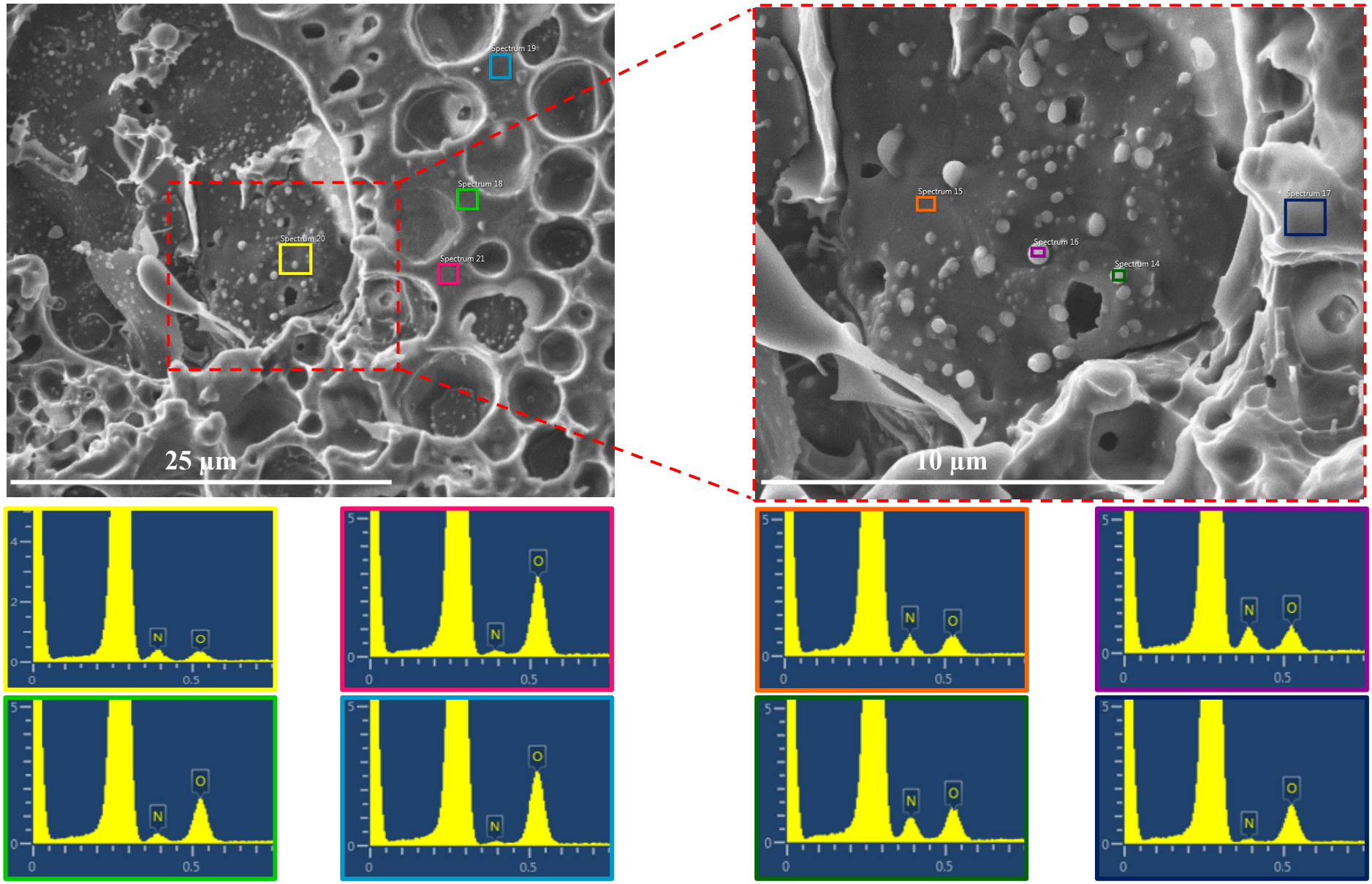


Figure 58. SEM pictures (top row) and corresponding EdX spectra (bottom row) of 47.5%-loaded lidocaine MPs. The SEM picture on the right is a zoom of a part of the one on the left.

First of all, it can be noticed that the MP's cross-section reveals different structures: Pores that seem to be lined with something in some cases, holes or channels for which the bottom is not visible, small spheres (more visible on the zoom on the right), smoother surfaces, etc. On the left picture, a focus is made on the material lining the cavities and that around the pores. As it can be seen on the pink, light green, and light blue spectra, the latter material is in great part PLGA, with a few lidocaine molecules, because the oxygen signal is much higher than the nitrogen one (please note that the peak to the left of the nitrogen signal is the one of carbon). According to the yellow spectra, the molecule that is found in this area is only lidocaine. Indeed, the oxygen and nitrogen signals are of the same intensity. This area highlights a crystal of lidocaine. In fact, at this high DL, lidocaine cannot be entirely solubilized in amorphous form in the polymer matrix, and this is confirmed by EdX. This means that some parts of the MPs will be rich in lidocaine due to the presence of crystals, but as nitrogen signals can be seen on all the spectra, this indicates that lidocaine is found throughout the MP. These types of crystalline structures in the porous cavities will strongly impact the release of lidocaine: Once the water has penetrated the pore network, the lidocaine crystals present in those pores will dissolve and diffuse into the release medium in greater quantities than if all the lidocaine was solubilized in the polymer. The fact that these crystals are found within the pores themselves makes them more accessible to water than if they were included in the polymer matrix, so this particular structuring will lead to improved drug release.

Thanks to the zoom, it was possible to analyze some areas more precisely. The orange zone confirms the presence of a lidocaine crystal, while the blue zone ensures that the material is lidocaine solubilized in PLGA (presence of nitrogen but with a more intense oxygen signal). The purple and black green zones were placed on the small spheres, and showed a spectrum characteristic of the presence of lidocaine alone, i.e. with the nitrogen signal of the same intensity as that of oxygen. Surprisingly, these little spheres don't have the appearance of crystals (as it should have been in the case of lidocaine), in other words, with geometric shapes such as needles. This means that these little spheres are probably clusters of lidocaine in amorphous form. As briefly mentioned in Chapter III, oversaturation of the polymer matrix can potentially occur during the MP production process. This amorphous drug oversaturation may be due to the rapid precipitation of organic phase droplets in the aqueous phase, trapping the amorphous drug in the matrix before it has had time to crystallize.

The previous DSC physical mixture analyses (Figure 57) showed that the PLGA matrix is saturated after more than about 15% ibuprofen or 20% lidocaine. As seen in Chapter III, some MPs loaded with more than 15% ibuprofen remained transparent, i.e., amorphous (for example, batch LAL-031, loaded with 20% ibuprofen). A closer examination will be conducted on the DSC thermograms of highly-loaded ibuprofen and lidocaine MPs. The thermograms of 47.5% drug-loaded MPs are shown in Figure 59.

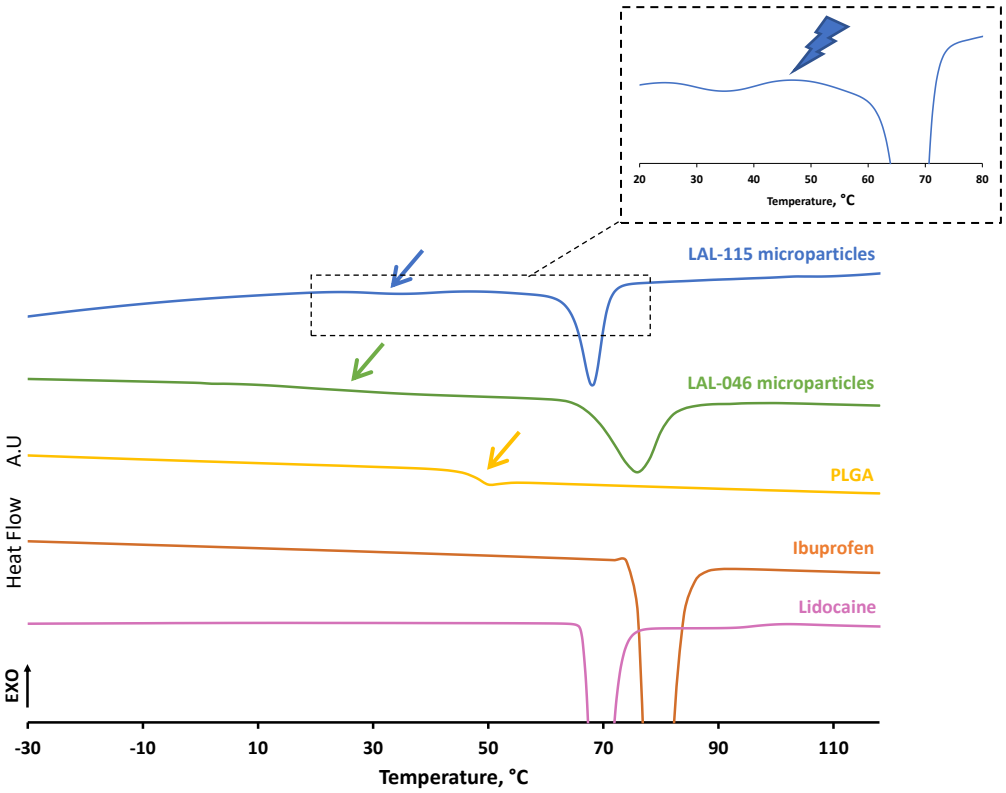


Figure 59. DSC thermograms of the LAL-046 (47.5% ibuprofen) and LAL-115 (47.5% lidocaine) microparticles (before exposure to the release medium, first heating cycle) and raw materials (PLGA, second heating cycle, and lidocaine & ibuprofen, first heating cycle).

In the case of ibuprofen MPs, the crystallinity, determined from the dissolution/melting peak (green curve) compared to the peak of pure fully crystalline ibuprofen (orange curve), was about 25%, meaning that more than 20% of the remaining ibuprofen is in an amorphous form in the PLGA matrix. However, it has been established that the maximum amount of ibuprofen that can be solubilized by PLGA is around 15% (Figure 57). This means that in the 47.5% ibuprofen-loaded MPs, 5% of the total ibuprofen is oversaturating the PLGA matrix. As mentioned in Chapter III, this will result in the recrystallization of ibuprofen over time within the MPs, because the system is unstable. For the 47.5% lidocaine-loaded MPs, another phenomenon occurred during the DSC analysis. This phenomenon is highlighted in the zoom

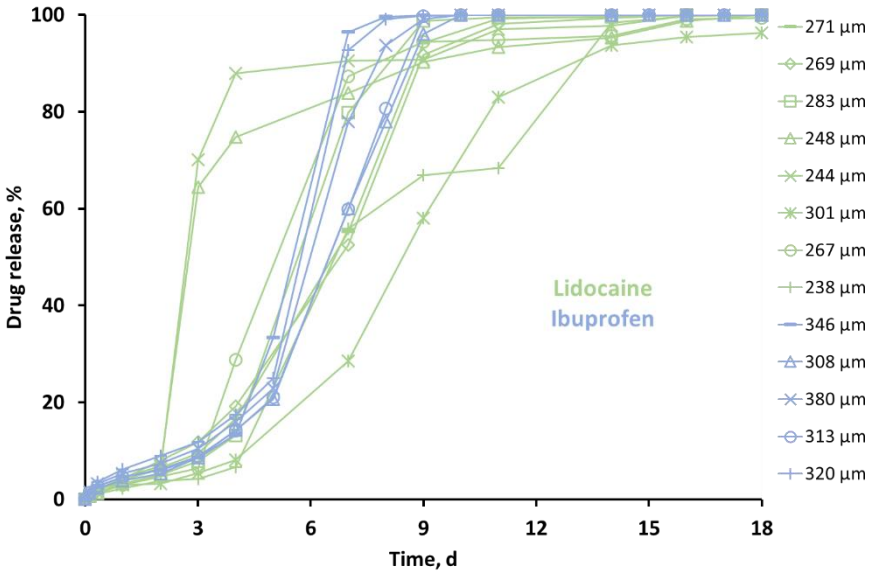
of Figure 59 by the lightning sign: It is a recrystallization of a fraction of the initially amorphous lidocaine solubilized in the matrix, which recrystallizes as the temperature rises during analysis. As a consequence, the 35% crystallinity previously measured with the dissolution/melting peak (blue curve) compared to the peak of pure fully crystalline lidocaine (pink curve), is not representative of the initial amount of crystalline lidocaine in the MPs, so the content of amorphous lidocaine present in these MPs cannot be determined exactly. Furthermore, lidocaine is known to recrystallize easily [215], unlike ibuprofen, explaining why this recrystallization phenomenon was not observed during the first heating cycle of ibuprofen MPs.

Therefore, the nature of the active ingredient has a major impact on the physicochemical characteristics of the resulting MPs. In the next section, it will be assessed whether these differences have an impact on drug release mechanisms.

II.2.2. Comparative release studies of ibuprofen- and lidocaine-loaded MPs

To better understand to what extent the active ingredient encapsulated in PLGA MPs can influence the release mechanisms, *in vitro* release studies were carried out on MPs with different loadings of the active ingredient. The release and swelling kinetics of MPs loaded with 8% ibuprofen and 8.5% lidocaine are compared in Figure 60 a and b respectively.

a)



b)

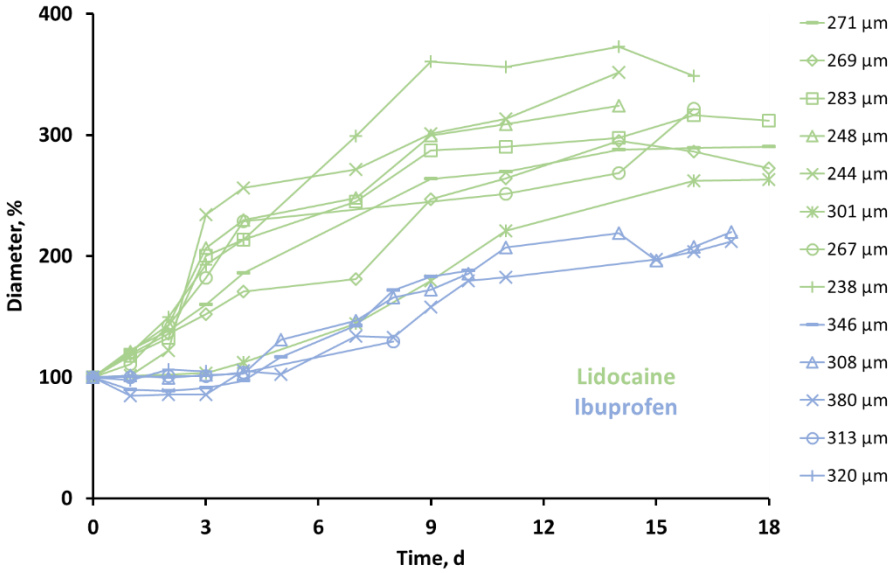


Figure 60. Single a) release and b) swelling kinetics of LAL-020 (8% ibuprofen) and LAL-086 (8.5% lidocaine) microparticles, upon exposure to phosphate buffer pH 7.4

Figure 60 a shows that the release kinetics are not very different for either active ingredient. It can only be noted that the release kinetics of ibuprofen MPs are more homogeneous than those of lidocaine. This better homogeneity in release could be due to a better homogeneity in swelling; Indeed, the swelling of ibuprofen MPs seems to be more regular than that of lidocaine MPs. Interestingly, lidocaine MPs swelled much more and earlier than ibuprofen MPs. The diameter is multiplied by three in the case of lidocaine MPs and only by two for ibuprofen MPs, and the onset of the substantial swelling appears on day 2 for lidocaine and on day 5 for ibuprofen. A closer look at the correlation between swelling and release has enabled us to note that the release of ibuprofen MPs also starts to be significant after 5 days of exposure to the release medium. This may reflect a close relationship between the PLGA swelling mechanism and ibuprofen release (this will be further investigated in Chapter VI). On the other hand, the swelling kinetics of lidocaine MPs are more heterogeneous, which might explain the more heterogeneous resulting release kinetics. If a focus is made on some MPs, it can be noted that the two MPs that swelled first (initial diameters of 244 and 248 µm) are also the first ones to release. Similarly, the MP that swells the least quickly (initial diameter of 301 µm) is the one that releases the latest. However, even if it is clear that in both cases swelling seems to play a major role amongst the release mechanisms, a difference in release rates should have been observed: As lidocaine MPs swelled earlier than ibuprofen MPs, they should have released earlier as well. Moreover, lidocaine MPs were a bit smaller than ibuprofen MPs (initial diameter in the left-hand side on Figure 60), and thus should have released a bit faster. In order

to facilitate the kinetics understanding, the averages of the single release kinetics were plotted on Figure 61.

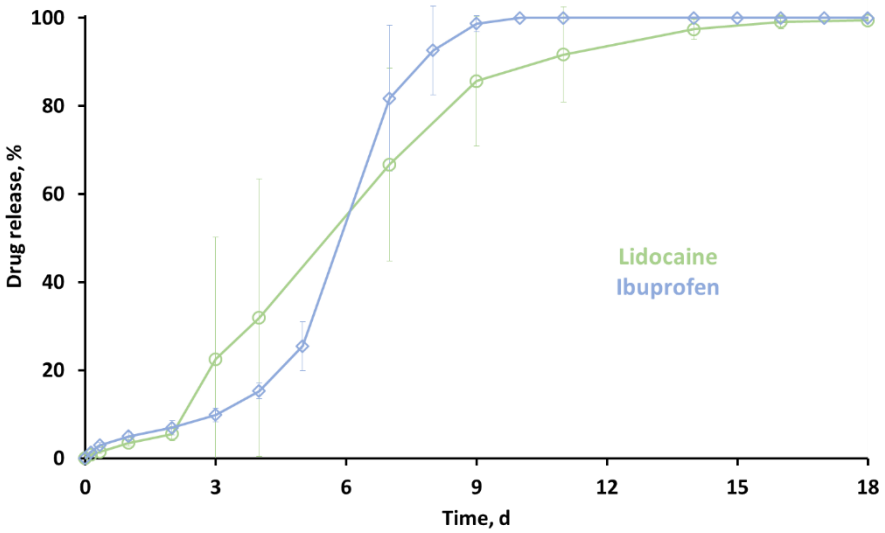


Figure 61. Averages of single releases of the LAL-020 (8% ibuprofen) and LAL-086 (8.5% lidocaine) microparticles upon exposure to phosphate buffer pH 7.4

A slightly clearer trend emerges from these averages, biphasic profiles appear, with an initial phase of varying length: Lidocaine MPs started to release after 2 d, which corresponds to the time it took for the majority of MPs to show substantial swelling, in contrast to ibuprofen MPs, which release very significantly after 5 d, again corresponding to the substantial swelling of the MPs. However, even though lidocaine MPs started releasing the active ingredient earlier, the total duration of release is a week longer.

Following on from the previous characterizations carried out, it was shown that for these batches, there was no significant difference in terms of internal or surface porosity, thus not explaining why the lidocaine MPs swelled earlier. It could have been suggested that the internal porosity of ibuprofen MPs was a bit more important (see Figure 55). A hypothesis could be that the plasticizing effect of lidocaine being greater than that of ibuprofen disorganized the PLGA chains more, thus making it easier for water to penetrate the MP and make it swell. This earlier swelling allows for the rapid diffusion of lidocaine present in the swollen polymer. However, this swelling is probably not sufficient to release the whole drug present in the MP, potentially because of the ionic attractive interactions existing between lidocaine and PLGA. In the case of ibuprofen, once swelling has begun, the drug is released quickly and easily because of the potential repulsive interactions between PLGA and ibuprofen. DSC analyses were performed in order to determine whether this theory of different plasticizing effects could be applied to

these MPs. The obtained thermograms of MPs and raw materials for reasons of comparison are shown in Figure 62.

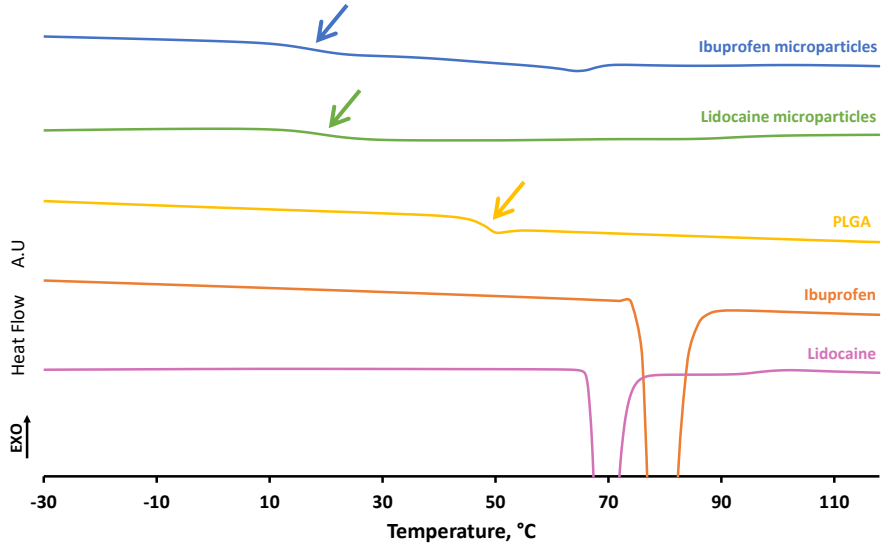


Figure 62. DSC thermograms of the: Investigated LAL-020 ibuprofen and LAL-086 lidocaine microparticles (first heating cycle) and raw materials (PLGA, second heating cycle & ibuprofen and lidocaine, first heating cycle)

Surprisingly, although it was seen in Figure 57 that lidocaine exerts a stronger plasticizing effect on PLGA than ibuprofen in the case of physical mixtures, this was not verified in the case of MPs. One explanation could be that a higher water content in ibuprofen MPs, which is also known to exhibit a strong plasticizing effect, is responsible for the lower T_g. In fact, the T_g is lowered to 16 °C in the case of ibuprofen MPs, and to only 19 °C for lidocaine MPs. If the aim is to get rid of this residual water, which would modify the T_g, the T_g at the 2nd heating cycle, i.e., after the evaporation of this water, can be examined. Once again, contradictory results to those expected were obtained: The T_g in the case of ibuprofen MPs is reduced to 32 °C *versus* 34 °C for lidocaine MPs (data not shown). This is probably due to a partial recrystallization of lidocaine during the cooling cycle, which led to a smaller amount of the plasticizing amorphous lidocaine in the matrix. The plasticizing effect cannot explain, in this case, why lidocaine MPs presented an earlier swelling.

In the case of these MPs, their initial relatively non-porous nature apparently played no role either in swelling or subsequent drug release. In order to monitor the porosity evolution within the MP during the release, XµCT analyses were performed on these investigated MPs. The obtained images before and after 2- and 7-d exposure to the release medium are shown in Figure 63.

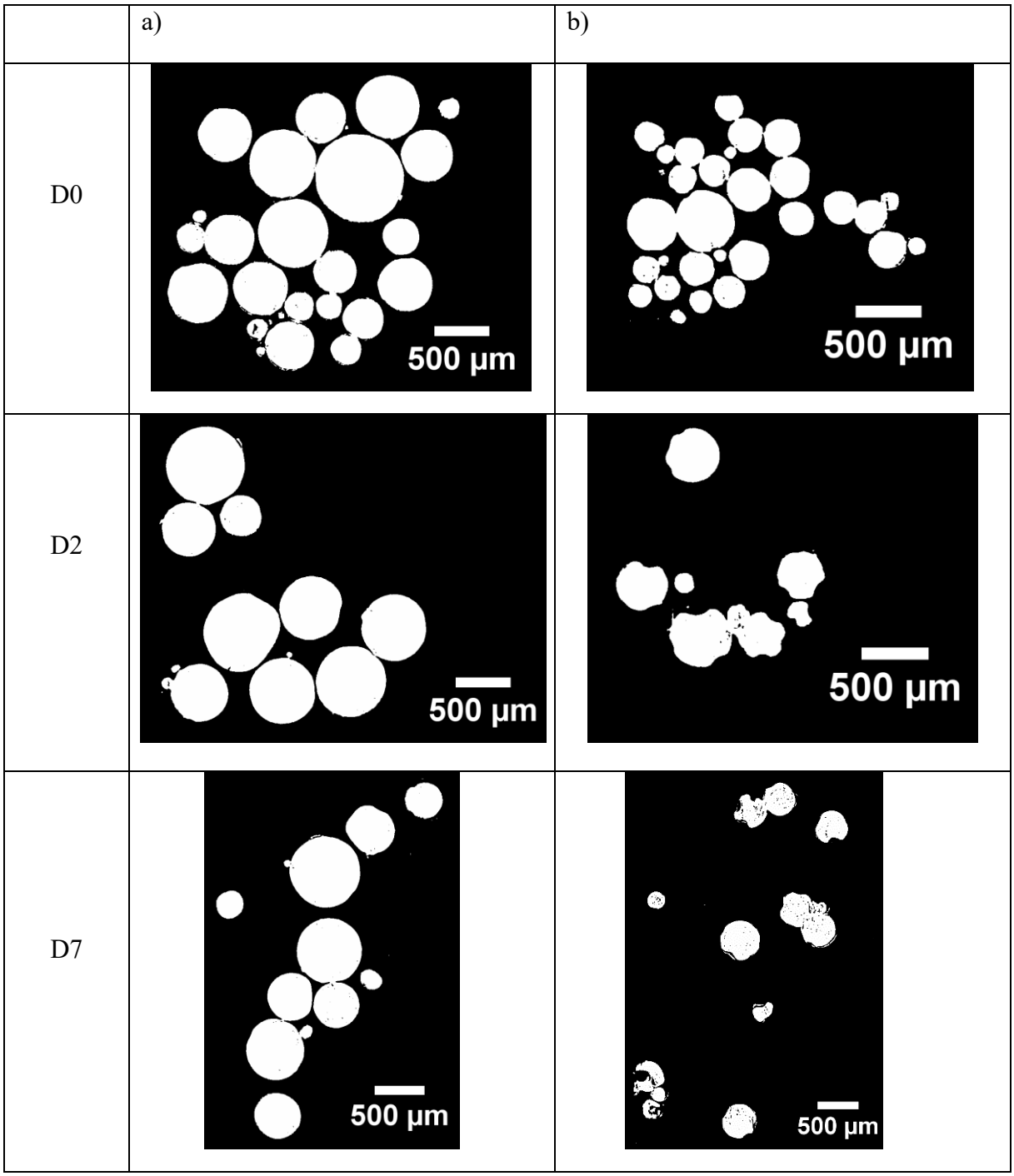


Figure 63. X-ray µCT virtual cross-sections of the a) LAL-020 (8% ibuprofen-loaded) and b) LAL-086 (8% lidocaine-loaded) microparticles, before and after 2- and 7-d exposure to release medium

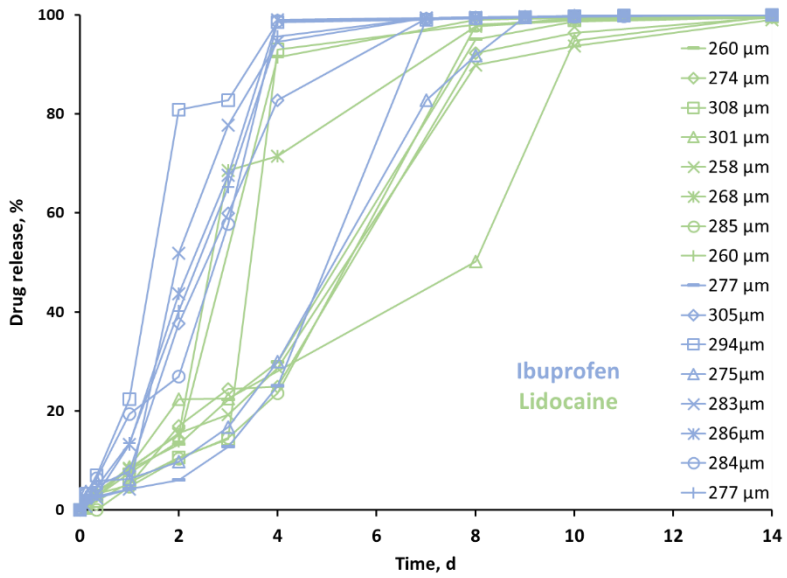
These XµCT analyses revealed unexpected differences between the MPs of ibuprofen and lidocaine during release. The MPs which were initially non-porous, or at least only slightly porous, for the two drugs, did not remain so in the case of lidocaine MPs. After 2 days in contact with the release medium, the ibuprofen MPs look basically the same as before exposure: Neither their shape nor their porosity has changed. On the other hand, lidocaine MPs are no longer as

spherical as they were before contact with the release medium. This is most probably due to their swelling, which begins after 2 days of release (Figure 60 b). Indeed, to carry out the analyses, the MPs were withdrawn from the PB, which meant that they were left for around 2 hours in a dry environment, which probably led to the shrinkage of the swollen polymer. Furthermore, their porosity does not seem to have changed after 2 days. After 7 days in contact with the PB, ibuprofen MPs should have already swollen for 2 days (Figure 60 b), so the same shrinkage phenomenon as for the lidocaine MPs after 2 days should have been observed. However, this is not the case: The ibuprofen MPs still don't seem to have changed, either in terms of sphericity or porosity. This could be explained by the fact that lidocaine MPs swelled much more than ibuprofen MPs, even after 7-day exposure to PB, as shown in Figure 60 b. Concerning the lidocaine MPs, after 7 days, their shape is similar to that which they had after 2 days in contact with the release medium, but their porosity has increased quite significantly. This increase in porosity is probably due to the lower matrix density attributable to the lower organic phase concentration in lidocaine MPs, pores are then more easily created. The fact that porosity increased during the release only for lidocaine MPs could explain why lidocaine MPs swelled more than ibuprofen ones. However, with an improved income of water in the matrix thanks to the increasing development of the porous network, lidocaine MPs should have released faster than the ibuprofen ones, but it was previously shown that the release was over one week after ibuprofen MPs.

All these observations suggest that with lidocaine, which presented a smaller MP size, an earlier and greater swelling, and an increased porosity during release, a more rapid release of lidocaine MPs should have been observed. Possible explanations for this observation are either that the attractive ionic interactions are indeed responsible for a longer residence time in the matrix for lidocaine, thus slowing down release, or that the acidic or basic nature of the active ingredient may also play a role in release mechanisms. As mentioned previously, ibuprofen is an acidic molecule, while lidocaine is a basic one. However, it has been observed that PLGA can be degraded by both acid and base catalysis. PLGA degradation products (PLA and PGA) are acidic, and once released in the medium, they will catalyze the breakage of the ester bonds of the polymer chains remaining on the MPs. If ibuprofen is in the medium, the pH will be even more acidic, and the degradation process will then be accelerated. If lidocaine is in the medium, even if it can catalyze the PLGA degradation, it will neutralize the pH of the medium, and the polymer degradation will be lowered. Unfortunately, pH measurements of the medium during release could not be carried out accurately in this study because the volumes of solution used were too small (100 µL).

It has been observed that, although understanding the role and extent of MP characteristics in release mechanisms is complex, the type of encapsulated active ingredient does appear to exert a non-negligible influence on PLGA release mechanisms. The purpose of the following comparative *in vitro* release studies will be to determine the impact of a more important loading rate of the MPs on their release mechanisms. Figure 64 a and b present, respectively, the release and swelling kinetics of 14%-loaded ibuprofen or lidocaine MPs.

a)



b)

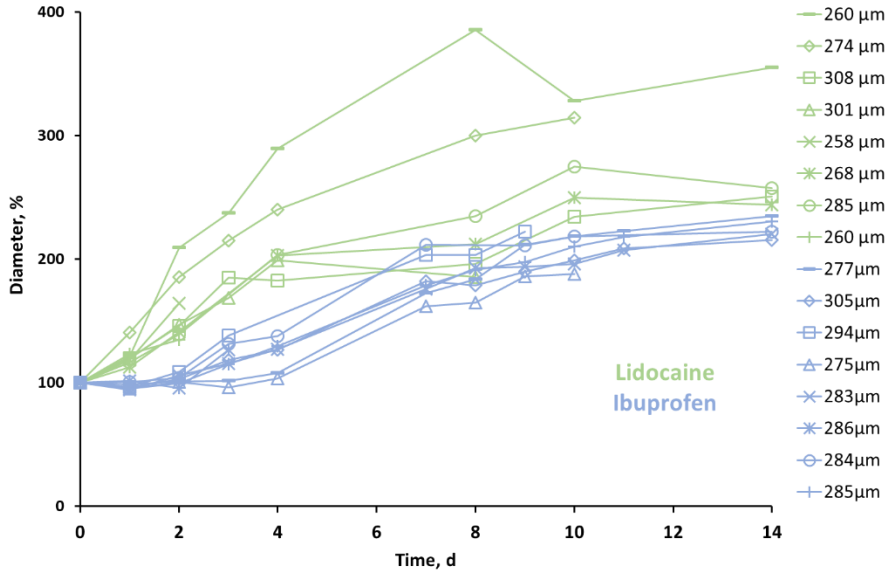


Figure 64. Single a) release and b) swelling kinetics of LAL-030 (14% ibuprofen) and LAL-087 (14% lidocaine) microparticles, upon exposure to phosphate buffer pH 7.4

Firstly, unlike the previous release study, the sizes of the MPs investigated here are more comparable, so there will be no impact of their size differences on the resulting release rates. Moreover, the release kinetics of ibuprofen MPs do not appear to be more homogeneous than those of lidocaine MPs, so the nature of the API is not in itself a factor affecting the homogeneity of the resulting releases. As in the case of the 8% ibuprofen and lidocaine batches studied previously, lidocaine MPs swell faster and to a greater extent than ibuprofen MPs, but their release is not accelerated as might be expected. This brings us again to the theory explained above, which is that: Either the acidic nature of ibuprofen catalyzes PLGA degradation to a greater extent than the basic catalysis provided by lidocaine; Or the ionic interactions between lidocaine and PLGA are indeed more important than those between ibuprofen and PLGA, leading to a slower release in the case of lidocaine, even if the MPs swell more and faster.

To better visualize the impact of the DL on MPs' release kinetics, individual MPs' averages are plotted in Figure 65.

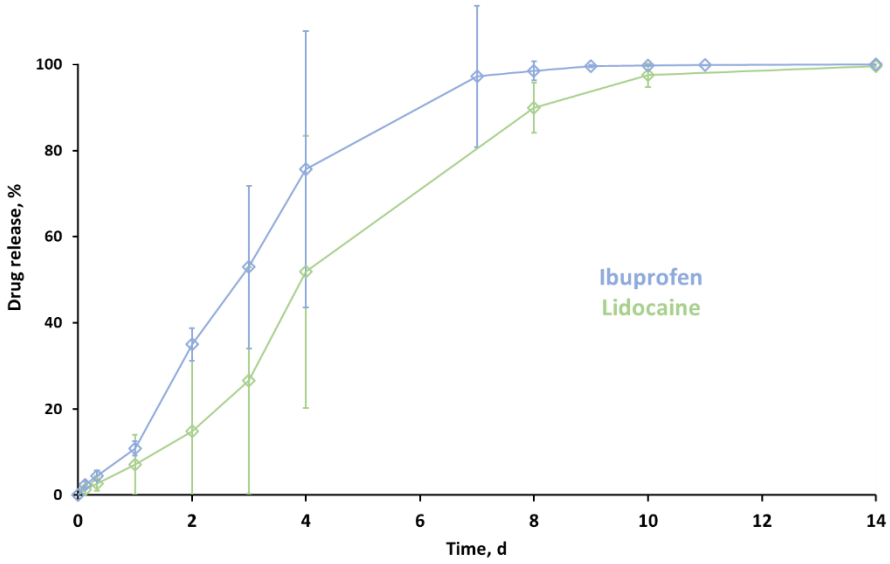


Figure 65. Averages of single releases of the LAL-030 (14% ibuprofen) and LAL-087 (14% lidocaine) microparticles upon exposure to phosphate buffer pH 7.4

MPs loaded with 8% active ingredient released all their drug in an average of 9 d for ibuprofen and around 14 d for lidocaine (Figure 61). In the case of 14%-loaded MPs, release, which always shows a biphasic profile, is slightly faster: 7 days on average for ibuprofen and 10 days for lidocaine. At the same time (7 days for ibuprofen and 10 days for lidocaine), MPs loaded with 8% drug had released only 80% of their total loading. This phenomenon can be explained by the fact that, once in contact with the release medium, the active ingredient located

close to the surface of the MP is rapidly released. Once in the release medium, the drug molecules will leave empty spaces in the MP, which will be rapidly filled with water. This penetration of water will accelerate the release of other drug molecules, gradually creating more and more diffusion pathways, increasing the influx of water into the MP, and thus speeding up release kinetics. In addition, the higher the DL, the less PLGA is present, so the matrix is less dense. This results in an increase in porosity. This porosity, confirmed in X-ray µCT and presented in Figure 66, is also responsible for more rapid water penetration and, consequently, accelerated drug release.

a) b)

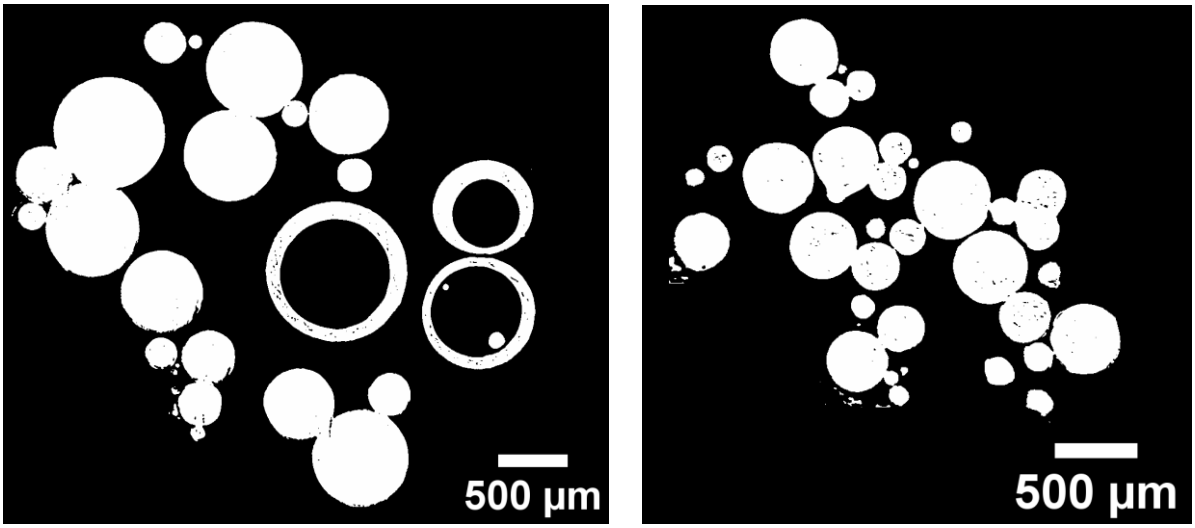
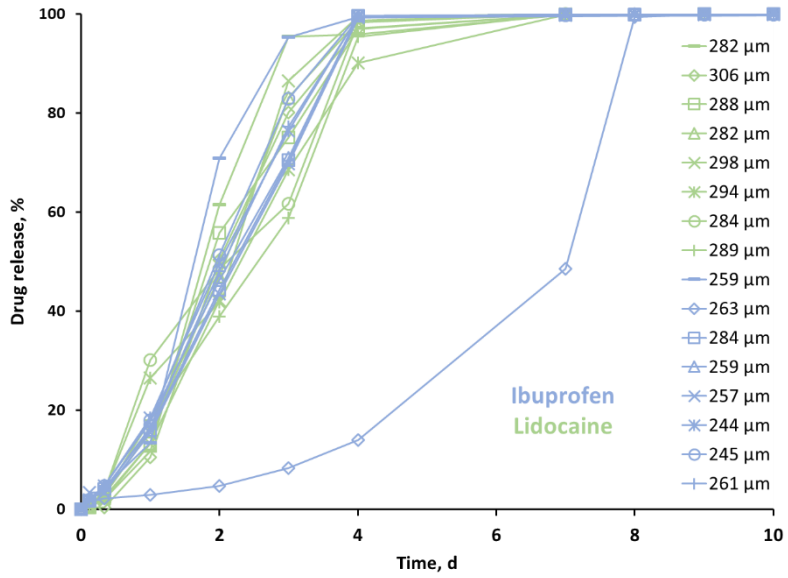


Figure 66. X-ray µCT virtual cross-sections of the a) LAL-030 (14% ibuprofen-loaded) and b) LAL-087 (14% lidocaine-loaded) microparticles, before exposure to release medium

The porosity of these 14%-loaded MPs is more important than for the previous 8%-loaded MPs (see Figure 63), and some ibuprofen MPs even feature hollow cavities (Figure 66 a). If this type of highly porous MP is investigated in release studies, the resulting release will be much more rapid than for non-hollow MPs, because once the water has passed through the thin wall, the cavity entirely fills with water and all the ibuprofen can be released. The higher porosity in lidocaine MPs corroborates the fact that a faster and more important swelling than for ibuprofen MPs (Figure 64 b) was observed.

By increasing the DL from 8 to 14%, it was observed that the release was accelerated by a few days. If the DL is increased again, this time to 20%, the resulting release should be even faster. The release and swelling kinetics of about 20% drug-loaded microparticles are shown in Figure 67 a and b respectively.

a)



b)

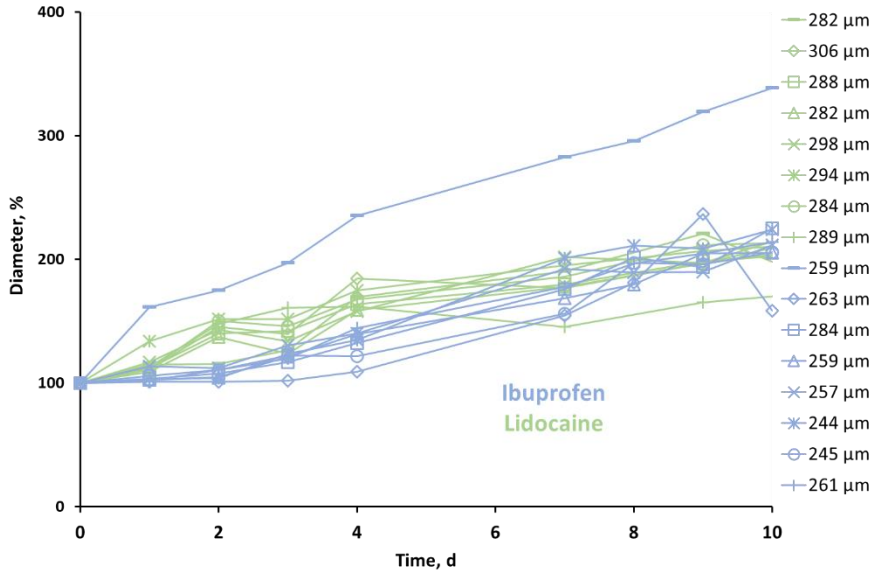


Figure 67. Single a) release and b) swelling kinetics of LAL-031 (20% ibuprofen) and LAL-117 (21% lidocaine) microparticles, upon exposure to phosphate buffer pH 7.4

Releases of MPs loaded with around 20% of active ingredient are, as expected, faster than releases of less loaded MPs. However, this time, the release of lidocaine MPs appears to be as fast as that of ibuprofen, whereas for the lower DLs, the release of lidocaine MPs was slightly slower. In both cases, releases are over after only 4 days of exposure to the release medium, compared to more than one week for less loaded MPs. Indeed, as shown in Figure 55, their porosity is much more important than that of lower DLs, allowing for water to penetrate more easily in the MP, causing a faster release. Surprisingly, in this case, ibuprofen MPs are

more porous than those of lidocaine (Figure 55 20-21%, *please note the different scale between the two pictures*), whereas previously it was shown that for the same DL, lidocaine MPs were more porous because they were prepared with a less concentrated organic phase. In addition, it can be noticed that these 21% lidocaine-loaded MPs swell less than the less loaded ones: Around 300% in the case of 8%-loaded MPs (Figure 60 b), 250% in the case of the 14%-loaded MPs (Figure 64 b), and 200% for the 21%-loaded MPs (Figure 67 b) after 10 days of exposure to the release medium. In comparison, ibuprofen MPs of equivalent DL always swell less than lidocaine MPs, and by around 200%, independent of the DL. Normally, the higher the DL, the less polymer there is, i.e., less polymer will be available for swelling, so the final swelling will be less important than that of less loaded MPs. On the other hand, as the matrix is more porous due to the lower amount of polymer, water will easily penetrate the MPs and make them swell rapidly. In our cases, for both drugs, swelling did not occur faster for more loaded MPs; It always occurred after around 4 days for ibuprofen and after 1 day for lidocaine. Nevertheless, the higher the DL of lidocaine, the lower the swelling, which is consistent with the phenomenon described above. However, this was not verified for ibuprofen MPs. One explanation of this could be that it has been observed that ibuprofen MPs are more fragile and break faster than lidocaine MPs, so they potentially have less time to swell as they erode faster, decreasing their diameter. Indeed, the measurement of swelling in this study is allowed by the measurement of the two-dimensional diameter of MPs obtained thanks to optical microscopy. It is then difficult to dissociate erosion from a less important swelling, as a MP that swells less is maybe more like a MP that swells in the same way but erodes more.

Furthermore, the encapsulated drug probably has an effect on the physicochemical characteristics of the MPs: If lidocaine interacts attractively with PLGA, this potentially strengthens the matrix and limits erosion in the bulk fluid, unlike ibuprofen which does not interact with PLGA, making the latter less resistant to erosion.

By following the previous reasoning, the greater the porosity, the faster and greater the swelling after contact with the release medium. However, in this particular case, ibuprofen MPs, being more porous, should have swollen faster than lidocaine MPs, but this is not the case. The first hypothesis is that the pores of ibuprofen MPs are less interconnected, which doesn't facilitate water diffusion through the MP, whereas for lidocaine MPs, although in this case the pore network seems less important, the pores are perhaps better interconnected, facilitating water penetration throughout the MP. The second hypothesis is that, with the plasticizing effect of lidocaine being more important, the PLGA chains were more likely to allow water to

penetrate the matrix, leading to faster swelling (the T_g of ibuprofen MPs was an average of 16.6 °C, against 12.6 °C for lidocaine MPs, data not shown).

Again, these elements converge towards a theoretically faster release of lidocaine than ibuprofen, but this was not what was observed. This leads us to the following conclusion: Either the physicochemical interactions between the drug and the polymer do indeed have consequences on the release mechanisms, inducing a modification of the release kinetics depending on whether the interactions are of an attractive or repulsive nature; or swelling finally has little impact on release. This latter hypothesis could explain why lidocaine MPs, which swell more and faster than ibuprofen MPs, would not release faster. For both scenarios, the effect of the pH medium should not be neglected. Indeed, if the pH of the medium decreases as the ibuprofen is released (before the phosphate buffer has had time to neutralize the charges), acid catalysis of the PLGA ester bonds will be accelerated, thereby increasing degradation and ibuprofen release. The release rate of ibuprofen will thus balance with the release of lidocaine due to faster swelling. If swelling plays a minor role in release mechanisms, perhaps acid/base catalysis of PLGA may be a major release mechanism, increasing polymer degradation and hence active ingredient release. This also correlates with the fact that the greater the DL, the greater the amounts of drugs released, the more acidic or basic the release medium will be before the buffer has neutralized the charges, and the faster the total release will be by increasing the rate of degradation.

III. Conclusion

The conclusion that can be drawn from these results is that the type of drug will impact the drug release mechanisms by modifying the physicochemical characteristics of the MPs. Lidocaine MPs appeared more homogeneous in terms of physical appearance (opaque or transparent) and also more porous in general than ibuprofen MPs, but this was totally or partially due to the different process parameters that had to be applied for lidocaine MPs (organic phase concentration and stirring speed). This more important porosity will lead to a faster release of lidocaine than ibuprofen. With the plasticizing effect of lidocaine being greater than that of ibuprofen, the mobility of lidocaine molecules will be improved, leading to a faster release. However, the ionic interactions existing between drug and PLGA being attractive in the case of lidocaine, coupled with the neutralization of degradation products by lidocaine, will result in reduced degradation, and the release of lidocaine will be delayed, resulting in a final release rate equivalent to that of ibuprofen. Thanks to DSC analyses, we were able to discover that the PLGA matrix can be oversaturated with amorphous drugs. In the case of these two APIs, all these differences in terms of the characteristics of the MPs obtained did not lead to very significant differences in release rate. Nevertheless, the encapsulation of other types of drugs, such as hydrophilic drugs, even more basic drugs than lidocaine, or drugs which do not play the role of PLGA plasticizer, would certainly show different release kinetics. This could be useful in the future to better understand the release mechanisms of PLGA MPs.

III. Conclusion

La conclusion que l'on peut tirer de ces résultats est que le type de substance active a un impact sur les mécanismes de libération en ayant modifié les caractéristiques physicochimiques des MPs. Les MPs de lidocaïne sont apparues plus homogènes en termes d'apparence physique (opaques ou transparentes) et également plus poreuses en général que les MPs d'ibuprofène, mais ceci est totalement ou partiellement dû aux différents paramètres de procédé qui ont dû être appliqués pour les MPs de lidocaïne (concentration de la phase organique et vitesse d'agitation). Cette porosité plus importante conduira à une libération plus rapide de la lidocaïne que de l'ibuprofène. L'effet plastifiant de la lidocaïne étant plus important que celui de l'ibuprofène, la mobilité des molécules de lidocaïne sera améliorée, conduisant à une libération plus rapide. Cependant, les interactions ioniques existant entre la substance active et le PLGA étant attractives dans le cas de la lidocaïne, couplées à la neutralisation des produits de dégradation par la lidocaïne, aura pour conséquence une dégradation diminuée et une libération

retardée de la lidocaïne, entraînant une vitesse de libération finale équivalente à celle de l'ibuprofène. Grâce aux analyses DSC, nous avons pu découvrir que la matrice PLGA peut être sursaturée en médicaments amorphes. Dans le cas de ces deux substances actives, toutes ces différences en termes de caractéristiques des MPs obtenues n'ont pas conduit à des différences très significatives en termes de taux de libération. Néanmoins, l'encapsulation d'autres types de médicaments, tels que des substances actives hydrophiles, encore plus basiques que la lidocaïne, ou qui ne jouent pas le rôle de plastifiant du PLGA, montrerait certainement des cinétiques de libération différentes. Cela pourrait être utile à l'avenir pour mieux comprendre les mécanismes de libération des MPs de PLGA.

CHAPTER VI – IMPACT OF THE *IN VITRO* RELEASE SETUP ON SWELLING AND DRUG RELEASE FROM SINGLE PLGA MICROPARTICLES

I. Objectives of the work

The aim of this work was to evaluate the impact of the type of *in vitro* release setup used on the release of ibuprofen and lidocaine from PLGA microparticles. This will allow us to further investigate the swelling behavior of microparticles, in order to better understand the importance of polymer swelling among the mechanisms involved in the drug release from PLGA-based DDS. Furthermore, this will also enable us to get closer to an environment comparable to that found in subcutaneous tissues, in the case of a potential future use of these microparticles in intraperitoneal drug delivery [216], [217]. As this type of gel was already used in the literature to mimic the intraperitoneal environment, it will therefore provide us with more consistent data with the possible application of microparticles.

As previously mentioned, the release studies were conducted on *single* microparticles, allowing us to better correlate a characteristic of a microparticle to its release behavior. This behavior can potentially strongly depend on the surrounding conditions and, thus, on the experimental setup used for drug release measurements [52], [218]. The most commonly used *in vitro* release setup is the one called the "bulk fluid setup", shown in Figure 22, where microparticles are in a phosphate buffer at physiological pH (7.4 in our case). In that study, releases of microparticles obtained in bulk fluid will be compared to releases of microparticles embedded in an agarose gel (Figure 23), mimicking patient tissue upon subcutaneous or intramuscular administration. This gel will create a sterical hindrance around the microparticle and will slow down and limit its swelling.

In those two setups, releases are conducted in a phosphate buffer (PB) pH 7.4 (the agarose gel is also made from the same PB), but in many studies, it can be noted that phosphate buffer saline (PBS) at pH 7.4 is frequently used due to its better simulation of the *in vivo* environment, by taking more into account the osmolarity of the medium. Comparative studies were therefore carried out in PBS (for the bulk fluid and the agarose gel setups) in order to determine if the type of medium would influence the microparticles' behavior.

Moreover, according to the theory that lidocaine is able to create ionic interactions with PLGA at pH 7.4 [83], a release of lidocaine microparticles in PBS was performed. Indeed, if lidocaine is really linked to the PLGA matrix by ionic bonds, delaying its release in PB, the salts present in the PBS should break these bonds, which would accelerate the release. The aim

of this work is to investigate further the possible influence of these ionic interactions on the release process. To ensure that the potential difference in release rates is not due to a difference in the solubility of lidocaine in PB and PBS, solubility studies were performed in both media.

II. Results and discussion

II.1. General characteristics of the investigated microparticles

The characteristics of the investigated microparticles in this study are listed in Table 5. The sample name, the encapsulated API, the preparation method, the average size of the microparticles in the corresponding batch, the polydispersity index, the average drug loading and encapsulation efficiency, as well as the initial average diameter of the MPs investigated in the release studies for each setup, are reported. In order to induce as little bias as possible in the comparison of *in vitro* release setups, the MPs selected are always of a similar size, to within a few tens of micrometers, and of a similar average drug loading.

Table 5. Characteristics of the investigated microparticles in this chapter

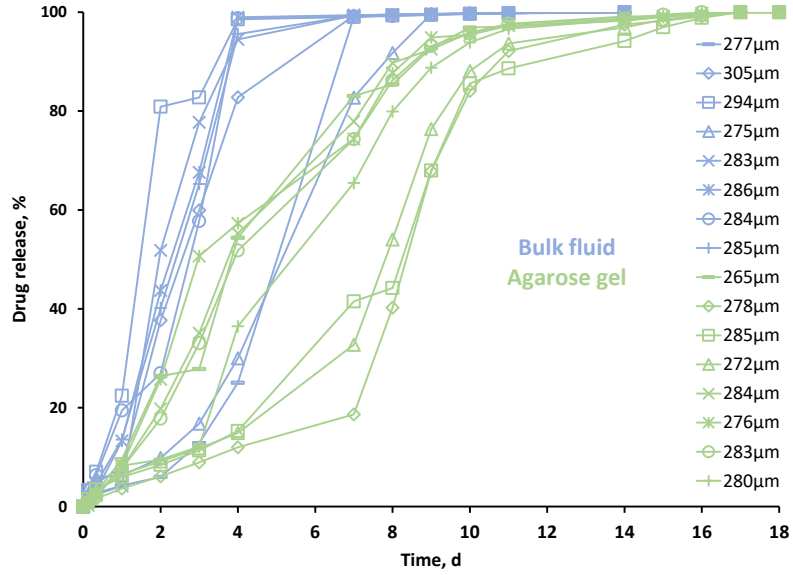
Sample name	Drug	Preparation method	Average diameter (µm)	PdI (%)	DL_{exp} ± SD (%)	EE (%)	Initial average diameter of investigated MPs during release (µm)
LAL-030	Ibuprofen	Standard	362	44	14±0.05	95	Bulk fluid PB: 286 Agarose: 278
LAL-031	Ibuprofen	Standard	269	41	20±0.71	98.5	Bulk fluid: 259 Agarose: 253
LAL-042	Ibuprofen	Microfluidics	326	20	23.5±0.64	78	Bulk fluid: 267 Agarose: 259
LAL-046	Ibuprofen	Standard	203	30	47.5±0.4	95	Bulk fluid: 237 Agarose: 282
LAL-074	Lidocaine	Microfluidics	273	20	7±0.4	72	Bulk fluid: 266 Agarose: 241
LAL-086	Lidocaine	Standard	275	16	8.5±1.23	86	Bulk fluid: 265 Agarose: 228
LAL-087	Lidocaine	Standard	257	27	14±1.88	96	Bulk fluid: 277 Agarose: 242
LAL-117	Lidocaine	Standard	298	39	21±0.75	84	Bulk fluid PB: 290 Bulk fluid PBS: 285 Agarose PB: 238 Agarose PBS: 236

II.2. Comparative release and swelling kinetics of the investigated microparticles in PB versus agarose

II.2.1. Release and swelling kinetics of standard microparticles of ibuprofen

Figure 68 details the release (a) and swelling (b) kinetics of each microparticle of batch LAL-030 upon exposure to phosphate buffer pH 7.4, either in the bulk fluid setup (represented by blue curves), or in the agarose gel setup (represented by green curves). These MPs contain 14% ibuprofen. The initial diameter of each MP is reported on the right-hand side of each graph.

a)



b)

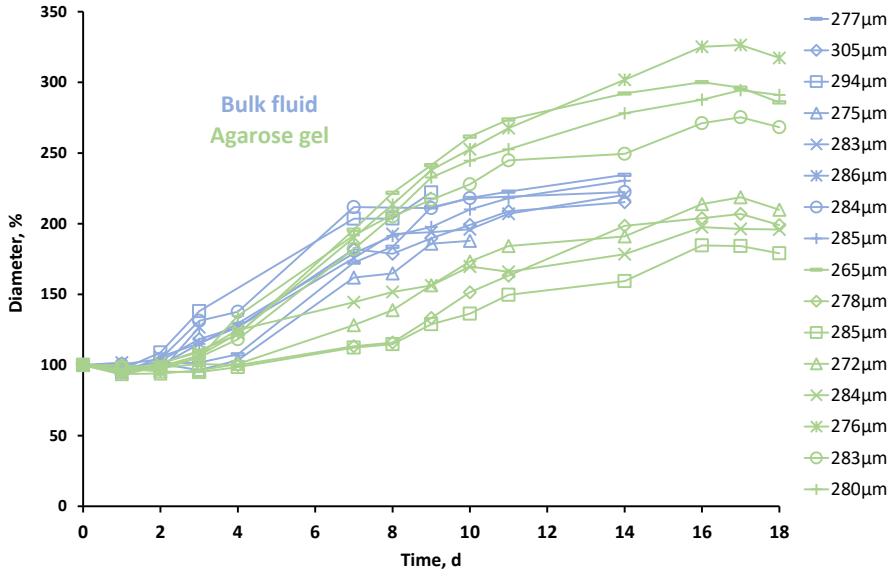


Figure 68. Ibuprofen single a) release and b) swelling behavior of the LAL-030 microparticles upon exposure to phosphate buffer pH 7.4 for the two different setups

It can be seen that the MPs present in the bulk fluid are releasing faster than the MPs entrapped in the agarose gel. It took between 4 and 9 days for all the MPs in the bulk fluid setup to release all their ibuprofen. In contrast, for the MPs entrapped in the agarose gel setup, it took between 11 and 16 days for them to release almost all the initially encapsulated ibuprofen.

By looking at the swelling kinetics, it can be seen that the swelling of the MPs in the bulk fluid is quite homogeneous, while the swelling of the MPs in the agarose is divided into two groups, where the swelling is homogeneous within each group. Swelling onset of the MPs in the bulk fluid starts on day 2 for the majority of them, except for two MPs, for which swelling starts after 4 days. The substantial swelling occurs after 4 days of release for all the MPs. At the end of the release, the MPs in the bulk fluid had doubled in size. For the MPs present in the gel, swelling occurs later than in the bulk fluid. The swelling onset appears after 3 days for a group of five MPs, whereas it starts rather after 4 days for the other group. The substantial swelling of the first group starts after 4 days, against around 8 days for the second group. Interestingly, the group of MPs that swell faster in agarose is also the one whose MPs tripled in diameter at the end of the release (except for one MP), whereas the group of MPs that swell later only doubled in diameter.

By correlating the swelling kinetics with the release kinetics, it can be clearly seen that the MPs that swell the fastest are also the ones that release the fastest, and vice versa. Indeed, it can be noted that two MPs present in the bulk fluid (initial diameter of 277 and 275 μm) swell less quickly than the others, and surprisingly, they are also those that release ibuprofen the slowest. Even more strikingly, for the MPs present in agarose, the three MPs swelling the least rapidly (initial diameter of 278, 285 and 272 μm) are also the ones releasing the latest. The swelling therefore appears to be closely related to the release of ibuprofen.

To facilitate a general understanding of the release and to better appreciate the shape of the release profile, the average releases of each MP were made for each setup and compared in Figure 72. It can be seen even more clearly that there is an average delay of 7 days between a full release for the bulk fluid setup and the agarose setup.

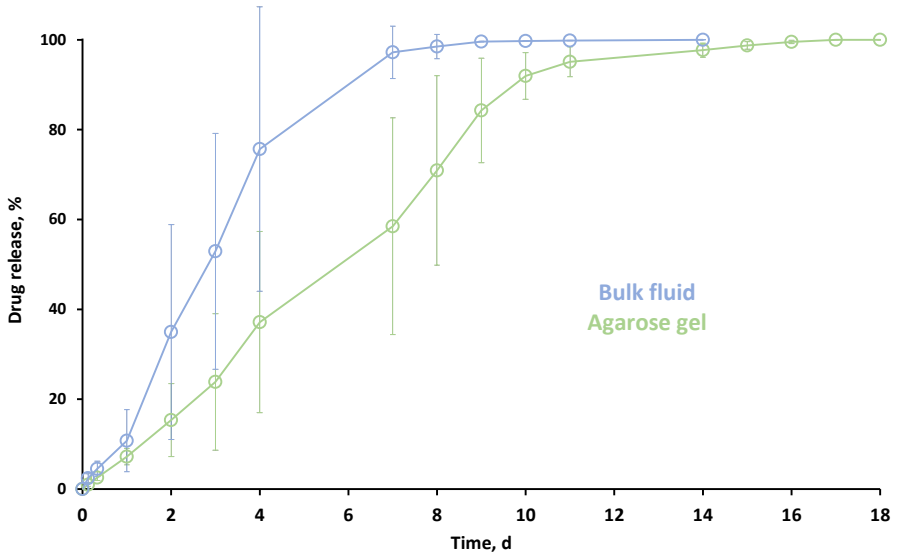
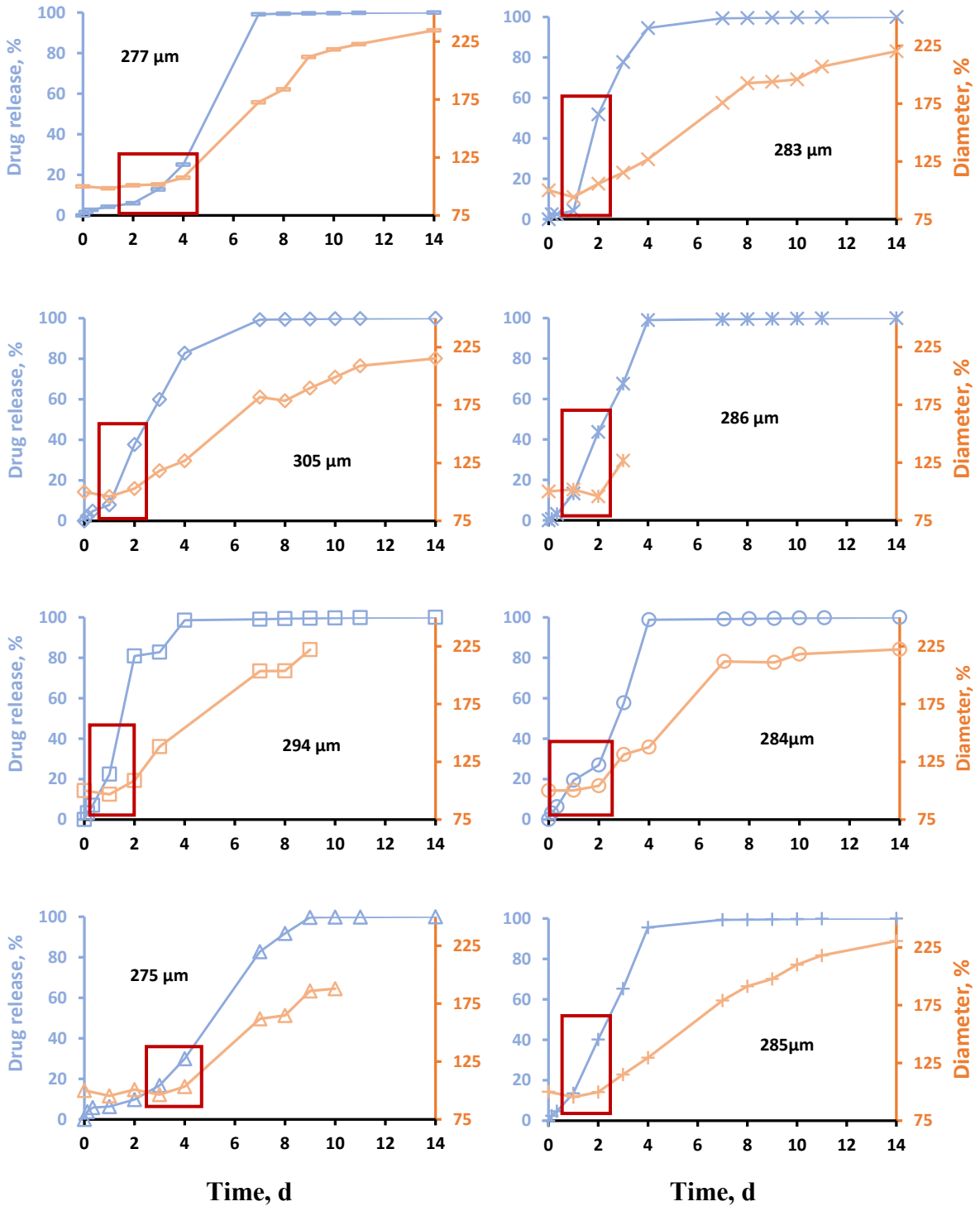


Figure 69. Averages of single releases of the LAL-030 microparticles upon exposure to phosphate buffer pH 7.4 for the two different setups

In order to further evaluate the correlation between swelling and release, the kinetics will be compared for MPs individually, as shown in Figure 70 a for MPs studied in the bulk fluid setup and in Figure 70 b for the ones in the agarose gel setup. The blue and green curves correspond to the left y-axes and show the observed drug release rates, while the orange curves correspond to the right y-axes and illustrate the swelling kinetics. In each diagram, the initial diameter of the investigated MP is reported, and the red square highlights the period during which the swelling of the MP begins. The same symbols are used as in Figure 68 thus, curves with the same color and symbol in Figure 68 a and b illustrate the release and swelling kinetics of the same microparticle.

a)


277 µm
283 µm
305 µm
286 µm
294 µm
284µm
275 µm
285µm
Drug release, %
Diameter, %
Time, d
Time, d

b)

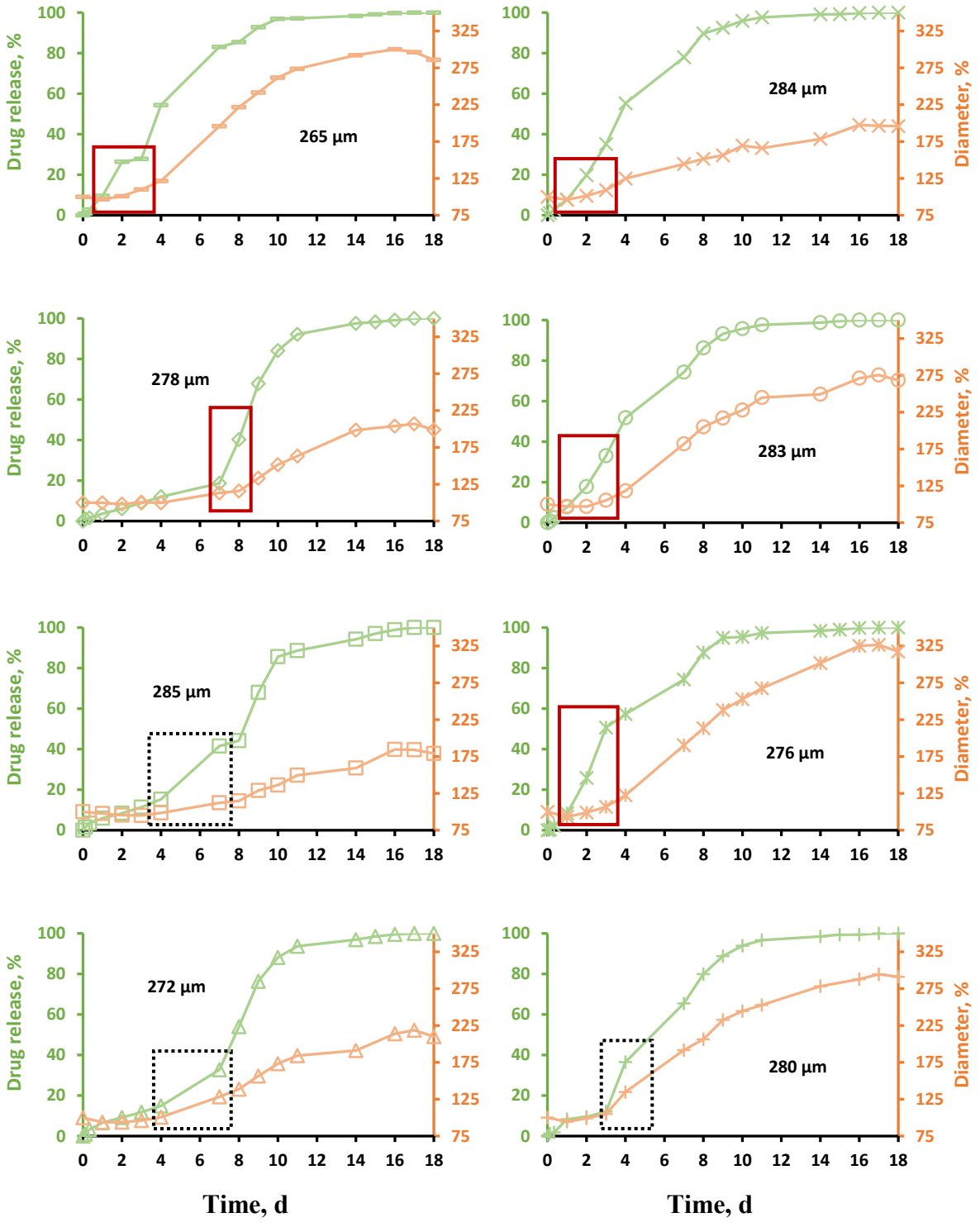


Figure 70. Individual release and swelling kinetics of the LAL-030 microparticles for the a) bulk fluid setup in PB, and the b) agarose gel setup

Thanks to these individual diagrams, it can be seen more clearly that in the bulk fluid setup (Figure 70 a), the ibuprofen release starts before the onset of system swelling. This phenomenon is highlighted by the red rectangles. Interestingly, in the agarose gel setup, the onset of the substantial swelling better coincides with the beginning of the more important ibuprofen release, as pointed out by the black dotted squares.

The substantial swelling of PLGA-based implants and microparticles has previously been hypothesized to be the primary cause of the onset of the final rapid drug release phase from these systems, leading to complete drug exhaust [65], [67]. When a system is exposed to an aqueous medium, water will rather rapidly penetrate through the system. However, the penetration rate of water into the device will depend on the polymer hydrophilicity or hydrophobicity, but even limited amounts of water will be sufficient to lead to hydrolytic bond cleavage of the PLGA. Knowing that each ester bond cleavage will generate two new groups (an -OH and a -COOH group), the PLGA matrix will become more and more water-loving. As a consequence, the polymer molecular weight will decrease, and the chain entanglement degree will decrease as well: Swelling will therefore be facilitated. In addition, the water-soluble degradation products created during this process will increase the osmotic pressure in the system, leading to a substantial swelling onset once a critical molecular weight is reached. Hence, the drug mobility through the polymer is increased, and the drug release becomes more important.

These previously described behaviors can be easily explained by the fact that MPs of this batch are porous, as can be seen in the SEM cross-section in Figure 71, meaning that they will quickly become filled with water, which will penetrate the MP and allow the polymer to swell, thus disentangling the PLGA chains and releasing ibuprofen. In the agarose gel setup, the water uptake also happens quickly, but the swelling is hindered by the sterical hindrance created around the MP by the gel, which is much denser than the bulk fluid. The PLGA chains will therefore have more difficulty disentangling, which will hinder the release of ibuprofen. Moreover, the mechanical stress induced by the surrounding medium is less pronounced in the agarose gel setup because of the absence of a convective flow. On the contrary, a MP in the well-agitated bulk fluid setup will be exposed to an important convective liquid flow around it, which will lead to premature surface erosion of the thin PLGA outer layer. The probability that direct surface access to the porous network is created is then higher in the bulk fluid than in the agarose gel setup.

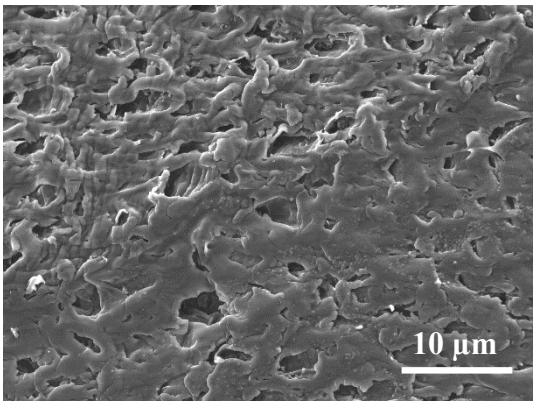
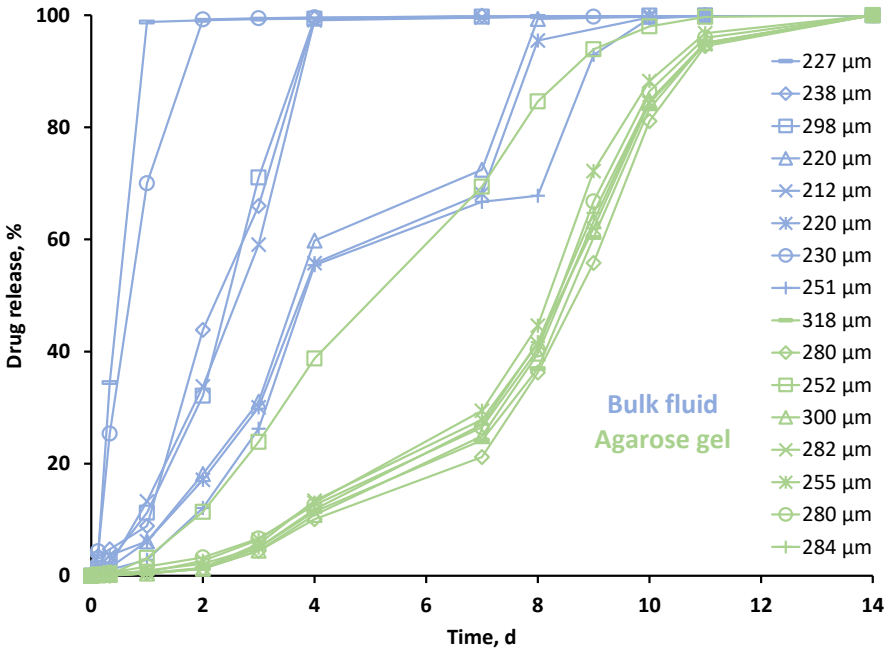


Figure 71. SEM picture of LAL-030 cross-section before exposure to the release medium

Figure 72 details the release (a) and swelling (b) kinetics of each microparticle of batch LAL-046 upon exposure to phosphate buffer pH 7.4, either in the bulk fluid setup, or in the agarose gel setup. These MPs are more loaded than the batch LAL-030 described above; their DL is 47.5% ibuprofen. In this way, the role of the drug loading in the ibuprofen release can also be determined.

a)



b)

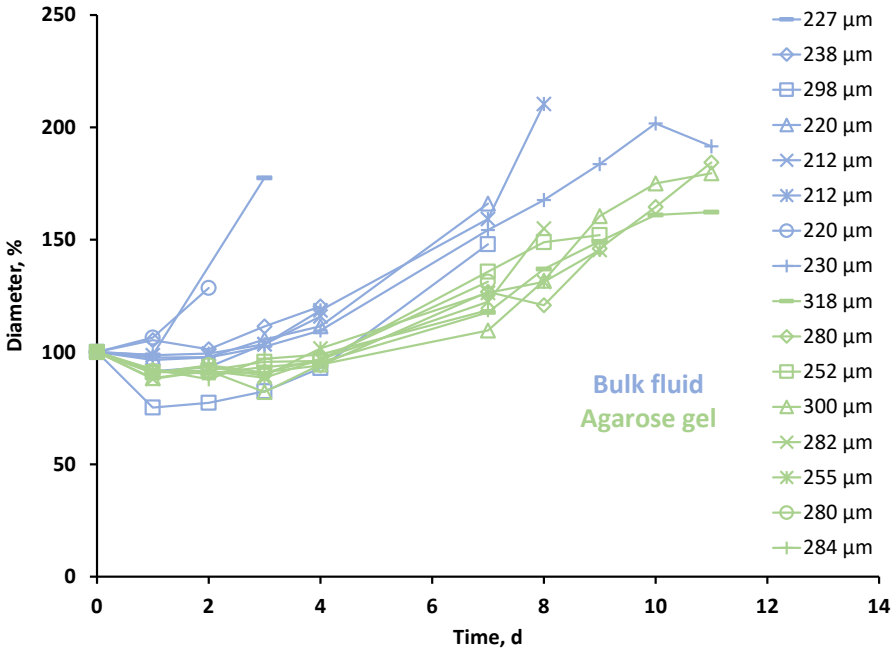


Figure 72. Ibuprofen single a) release and b) swelling behavior of the LAL-046 microparticles upon exposure to phosphate buffer pH 7.4 for the two different setups

Clearly, the observed drug release rates were substantially different, although the microparticle samples were from the same batch: Ibuprofen release was much faster into the well-agitated bulk fluid compared to the agarose gel. In addition, the variability in drug release was more pronounced upon direct exposure to the phosphate buffer compared to inclusion into a hydrogel (mimicking patient tissue).

At this high drug loading, it is not surprising that release in the bulk fluid is much more heterogeneous than for batch LAL-030 loaded with only 14% ibuprofen. Indeed, with 14% ibuprofen, all the drug is dissolved in an amorphous form in the matrix of PLGA, whereas at 47.5%, the matrix is saturated by ibuprofen, which forms crystal clusters. The physical state of ibuprofen in the MPs was confirmed by X-ray diffractograms, represented in Figure 73.

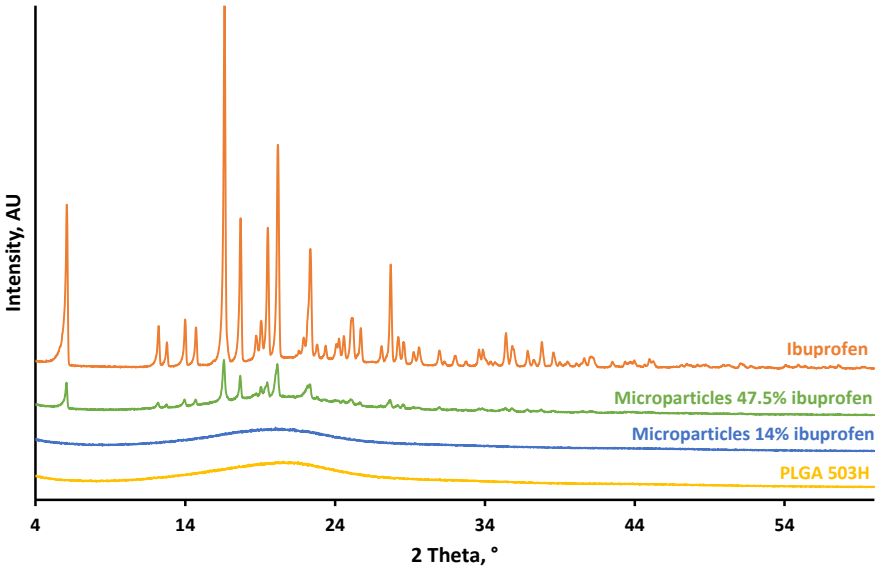


Figure 73. X-ray diffraction patterns of the investigated ibuprofen-loaded PLGA MPs, and raw materials (PLGA & ibuprofen). The batch LAL-030 is represented in blue, and the batch LAL-046 in green

The diffractogram of the batch loaded with 14% ibuprofen is completely amorphous, so all the ibuprofen is dissolved in the PLGA, also amorphous, while that of the batch loaded with 47.5% ibuprofen shows diffraction patterns, corresponding to the patterns of pure ibuprofen, which is fully crystalline.

As a consequence, ibuprofen, remaining in crystalline form, aggregates and builds up into larger crystals dispersed in the polymer. During the release, the dissolution of these crystal aggregates will induce an important release of ibuprofen in the medium. On the other hand, if ibuprofen is dissolved in the polymer, its release will be more gradual. However, depending on the crystal's size and location, the release time may vary: Either the crystals are on the surface of the MP and the release is fast, or they are in the core of the MP and the release is slower, because of the water-diffusion time throughout the matrix. This is part of the reason why the releases in bulk fluid are heterogeneous for high-loaded MPs. In addition, it can be noted that the most rapidly releasing MPs (initial diameters of 227 and 230 µm) are the ones swelling at the very beginning of the release, proving, once again, the close relation between swelling and release.

In the case of the agarose gel setup, the diffusion of ibuprofen through the medium is more difficult because of the limited swollen polymer, so even if the crystals are broken at the same speed as for the bulk fluid setup, the entanglement of the PLGA chains remains too high to let ibuprofen diffuse in the release medium. Moreover, swelling seems to be more controlled when the MP is entrapped in agarose. This results in more homogeneous releases in agarose, as illustrated by Figure 74.

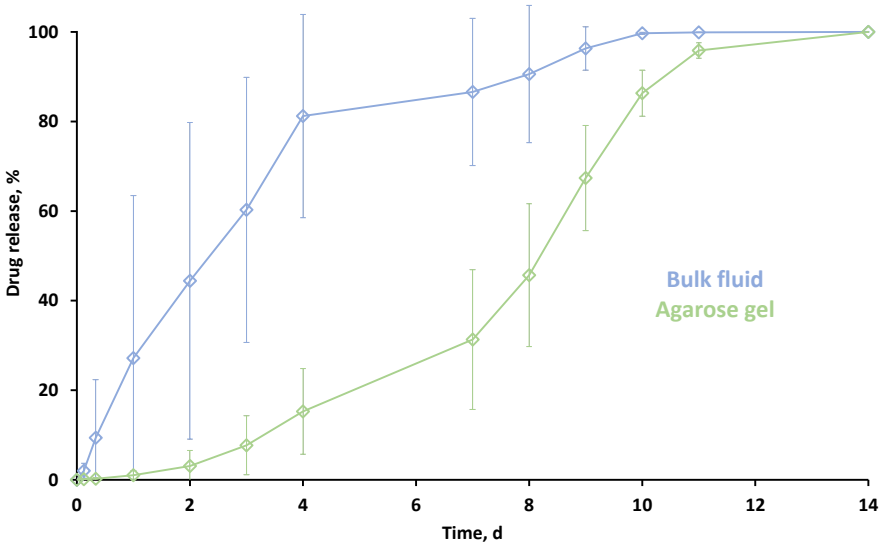


Figure 74. Averages of single releases of the LAL-046 microparticles upon exposure to phosphate buffer pH 7.4 for the two different setups

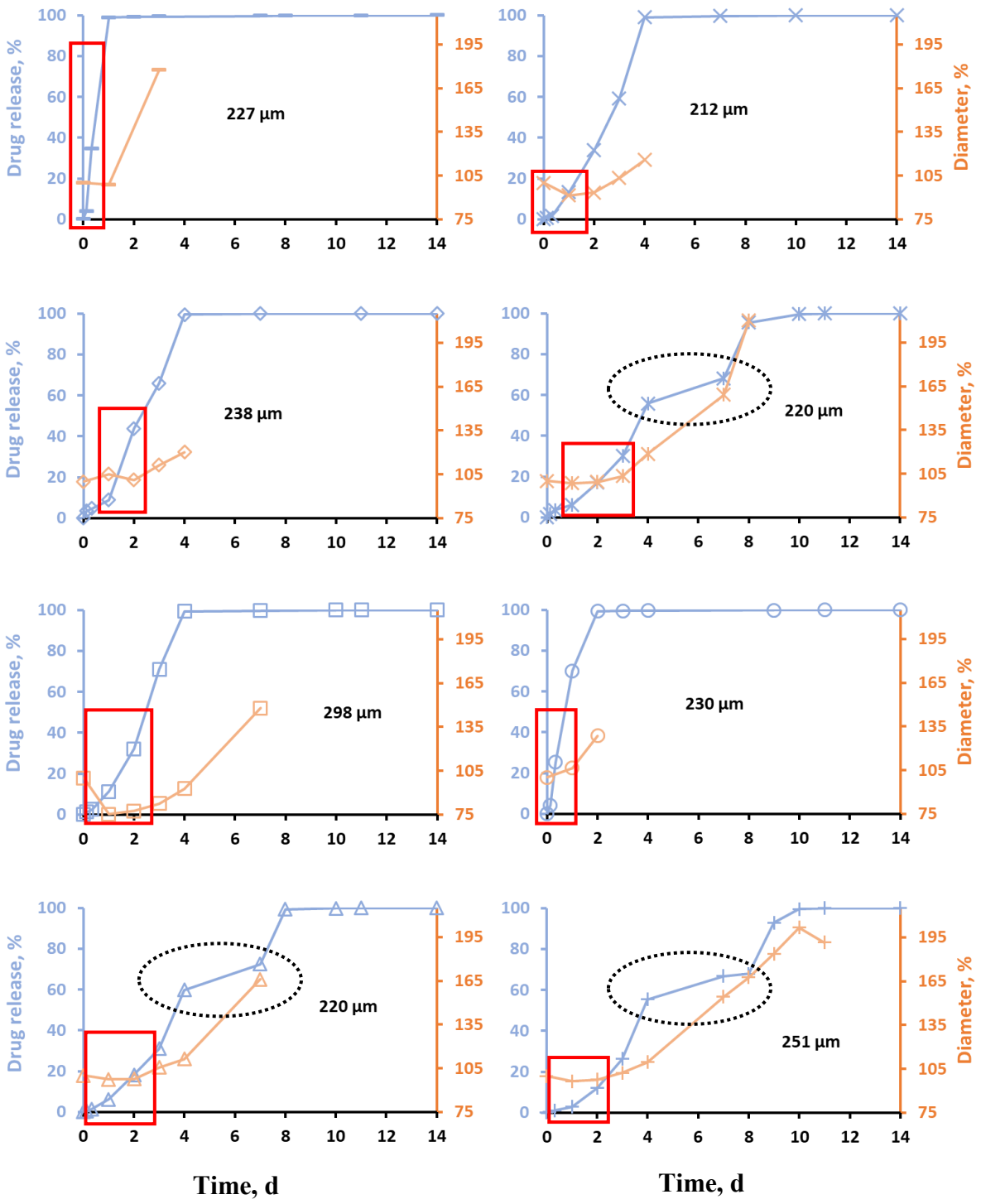
As it can be seen, bi-phasic drug release kinetics were observed for the bulk fluid setup microparticles, whereas a tri-phasic drug release was observed in the case of the agarose gel setup. Concerning the bulk fluid setup, an initial rapid drug release phase during the first four days was followed by a second release phase, slower than the first one, leading to complete drug exhaust. Concerning the agarose gel setup, an initial slow release phase was observed during the first two days of release, followed by a second phase where the release rate increased until day 7, where finally, a more rapid final ibuprofen release occurred.

This difference in release profile is probably due to the difference in swelling rates. Indeed, in the bulk fluid setup, a region-limited swelling of the polymer at the surface of the microparticle starts on the first days of release, and allows the diffusion and release of the drug through the swollen polymer. On the other hand, in the agarose gel setup, the swelling is hindered by the gel, and as a consequence, only a small amount of drug can diffuse in the release

medium. The more important release of ibuprofen after two days is allowed by the onset of the swelling, which leaves space between the polymer chains and enables the drug to diffuse.

This release hypothesis seems to be further verified by following the swelling and release of MPs individually, as shown in Figure 75 a and b for the bulk fluid and the agarose setups, respectively.

a)


Drug release, %
Diameter, %
227 µm
212 µm
238 µm
220 µm
298 µm
230 µm
220 µm
251 µm
Time, d
Time, d

b)

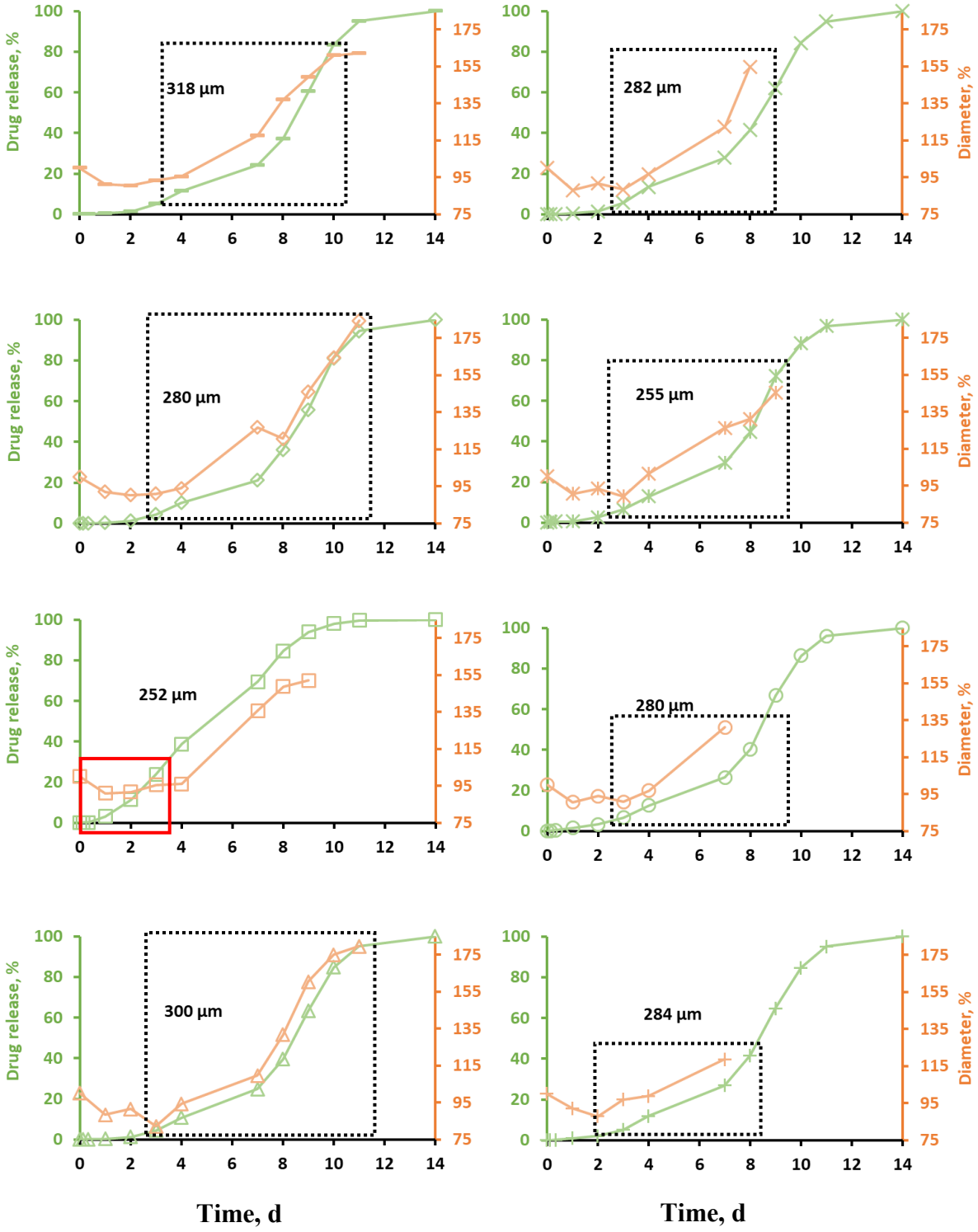


Figure 75. Individual release and swelling kinetics of the LAL-046 microparticles for the a) bulk fluid setup in PB, and the b) agarose gel setup

In the bulk fluid setup (Figure 75 a), it can be seen that the drug release started before the onset of the substantial MP swelling. This might be explained by the fact that a significant amount of water was able to rapidly penetrate the system through the pores, thanks to direct surface access to the porous network. The dissolution and then diffusion of ibuprofen crystals are thus rapid through the water-filled channels. However, in some cases, this water penetration through the porous network is not sufficient to lead to complete drug exhaust. Indeed, if the pores are less well connected to each other in some MPs, water cannot diffuse through the entire system. The drug located in these inaccessible regions will only be released when the swelling becomes more important, and the mobility of the polymer chains increases, allowing the drug to diffuse into the release medium. In these cases, a sort of plateau in the release kinetics (highlighted by the dotted black ovals in Figure 75 a) can be observed, and then the release continues at a higher rate once a certain MP swelling has been reached. These differences in release behaviors in this experimental setup might be explained by the high variability of the MP porous networks.

These behaviors also correlate with the highly porous network of this batch, as Figure 76 clearly shows. This was probably one of the MPs of the batch with well-connected pores, leading to a rapid, complete drug exhaust.

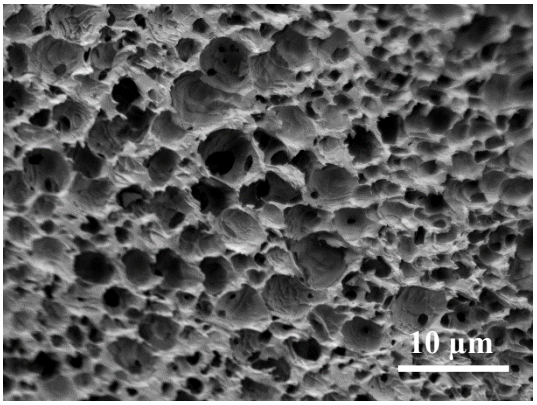


Figure 76. SEM picture of LAL-046 cross-section before exposure to the release medium

This highly porous network might also explain the behavior of the two MPs that show an early important swelling in the bulk fluid setup (MPs corresponding to the blue horizontal dashes and open circles in Figure 75 a. Their drug release began almost immediately after exposure to the release medium, thanks to probable direct surface access and interconnected pores, leading to rapid water penetration and then drug release. Consequently, the important amount of water present in the MP caused a faster hydrolytic cleavage of the PLGA ester bonds in these 2 MPs, leading to a decrease in the polymer molecular weight and resulting in a faster

system swelling. Hence, for these 2 MPs, swelling was not the main reason for the ibuprofen release, but in contrast, the early and complete drug release accelerated the swelling process of the MPs.

Compared to the porosity of batch LAL-030 (Figure 71), the porosity of batch LAL-046 (Figure 76) is much higher. The latter being more loaded with ibuprofen, it contains less PLGA, which makes the MP matrix less dense and therefore more porous. This will result in improved water penetration and thereby facilitate the diffusion of ibuprofen even faster.

Concerning MPs embedded in the gel (Figure 75 b), the swelling seems to be more controlled, with an onset at about 4 d exposure. Interestingly, this period coincided with the substantial release onset, except for 1 MP (highlighted by the red rectangle), for which the release started before the substantial swelling of the MP. As mentioned before, this might be due to the fact that this MP in particular had a well-connected, highly porous network with direct surface access, leading to rapid water penetration and thus drug release. It can also be assumed that this MP was hollow and broke under the effect of the stress exerted by the surrounding gel, leading to a rapid release of ibuprofen.

Moreover, upon exposure to well-agitated PB, access to the continuous pore network is facilitated compared to agarose gel. Indeed, the mechanical stress undergone by the thin layer of PLGA at the surface of the MP is higher in the case of well-agitated bulk fluid, because of the convective liquid flow around it. In contrast, MPs embedded in the middle of the agarose are not subject to this mechanical stress because they are isolated from the convective PB flow above the gel. SEM pictures of ibuprofen-loaded MPs' surfaces, which has been exposed to agarose gel or well-agitated PB for 1 d, seem to confirm this hypothesis: As it can be seen in Figure 77, numerous tiny pores are visible at the surface of a MP, which had been exposed to well-agitated bulk fluid, whereas less evidence for surface pores is apparent upon exposure to an agarose gel. Please note that the MPs were freeze-dried before analysis, which led to artifacts such as the shrinkage of the swollen polymer surface layers.

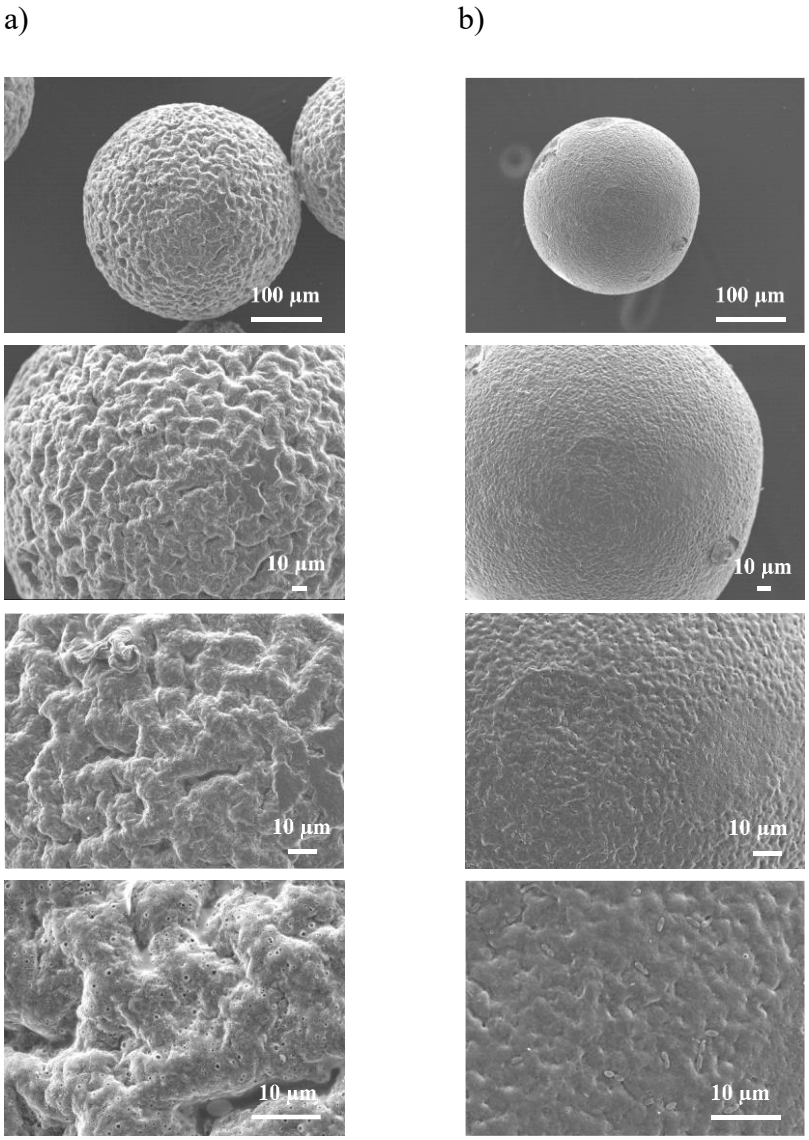
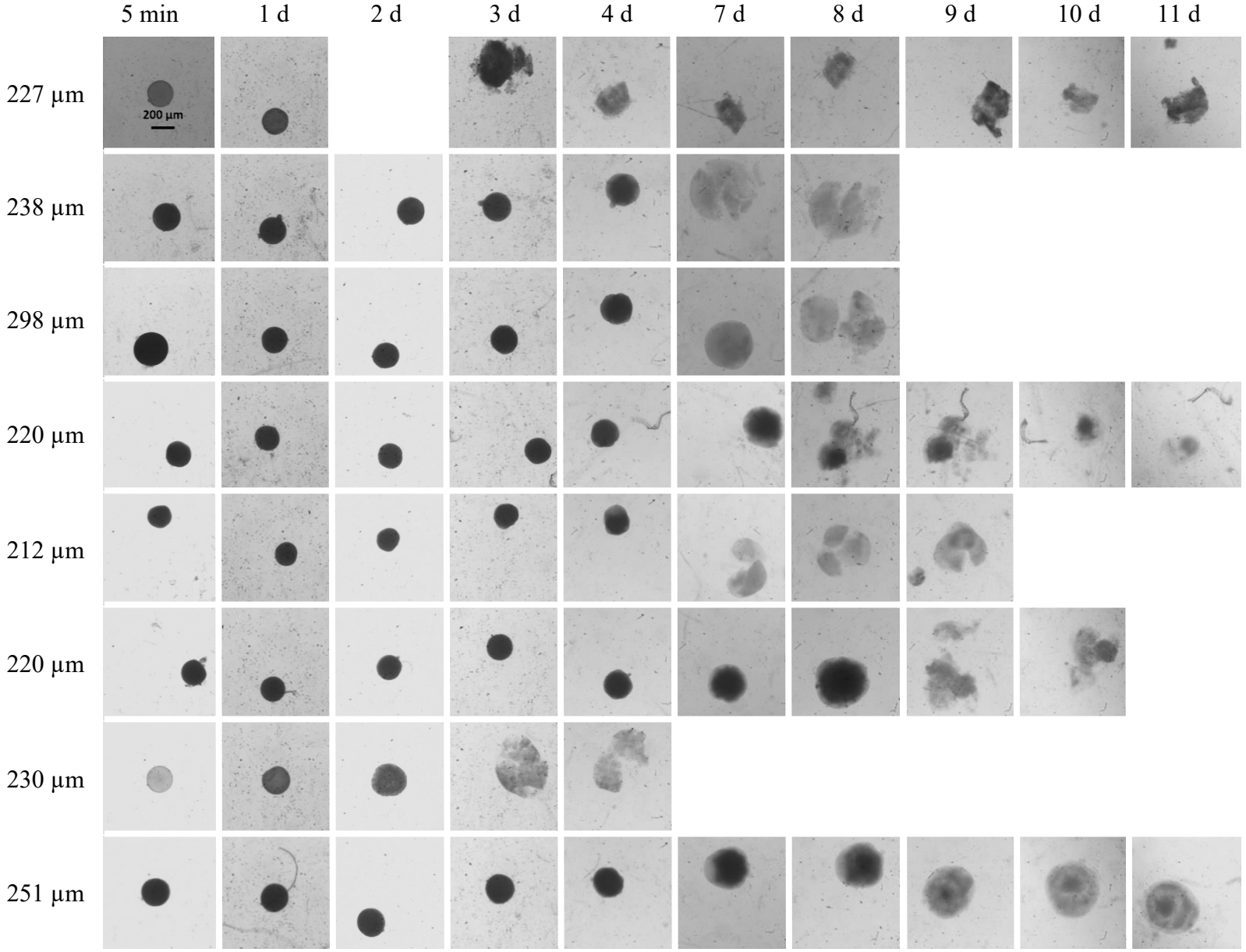


Figure 77. SEM pictures of surfaces of ibuprofen-loaded LAL-046 microparticles after 1 d exposure to well-agitated a) bulk fluid or b) agarose gel.

The hypothesis of MPs in well-agitated bulk fluid undergoing more mechanical stress leading to a faster release is also confirmed with the microscopic pictures of MPs taken during the release in order to monitor their diameter, but also their shape and physical appearance. The pictures of MPs studied in well-agitated bulk fluid and in agarose gel are presented in Figure 78 a and b respectively. *Please note that the little dots are dust particles on the lens of the microscope.*

a)


5 min
1 d
2 d
3 d
4 d
7 d
8 d
9 d
10 d
11 d
227 µm
238 µm
298 µm
220 µm
212 µm
220 µm
230 µm
251 µm
200 µm

b)

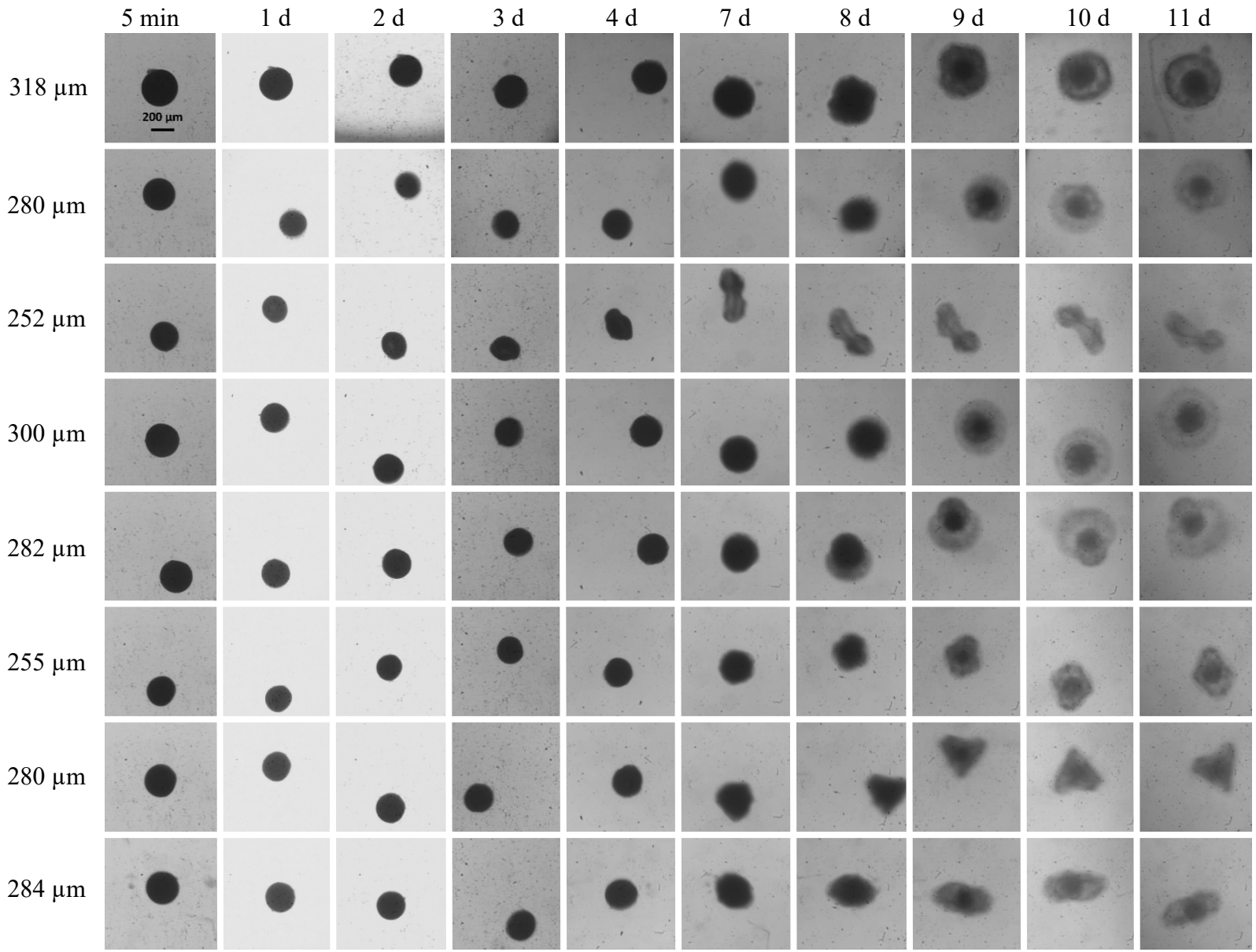


Figure 78. Optical microscopy pictures of ibuprofen-loaded LAL-046 MPs upon exposure to: a) well-agitated phosphate buffer pH 7.4, b) agarose gel. The exposure times are indicated at the top, the initial MP diameters on the left-hand side.

Thanks to this microscopic monitoring during release, it can be determined, as mentioned above, not only when the MP starts to swell but also whether it breaks. This is the case for the majority of MPs present in the bulk fluid, which eventually break into several pieces, whereas the MPs trapped in the agarose all remain in one piece. This clearly shows that mechanical stress has had a negative effect on the MPs in the bulk fluid and made them break, whereas it had no impact on the MPs in the agarose. It might be specified that when the MPs started to break, their release was already complete, which means that the breaking of the MPs did not affect the release.

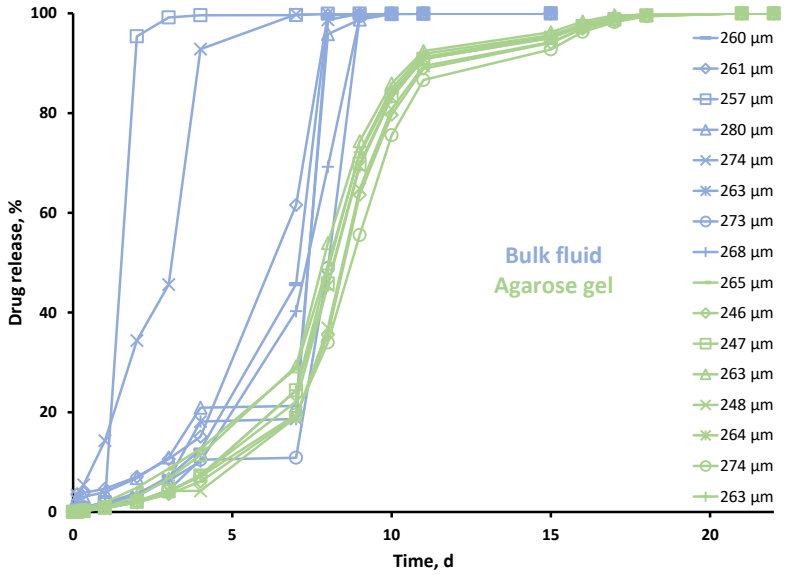
By hindering swelling, agarose highlighted its role in the release process of ibuprofen. Following these results, it has been proven that PLGA swelling is a more important release mechanism than has been described in the literature to date. It also proved that for MPs of the same batch, depending on the type of setup used, there could be differences in release, and that therefore the release setup is an important element to consider before doing *in vitro* release studies: It must be as close as possible to the physiological conditions in which the MPs will be found, in order to obtain the most reliable results.

So far, these differences in the behavior of MPs in release studies as a function of the *in vitro* release setup have been demonstrated for MPs produced in a standard way. However, as mentioned previously, the preparation method has a significant impact on the characteristics of the MPs. The same comparative study of setups will be carried out now, but this time, the focus will be on ibuprofen MPs produced in microfluidics.

II.2.2. Release and swelling kinetics of microfluidic ibuprofen microparticles

The batch studied now is a batch loaded with 23.5% of ibuprofen, produced with a microfluidics device (as described in the methods, *Section II.1.2.*). The swelling and release kinetics obtained for this batch are shown in Figure 79 a and b, respectively.

a)



b)

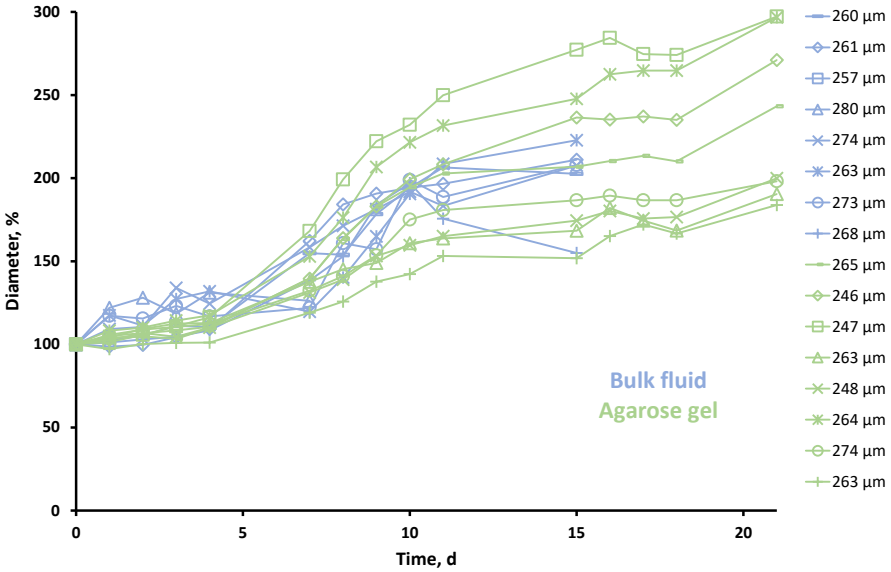
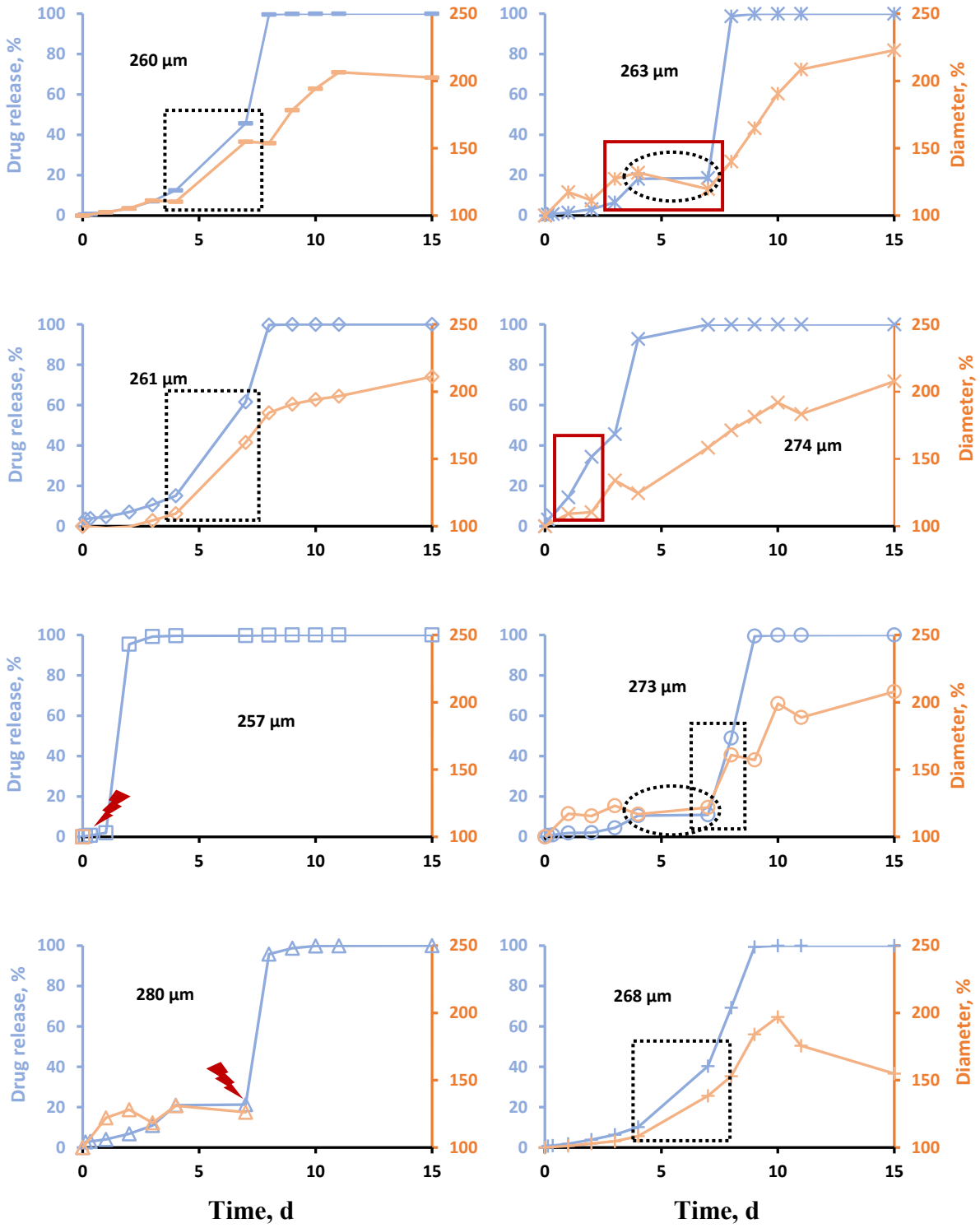


Figure 79. Ibuprofen single a) release and b) swelling behavior of the LAL-042 microparticles upon exposure to phosphate buffer pH 7.4 for the two different setups

As might be expected, the release in bulk fluid is also faster than in agarose, but the difference is maybe a bit less pronounced than for the previous standard batches. Besides, the difference in swelling kinetics is also less pronounced between the two setups than for the microparticles produced by the standard method. This again demonstrates that if the swelling is not delayed by the agarose, the release will not be delayed with respect to the bulk fluid either.

On Figure 80, individual swelling and release kinetics are represented. Please note that the red lightning sign is added when the MP is broken and its diameter can no longer be tracked.

a)


260 µm
263 µm
261 µm
274 µm
257 µm
273 µm
280 µm
268 µm
Drug release, %
Diameter, %
Time, d
0
5
10
15
0
20
40
60
80
100
100
150
200
250

b)

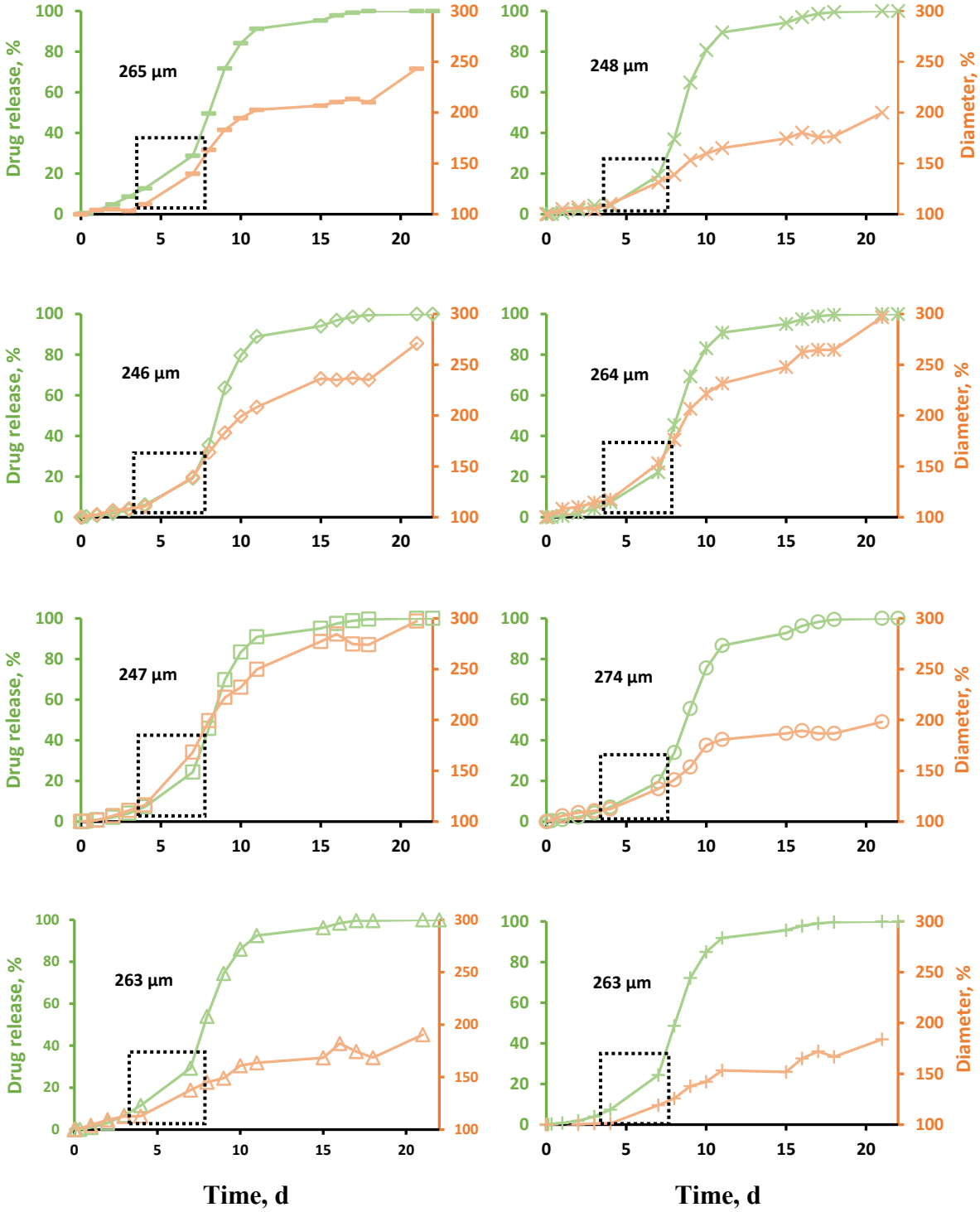


Figure 80. Individual release and swelling kinetics of the LAL-042 microparticles for the a) bulk fluid setup in PB, and the b) agarose gel setup

Figure 80 illustrates how strong the convective force undergone by the MP can be, damaging the MPs to such an extent that two of them broke in the first few days of release. This breaking of the MPs leads to a rapid complete drug release, because all the pieces of the MPs are exposed to the release medium, and when the surface contact is increased, the release is faster. The plateau phenomenon described previously also appears for 2 MPs in the bulk fluid (identified with dotted ovals in Figure 80 a), and shows that in that batch, different porous structures can be found as well, with more or less connected pores.

Again, in the agarose setup (Figure 80 b), the swelling and release kinetics follow each other perfectly. However, in that case, the swelling was in general more important in the agarose than in the bulk fluid setup. Indeed, the diameters of bulk fluid MPs had at most doubled when the diameters of MPs embedded in agarose tripled for three of them. It was previously seen that the gel should normally create a steric gene, which limits swelling. It should be noted that the release studies in agarose were performed for longer, in order to be sure that the releases, which are slower, are over. They are then followed for three weeks instead of two. This gives the MPs more time to swell until the end, also because the MPs embedded in the gel didn't break thanks to the protection from the agitated PB on its top. In fact, until 15 days, the MPs had at most doubled in diameter, as in the bulk fluid setup.

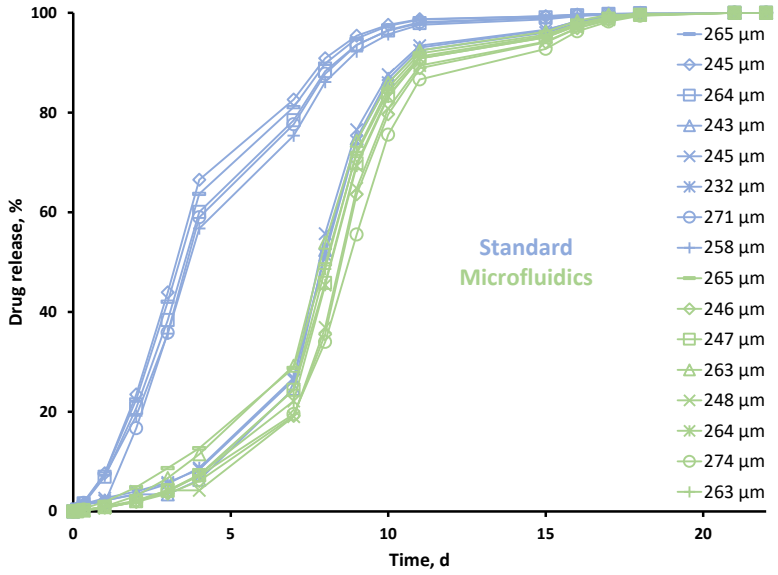
Therefore, it has been shown that irrespective of the preparation method of MPs (standard or microfluidics), swelling is a mechanism that plays a major role in the release of ibuprofen from PLGA-based MPs.

As shown in Chapter IV, for the same DL, the release in the bulk fluid of MPs produced in microfluidics is quite different from that of MPs produced in a standard way. The trend tended rather towards a faster release of MPs produced *via* the beaker method. The purpose of the next study will be to determine if this same trend is also verified when standard and microfluidic MPs are compared in the agarose gel setup.

II.2.3. Release and swelling kinetics of standard and microfluidic ibuprofen microparticles in agarose

The two batches compared in the following graphs are loaded with both 14% of ibuprofen. Figure 81 a and b represent the release and swelling kinetics of MPs in this batch, respectively, obtained in the agarose gel setup.

a)



b)

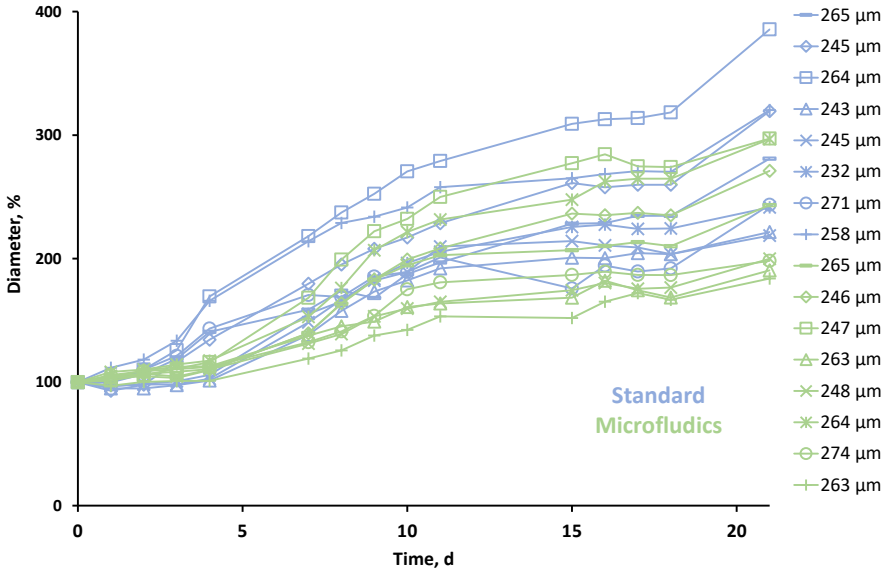


Figure 81. Ibuprofen single a) release and b) swelling behavior of the LAL-031 and LAL-042 microparticles, upon exposure to phosphate buffer pH 7.4 for the agarose gel setup

As it can be seen in Figure 81 a, the release in agarose was faster for the majority of the MPs produced in a standard way, as was the trend observed with the bulk fluid setup (Figure 47). This difference is even more clear when comparing the averages of the MPs, as shown in Figure 82.

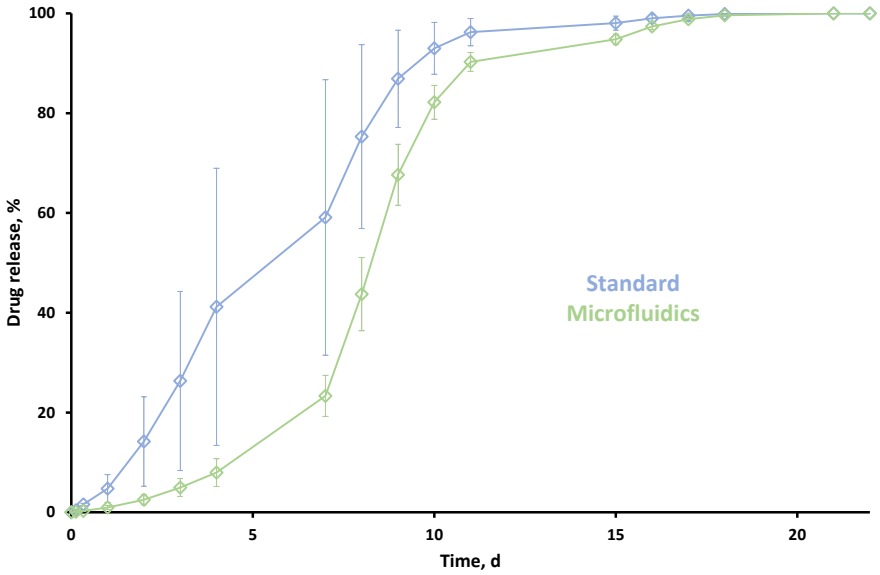


Figure 82. Averages of single releases of the LAL-031 and LAL-042 microparticles upon exposure to phosphate buffer pH 7.4 for the agarose gel setup

Interestingly, detailing the swelling kinetics, some MPs are swelling faster than the others, e.g. after only 3 days, against around 5 days for all the other MPs. These MPs, belonging to the group of MPs produced with the standard method, are also the ones releasing faster. However, some standard MPs are following the exact same release profile as the microfluidic MPs. This is easily explained by the fact that they swell at the same time as the MPs produced in microfluidics. Nevertheless, it is more difficult to find an explanation why some MPs are swelling differently from the others, and why in the agarose gel setup, the standard MPs are releasing faster than the microfluidics. Possible explanations of this phenomenon could be that within the same batch, some MPs are more porous than the others, or the pores are more connected between them, allowing for better water penetration and then faster swelling, leading to a faster drug release. One of the other hypotheses, which was suggested in Chapter IV, was that for MPs produced using the beaker method, the distribution of ibuprofen was not homogeneous within the MPs and was preferentially located on the surface. This distribution could have induced rapid release due to easier accessibility. On the other hand, for MPs produced using microfluidics, due to a more homogeneous distribution, ibuprofen present in the center of the MPs would take longer to be released. The other hypothesis was that the autocatalytic was more important in the beaker-produced MPs, due to a weaker elimination through the pores of the acidic degradation products. As MPs have unique intrinsic characteristics that are difficult to study with non-destructive techniques, there is variability in selecting MPs with a specific characteristic before performing the release studies.

Independently from the way MPs were produced, the type of setup used has a strong influence on the release of ibuprofen. In chapter V, the impact of the type of drug on the drug release for the bulk fluid setup was described. After having seen that the type of release setup is impacting the ibuprofen release, its impact on lidocaine MPs has to be investigated.

II.2.4. Release and swelling kinetics of standard microparticles of lidocaine

The MPs studied in this part are loaded with 8.5% lidocaine. Its release and swelling kinetics are reported in Figure 83 a and b, respectively.

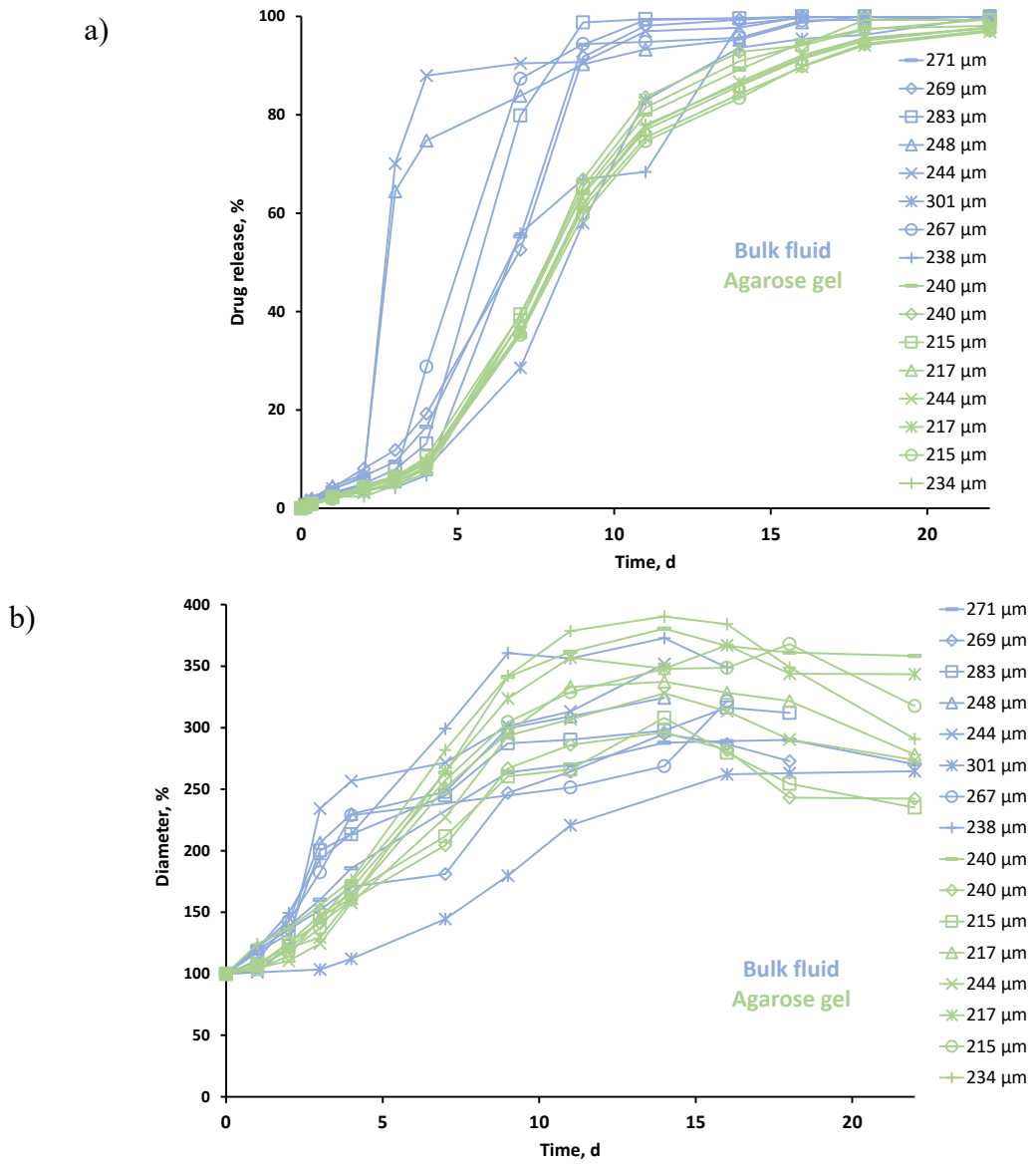
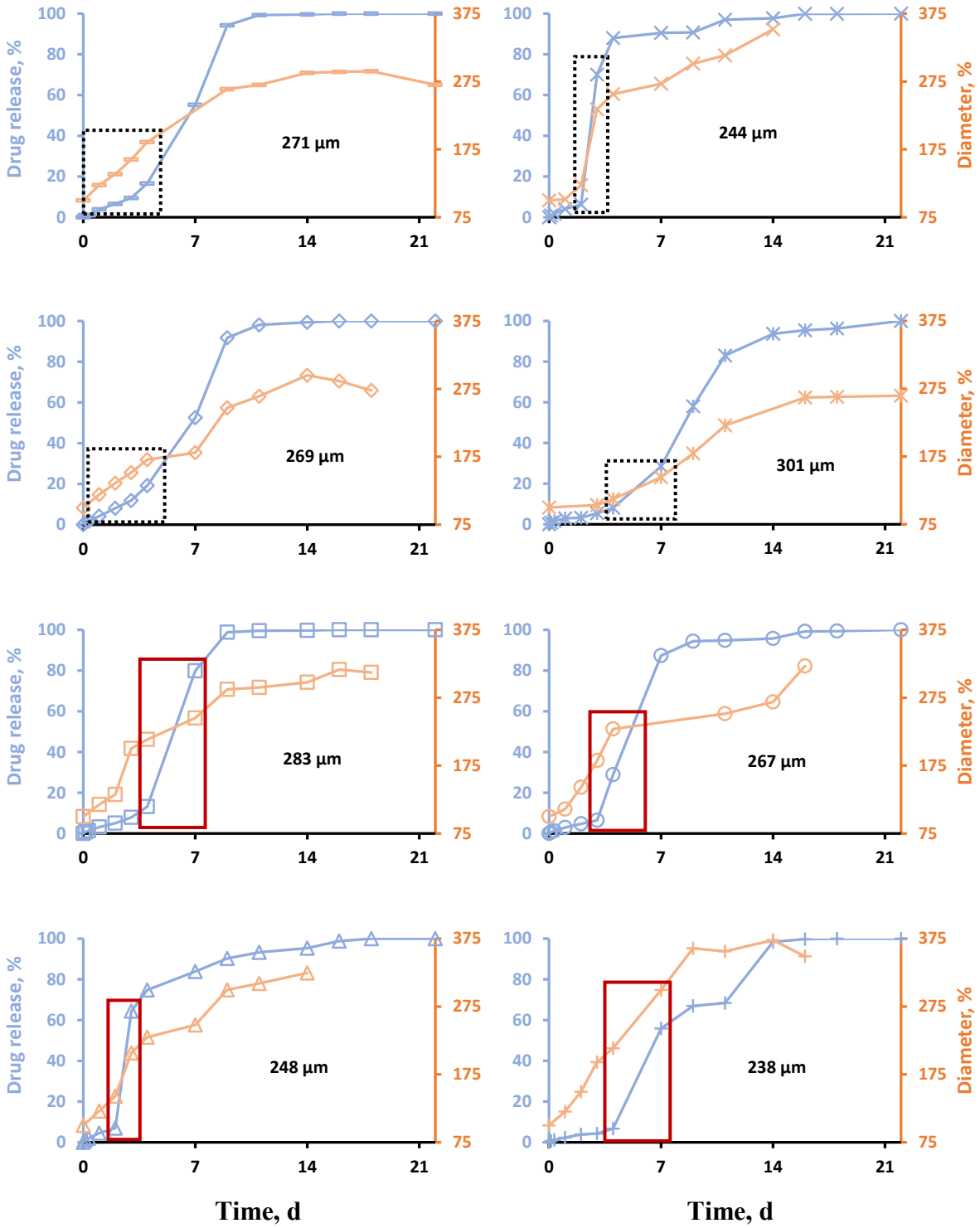


Figure 83. Lidocaine single a) release and b) swelling behavior of the LAL-086 microparticles upon exposure to phosphate buffer pH 7.4 for the two different setups

As expected, lidocaine is also released less quickly from the MPs when the latter are in the agarose setup than in the bulk fluid. Indeed, in that case as well, the swelling is delayed by the gel, as can be seen in Figure 83 b. However, one MP of the bulk fluid (initial diameter of 301 µm) took more than 4 days to swell, whereas the other MPs swelled from the first day of exposure to the bulk fluid. This MP swelled even less and less quickly than the MPs in the gel. This might be explained by the fact that this MP was the biggest of the investigated MPs. In that case, the diffusion paths are longer, so water will take longer to penetrate the whole MP. Thus, the swelling onset will appear later, and the release will be slower because lidocaine has to be transported over a longer distance before reaching the release medium. By looking back at the release kinetics in Figure 83 a, it can be noticed that this same MP is also the slowest to release. Once again, it has been proven that swelling is closely linked to the release of APIs from PLGA-based MPs.

On Figure 84 a and b, individual swelling and release kinetics of MPs in the bulk fluid or the agarose gel setup are respectively represented.

a)


Drug release, %
Diameter, %
271 µm
244 µm
269 µm
301 µm
283 µm
267 µm
248 µm
238 µm
Time, d
Time, d

b)

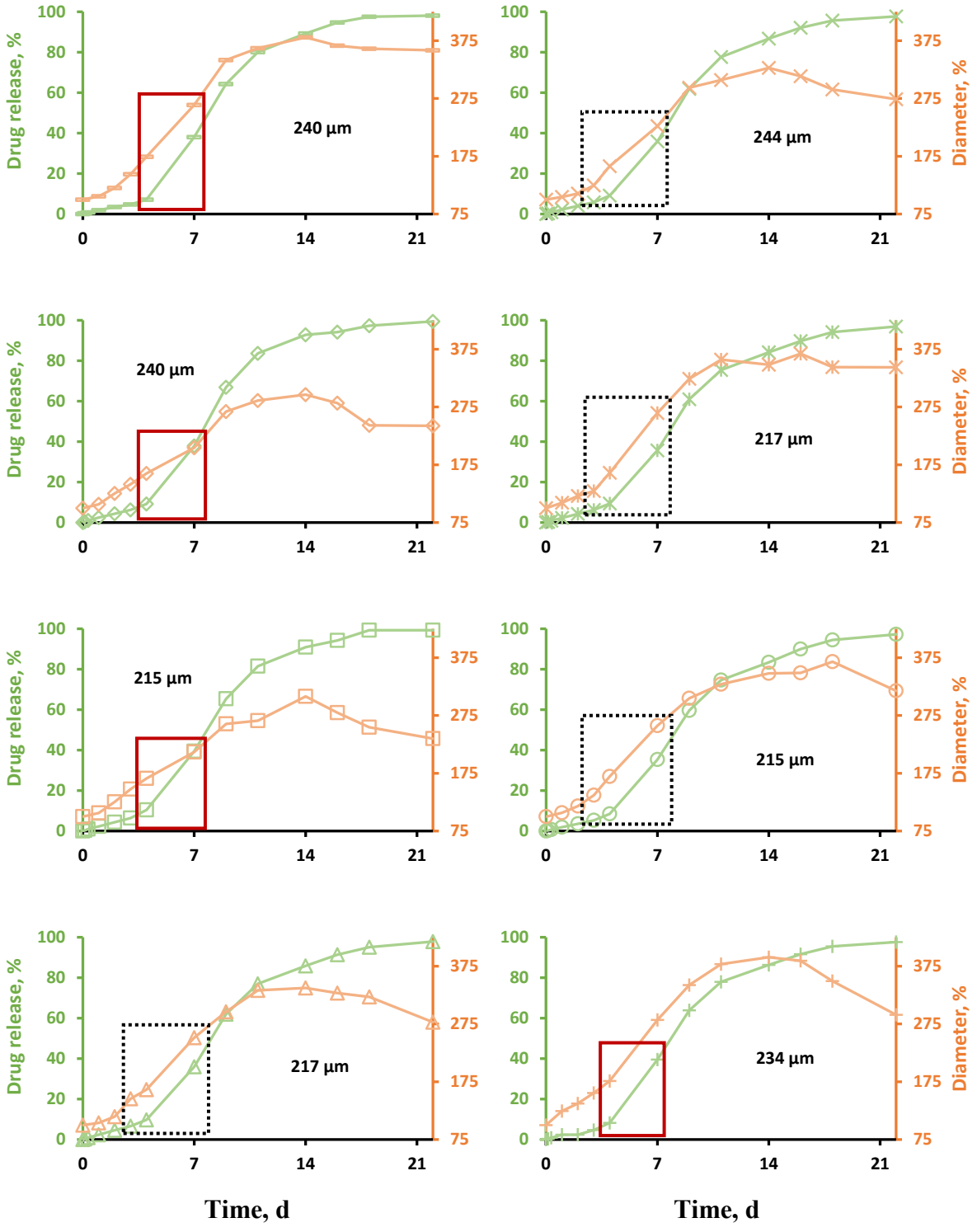


Figure 84. Individual release and swelling kinetics of the LAL-086 microparticles for the a) bulk fluid setup in PB, and the b) agarose gel setup

In Chapter V, it was shown that, contrary to ibuprofen, swelling of lidocaine MPs occurred always before the release, while the releases of ibuprofen often occurred before the substantial swelling. It is interesting to see that the same phenomenon occurs even in agarose.

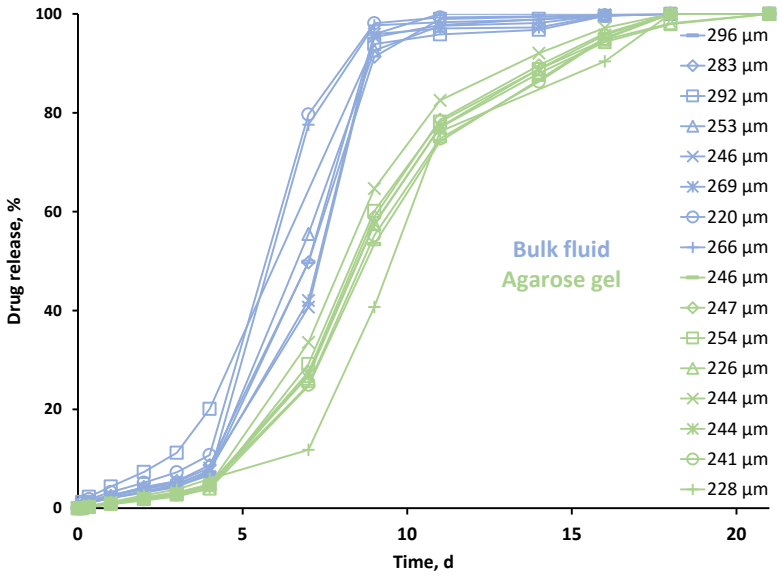
This could be explained by the fact that lidocaine has a more important plasticizing effect on PLGA, as demonstrated in Chapter V, Figure 57. Lidocaine has thus a more important capacity for disentangling the PLGA chains, leading to easier water penetration in the matrix by these less tightly linked chains, and hence rapid swelling. Moreover, the solubility of lidocaine being less important than ibuprofen in PB means that even if the swelling is rapid, it will take more time for lidocaine to be solubilized in the PB and then quantified as released.

As previously done for ibuprofen, an investigation will now be conducted to ascertain whether the release behavior of lidocaine MPs is similarly influenced by the preparation method when exposed to the bulk fluid or the agarose gel setup.

II.2.5. Release and swelling kinetics of microfluidics lidocaine microparticles

The following investigated MPs are produced *via* microfluidics and are loaded with 7% lidocaine. Its release and swelling kinetics are reported in Figure 85 a and b, respectively.

a)



b)

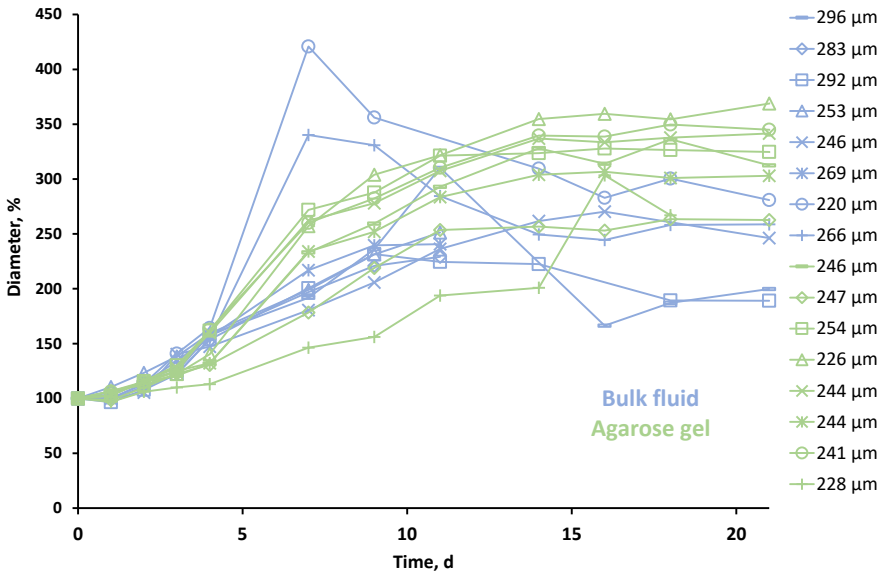
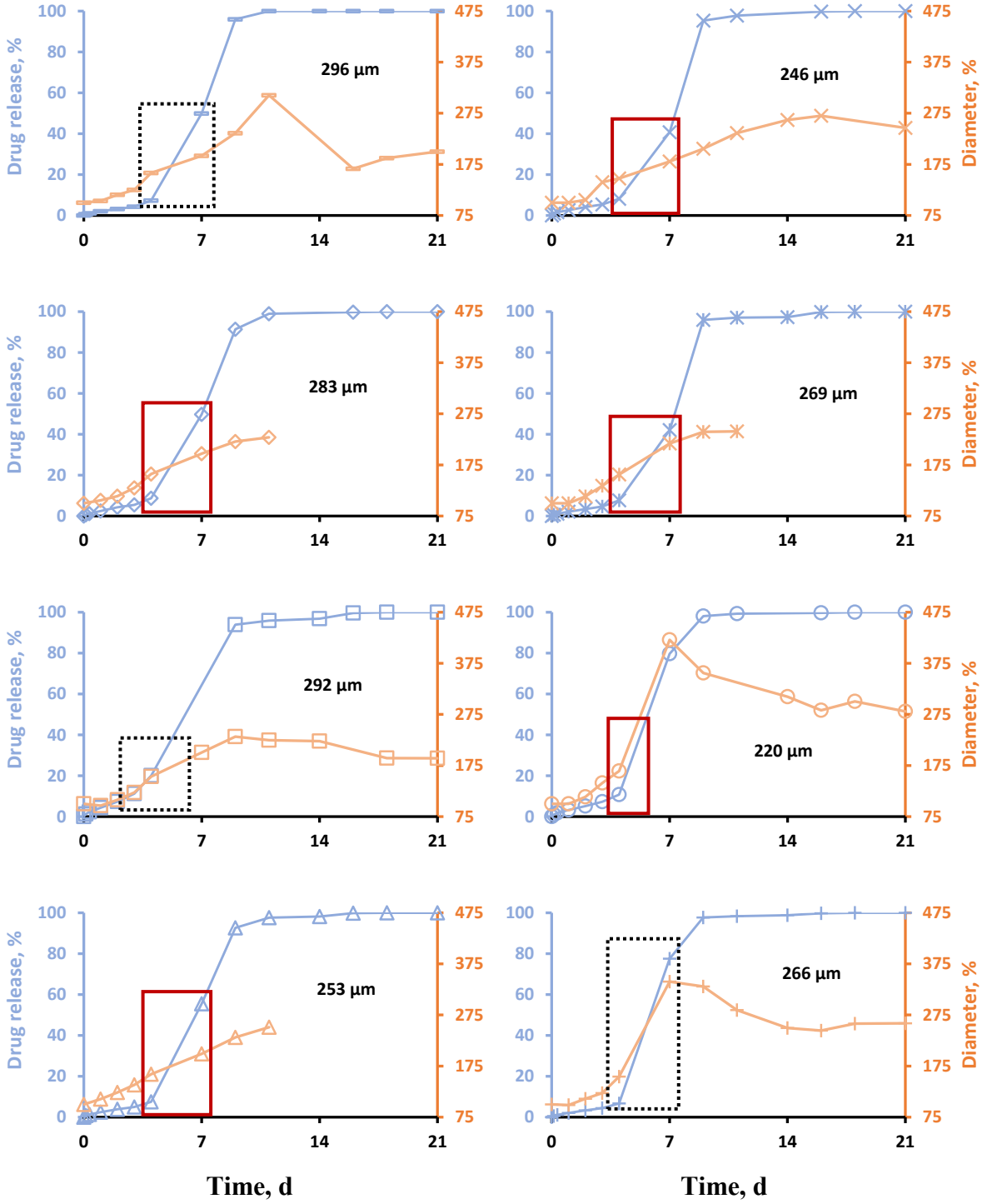


Figure 85. Lidocaine single a) release and b) swelling behavior of the LAL-074 microparticles upon exposure to phosphate buffer pH 7.4 for the two different setups

Figure 85 a shows that the releases are homogeneous in both setups, while they are heterogeneous for standard lidocaine in bulk fluid (Figure 83 a). Microfluidics appears to be a preparation method that allows the production of more homogeneous MPs within the same batch, leading to less release behavior variability in that case. Interestingly, the swelling doesn't seem to be impacted by the presence of the gel around MPs; The onset of the substantial swelling starts after 4 days of exposure in both setups. One MP behaves differently from the others in agarose (initial diameter of 228 µm): It swells later and releases later as well, supporting the idea that swelling is largely responsible for the release of APIs. Furthermore, the DL of these MPs is similar to the DL of the previous batch studied (7-8.5% for LAL-074 and LAL-086, respectively), and the release duration is the same: About 8 days in bulk fluid and 17 days in agarose.

On Figure 86 a and b, individual swelling and release kinetics of MPs in the bulk fluid or the agarose gel setup are respectively represented.

a)


Drug release, %
Diameter, %
296 µm
246 µm
283 µm
269 µm
292 µm
220 µm
253 µm
266 µm
Time, d
Time, d

b)

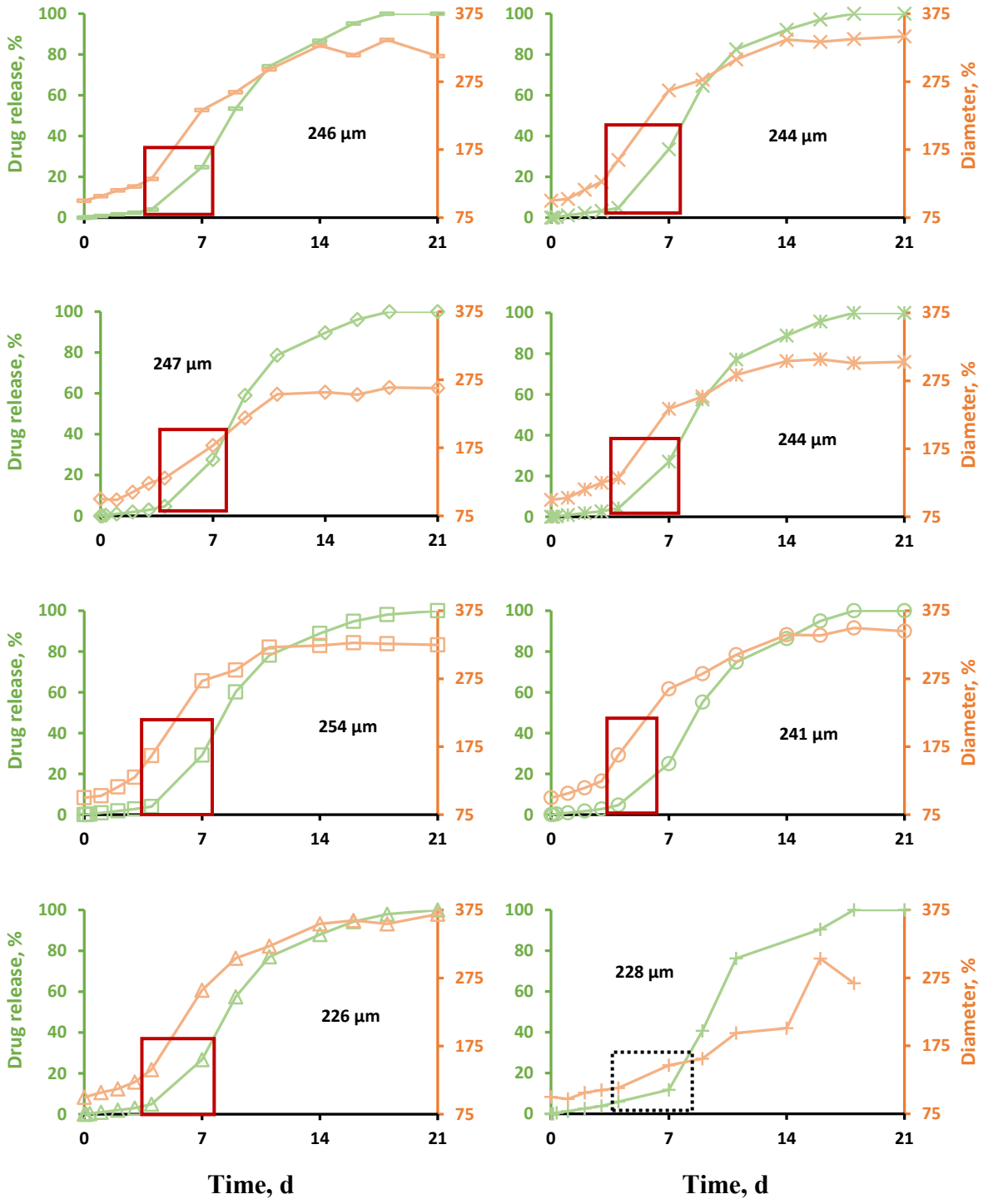


Figure 86. Individual release and swelling kinetics of the LAL-074 microparticles for the a) bulk fluid setup in PB, and the b) agarose gel setup

Lidocaine MPs produced in microfluidics also showed swelling prior to release in most of the cases, supporting here again the hypothesis that the important plasticizing effect of

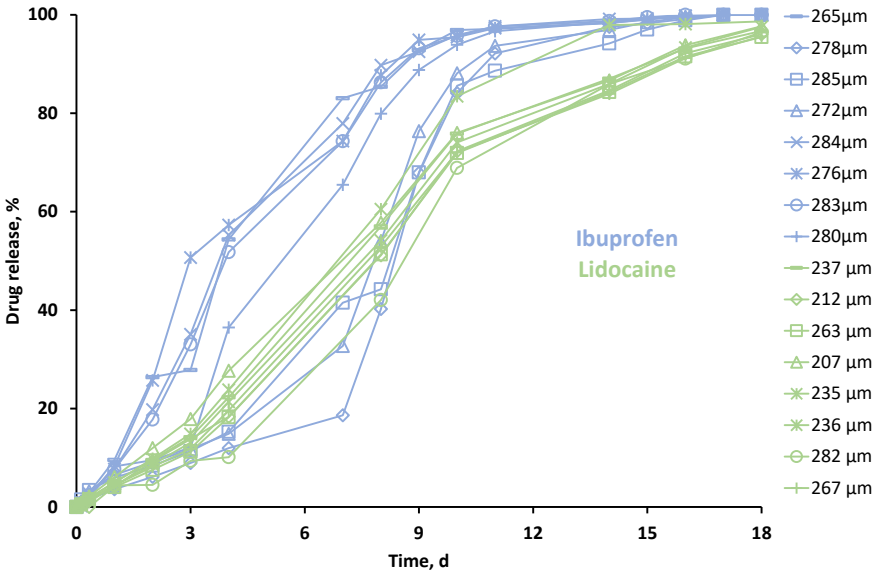
lidocaine disorganized the PLGA chains, allowing for fast water penetration and swelling before lidocaine has had time to solubilize in the release medium.

Having observed that the type of *in vitro* release setup has an impact on release, whether for ibuprofen or lidocaine, for MPs produced in the standard way or in microfluidics, the next step will involve assessing whether there is a difference in release in agarose between ibuprofen and lidocaine.

II.2.6. Release and swelling kinetics of standard ibuprofen and lidocaine microparticles in agarose

The following investigated MPs are loaded with 14% ibuprofen or lidocaine, their release and swelling kinetics in the agarose gel setup are compared, and represented in Figure 87 a and b, respectively.

a)



b)

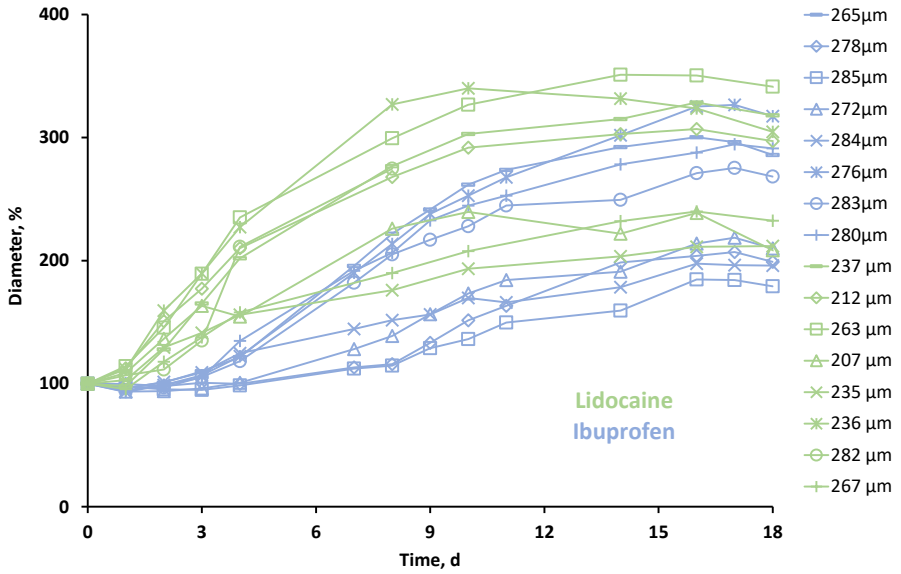


Figure 87. Ibuprofen and lidocaine single a) release and b) swelling behavior of the LAL-030 and LAL-087 microparticles respectively, upon exposure to phosphate buffer pH 7.4 for the agarose gel setup

In Chapter V, it was observed that for MPs loaded with the same amount of drug, with a similar initial diameter, the release of lidocaine was a bit slower compared to the release of ibuprofen in the bulk fluid setup. Unsurprisingly, in the agarose gel setup, the same observation can be made: Ibuprofen releases faster than lidocaine. It can be seen in Figure 87 a that the ibuprofen release is over after about two weeks, compared to three weeks for lidocaine. As in the bulk fluid setup, swelling is faster and more significant for lidocaine MPs (Figure 87b), despite the presence of the gel, which is supposed to hinder swelling.

Figure 88 represents the average release profile in the agarose gel of these investigated batches.

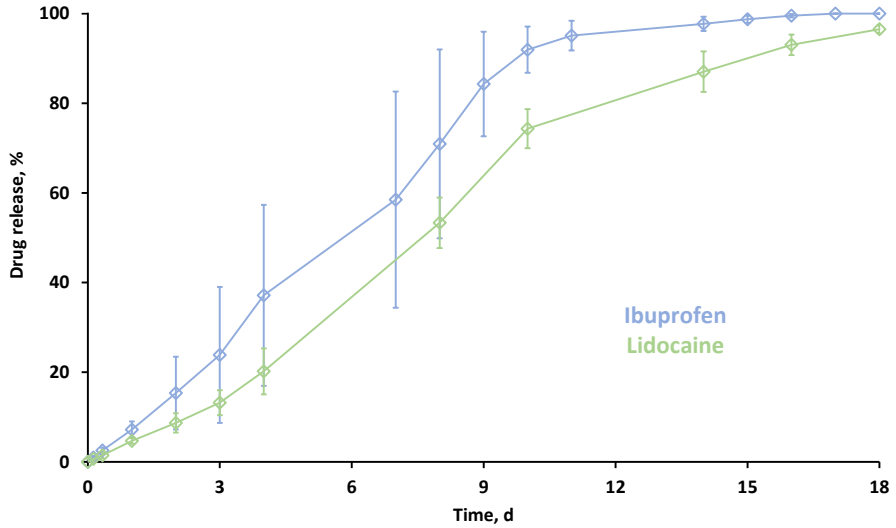


Figure 88. Averages of single releases of the LAL-030 and LAL-087 microparticles upon exposure to phosphate buffer pH 7.4 for the agarose gel setup

As it can be seen in Figure 88, this difference is not excessively pronounced, but it is important to remember that the solubility of ibuprofen in PB pH 7.4 is much lower than the solubility of lidocaine in this same medium (25 mg/L for ibuprofen against 5 g/L for lidocaine). As a result, if the release rates were similar for the 2 APIs, this greater solubility of lidocaine would support faster quantification in the release medium, but this is not the case. Therefore, if this solubilization rate in the medium is considered, the difference in terms of release would be even more marked between their release kinetics.

II.3. Extraction and release studies of loaded agarose gels

According to the previous results, swelling appears to be a predominant mechanism in the release of APIs from PLGA microparticles. However, before this hypothesis is confirmed, it needs to be ensured that the delay in the release of the drug is not simply due to the additional diffusion step of the drug into the agarose, prior to being quantified in the phosphate buffer. One of the reasons for the lower release rate observed when using the agarose gel setup is the following: The drug, which is released from the microparticles, is not immediately detected as "being released": It first has to diffuse through the hydrogel to get into the phosphate buffer, which is sampled and analyzed for its drug content. Thus, at least parts of the released drug remain "undetected". In fact, if the drug is released at the same rate in the "agarose gel setup" than in the "bulk fluid setup", and just requires more time before being quantified in the phosphate buffer due to the diffusion time through the gel, this indicates that swelling is not the

release mechanism responsible for the largest release phase of the API. On the other hand, if the diffusion time of the drug through the gel is negligible, then the hypothesis that swelling is a key release mechanism for PLGA microparticles can be confirmed.

To determine if the diffusion step of the drug is significant, it was decided to measure the amount of drug at a determined timepoint during a release. In order to do this, a release of batch LAL-046 was launched with the "agarose gel setup" and stopped on day 9. This was the day with the highest release rate of ibuprofen (highest slope in Figure 72). Since drug release was the fastest on this day, the amount of ibuprofen diffusing through the hydrogel can be expected to be higher. Samplings are dosed, each microparticle is removed from the gel and its content is dosed after dissolution in acetonitrile, and the agarose extraction protocol is applied (protocol described in the methods, *Section II.5.*). This day 9 corresponds both to the maximum release observed in the previous results, and therefore, to the maximum chance of dosing ibuprofen in the agarose, but it also corresponds to a day when the release is over in the bulk fluid setup, and therefore if there is no difference in release but just a delay in the measurements, no ibuprofen should be found in the remaining microparticle. The percentage of drug detected in the agarose gel at this sampling time point was 17.8 +/- 7.5 % (n = 8), referring to the total drug loading of the microparticle. This corresponds to an average of 1.4 µg of ibuprofen found in the gel, so this means that the diffusion of ibuprofen through the gel is not immediate, but can take some time. The average microparticles' content after 9 days was 2 µg of ibuprofen, so this means that the release was not over after 9 days as for the bulk fluid setup. In accordance with these results, the diffusion step has to be considered because it can be a part of the release's delay explanation, but it is not the only reason because the release was not complete after 9 days.

In order to monitor the diffusion time of ibuprofen through the layer of agarose, it has been decided to develop a different setup, already illustrated in the methods, *Section II.4.* Figure 24. This setup is composed of a gel loaded with approximately either 6 µg of ibuprofen, which corresponds to the bigger amount that can be released by a microparticle of a batch loaded with 47.5% ibuprofen (LAL-046) for a total weight of 13 µg (average of the individual microparticle masses of this batch), or 1.5 µg of ibuprofen, which corresponds to the amount recovered in the gel after extraction at day 9. This will allow us to determine how long it took for this quantity to diffuse into the gel and to be dosed into the samples. The results obtained are shown in Figure 89.

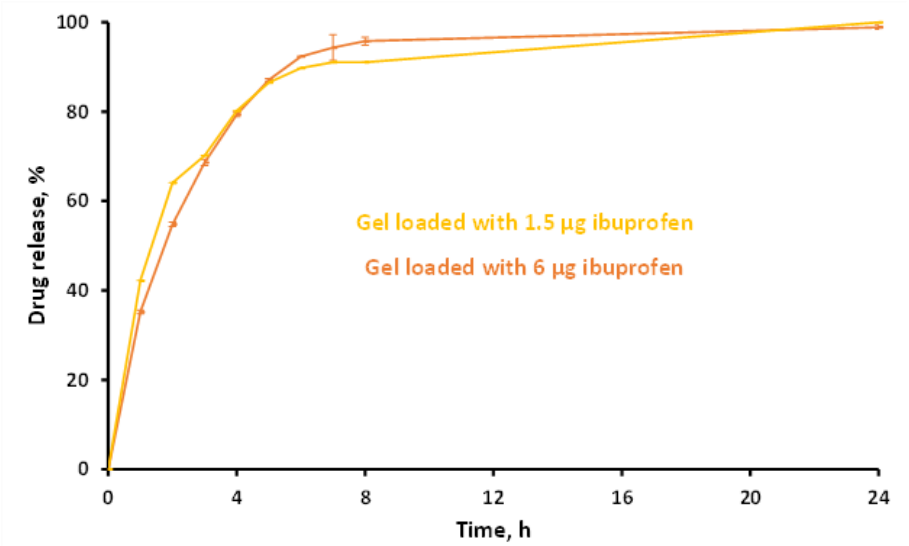


Figure 89. Ibuprofen release of differently loaded gel upon exposure to phosphate buffer pH 7.4

As it can be seen, the release kinetics were similar for both initial drug concentrations, and ibuprofen release was complete within about 8 h. This clearly indicates that the observed major differences in the release rates of ibuprofen from the investigated microparticles using the bulk fluid versus agarose gel setup can only, to a minor or moderate extent, be explained by the additional mass transport step through the hydrogel: The difference in drug release is in the order of several days, which compares to less than 8 hours for complete release from the hydrogel.

Moreover, the 1.4 µg of ibuprofen found after 9 days of release following extraction corresponds exactly to the average amount found in the samplings at day 10 for the release performed until the end, proving that the diffusion only takes a few hours.

The exact same protocol of release from loaded gels was applied to lidocaine. Gels containing 1.5 or 6 µg of lidocaine were exposed to PB pH 7.4, and the release rates were determined. The results are shown in Figure 90.

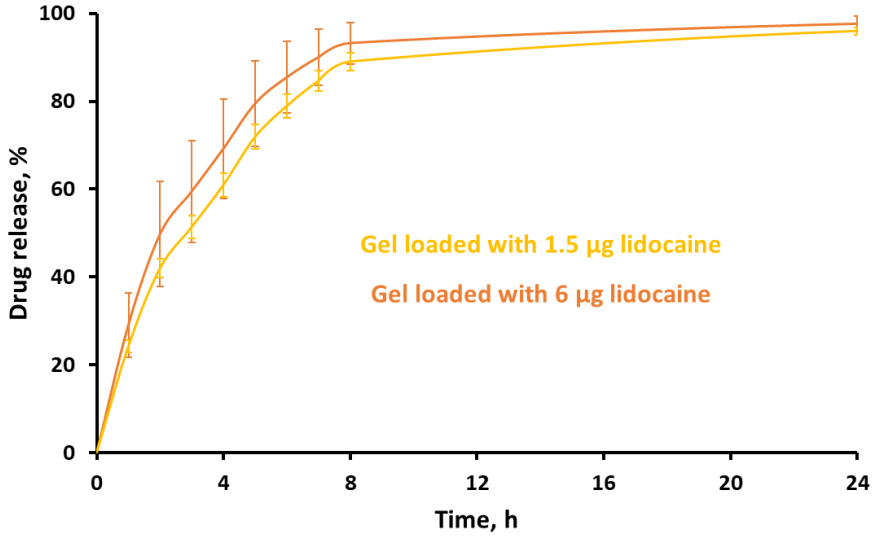


Figure 90. Lidocaine release of differently loaded gel upon exposure to phosphate buffer pH 7.4

With lidocaine, the same results as for ibuprofen were obtained: In 8 hours the plateau is reached, which confirms that the diffusion process of these two APIs through agarose is fast.

Thanks to these results, it can be stated that in a maximum of 1 day, all the drug present in the gel is recovered in the phosphate buffer, and as a consequence, the diffusion step through the gel is not the reason for the delay observed in release.

II.4. Solubility studies of lidocaine in PB and PBS pH 7.4

It was shown in Chapter V that the type of encapsulated drug has an impact on the PLGA release mechanisms: The release of lidocaine was slightly slower compared to ibuprofen, for the same drug loading. In order to more accurately determine whether the theory that lidocaine creates more ionic interactions with PLGA than ibuprofen, thus explaining why it takes longer to release, it was decided to perform a comparative release in a saline medium. In fact, salts will break the ionic bonds, and lidocaine, which will no longer be bound to PLGA, will release more rapidly.

To be sure that if there is a difference in release between the two media, it is not due to a lower solubility of lidocaine in one of the two media, a solubility study of lidocaine was

carried out in a phosphate buffer and a phosphate buffer saline, both pH 7.4. This will also allow us to confirm that the releases are indeed performed under sink or perfect sink conditions.

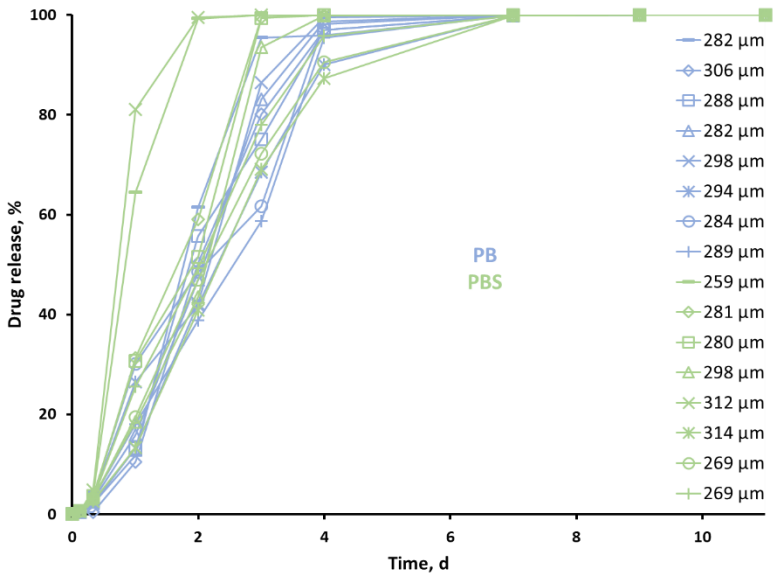
Moreover, as mentioned in the objectives of this work, release studies are usually conducted in phosphate buffered saline at pH 7.4, in order to simulate the *in vivo* environment, and thus have greater consistency between *in vitro* and *in vivo* results. By comparing these two media, it will be possible to determine whether they can impact the release behavior of MPs.

The results obtained after 21 days of solubility study are as follows: The solubility of lidocaine in PB pH 7.4 at 37°C is 5 g/L, while that in PBS pH 7.4 is 3.5 g/L. The solubility in PB is then much better than in PBS, but in both cases, release studies were conducted under perfect sink conditions, meaning that the solubility differences existing between the two media will not influence the release rate of lidocaine.

II.5. Comparative release studies of lidocaine MPs in PB and PBS pH 7.4

The release and swelling kinetics of LAL-117 MPs loaded with 21% lidocaine, either in PB pH 7.4 or in PBS pH 7.4 (bulk fluid setup), are presented in Figure 91 a and b, respectively.

a)



b)

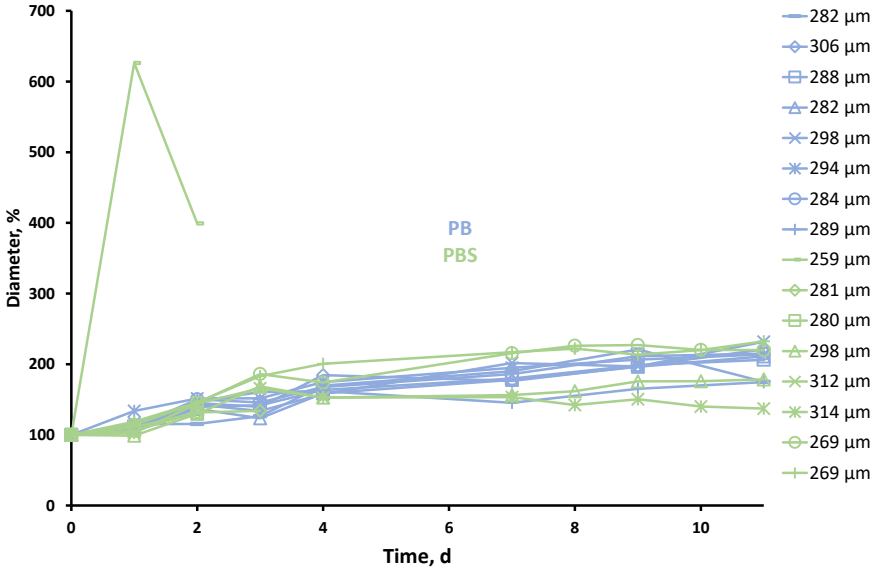


Figure 91. Lidocaine single a) release and b) swelling behavior of the LAL-117 microparticles, upon exposure to phosphate buffer or phosphate buffer saline pH 7.4 for the bulk fluid setup

The releases obtained in PB and PBS are very similar, as are their swelling kinetics. The two release kinetics, which are slightly faster than the others in PBS, correspond to a MP which was broken on the first day during handling (initial diameter of 312 µm), which explains why all the lidocaine was then released, and to a MP (initial diameter of 259 µm) which swelled considerably on the first day, and which therefore released more rapidly than the others. To get a better idea of the difference in release between the two media, these releases were averaged and are shown in Figure 92. The MP in PBS that was broken during handling was deliberately removed from the calculation of this average.

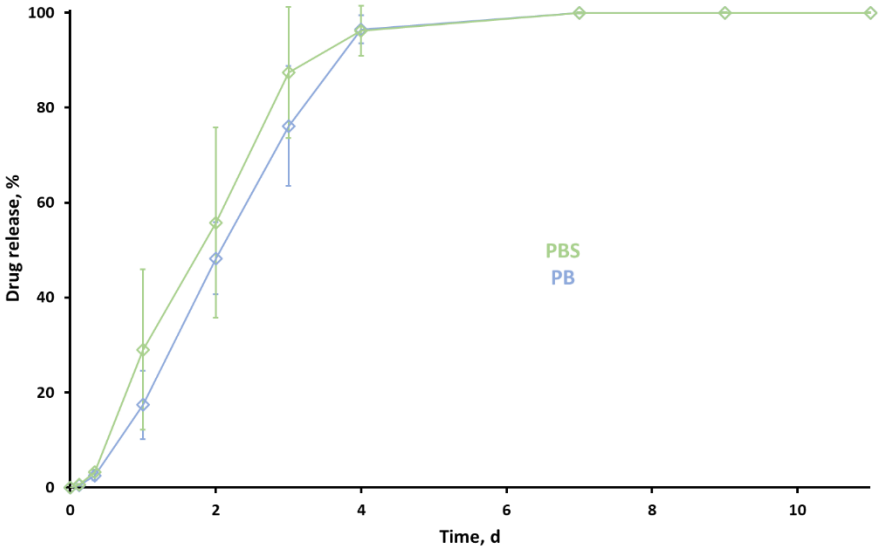


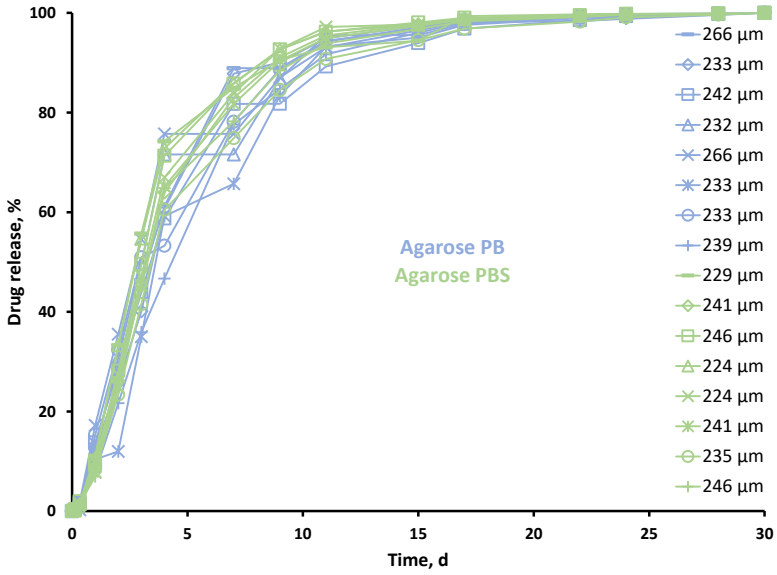
Figure 92. Averages of single releases of the LAL-117 microparticles upon exposure to phosphate buffer or phosphate buffer saline pH 7.4 for the bulk fluid setup

This type of curve makes it all the more clear that there is absolutely no impact of the release medium on the kinetics of the investigated lidocaine MPs. However, it is important to ensure that the same conclusions can be drawn in the agarose setup, which is the point of the following part.

II.6. Comparative release studies of lidocaine MPs in agarose upon exposure to PB and PBS pH 7.4

Release and swelling kinetics of the previously investigated batch (LAL-117, 21% lidocaine produced with the beaker method) in agarose gels prepared either with PB pH 7.4 or PBS pH 7.4 with PB and PBS as respective release media, are shown in Figure 93 a and b, respectively.

a)



b)

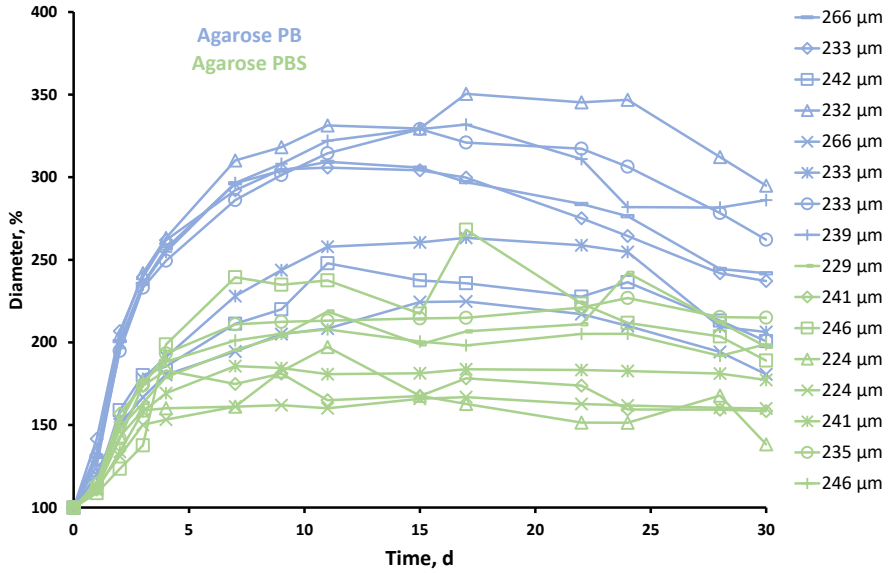


Figure 93. Lidocaine single a) release and b) swelling behavior of the LAL-117 microparticles, upon exposure to phosphate buffer or phosphate buffer saline pH 7.4 for the agarose gel setup

Firstly, it was observed, as previously for all other release studies, that MP releases embedded in agarose gel are slower than those present in bulk fluid. Whatever the medium (PB or PBS gel), release is delayed by about one week compared with bulk fluid.

Very clearly, it can be seen in Figure 93 a that there is no difference in release between MPs embedded in an agarose gel prepared from PB and those in a gel prepared from PBS. Curiously, the swelling kinetics do not follow the same trend. Indeed, there is an obvious difference between MPs in the gel made of PB, which presented a more important swelling than MPs in the gel made of PBS. Moreover, it can be noted that the maximum swelling of MPs in

PB or PBS in the bulk fluid setup was only two times the initial diameter, whereas it exceeded three times for MPs in the gel made of PB. However, it was previously mentioned that the agarose was supposed to limit swelling. This also contradicts previous findings that the faster a MP swells, the faster it will release: MPs in the gel prepared with PB do not release faster than MPs in the gel prepared with PBS. Following this, the hypothesis that emerged is that the preparation of the setup from two gel layers (one agarose layer cast into the well, MP placed in the center, then covered with another layer of agarose) could be the reason for a spreading of the swollen polymer at the interface of these two layers, instead of a homogeneous swelling all around the MP. A MP swelling homogeneously (in the bulk fluid setup), is represented in Figure 94 a, and a MP presenting a spread-out swelling (in the agarose gel) is illustrated in Figure 94 b.

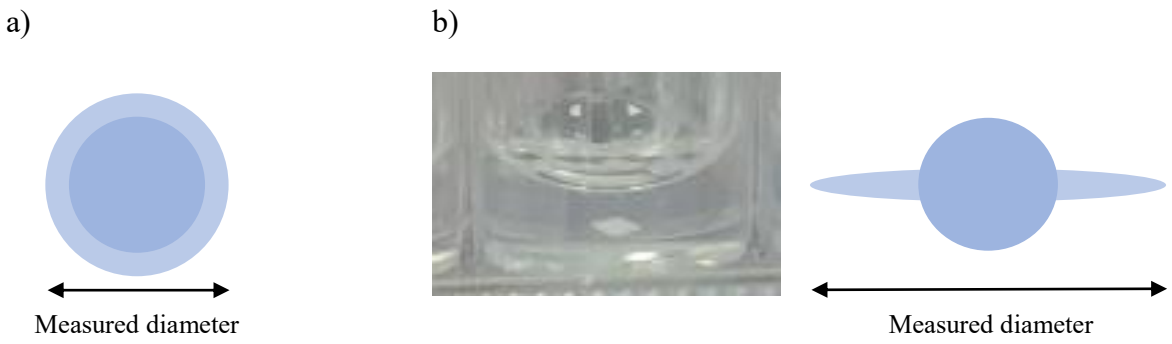


Figure 94. Swelling behaviors of MPs in a) bulk fluid or in b) agarose gel, upon exposure to PB pH 7.4

As described in this figure, the measured diameter of a MP present in the gel will be higher than the diameter of a MP in the bulk fluid setup, even if the PLGA swelled less in agarose. In addition to this, there was probably a difference in the agarose properties of the gel made of PB and the one made of PBS, because the swelling in the gel made of PBS was less important than in the gel made of PB (diameter was two times more important compared to the initial diameter instead of three for PB). The salts present in PBS probably modified the physical properties of the resulting gel because the phenomenon described in Figure 94 b was less pronounced. Indeed, the ions formed by the presence of salts can impact the cross-linking process during gel formation and strengthen intermolecular bonds, making the gel more resistant and further limiting swelling and then spreading between the two layers.

Thanks to this comparative study, it has been shown that there was no difference, in terms of release as well as swelling behaviors between lidocaine MPs in PB- or PBS-based media. Nevertheless, this conclusion is only valid for the drug studied here, lidocaine, at the

loading rate of 21%, for our type of drug delivery system which are microparticles. Another possible hypothesis that could be formulated is that the salts present in the PBS are not strong enough to break the ionic bonds between lidocaine and PLGA, explaining why the kinetics are the same in PB and PBS.

This type of study would need to be carried out at different lidocaine loading rates and also for other drugs. In fact, depending on the amount of drug released into the medium, the latter will not necessarily react in the same way, particularly in terms of the charge neutralization rate. A study from Faisant et al. (2006) [176] proved that, in function of the pH release medium and its osmolarity (amongst others), different drug release patterns, such as mono-, bi- and tri-phasic patterns, were observed in PLGA-based MPs.

III. Conclusion

Studying single microparticles instead of groups allowed us to better monitor the correlation between a specific characteristic of the microparticle and its release behavior. Thanks to this specific way of investigation, we were able to better assess the role of polymer swelling: Amongst the several mechanisms involved in the drug release from PLGA-based microparticles, the swelling of PLGA plays a major role. Furthermore, this type of study can help in the development of *in vitro* release setups that are more in accordance with *in vivo* environments, because despite the growing interest in the administration of PLGA-based drug delivery systems, there is still a lack of established regulatory guidelines for their *in vitro* studies. Nevertheless, *in vitro* release measurements should be viewed with great care because the underlying drug release mechanisms are complex and can be significantly influenced by experimental conditions.

III. Conclusion

L'étude de microparticules individuelles au lieu de groupes nous permet de mieux contrôler la corrélation entre une caractéristique spécifique de la microparticule et son comportement de libération. Grâce à cette méthode d'investigation spécifique, nous avons pu mieux évaluer le rôle du gonflement du polymère : parmi les différents mécanismes impliqués dans la libération de la substance active à partir de microparticules à base de PLGA, le gonflement du PLGA joue un rôle majeur. Par ailleurs, ce type d'étude peut contribuer au développement de setups de libération *in vitro* plus conformes aux conditions que l'on peut retrouver *in vivo*, car malgré l'intérêt croissant pour l'administration de systèmes de libération à base de PLGA, il n'existe toujours pas de lignes directrices réglementaires établies pour leurs études *in vitro*. Néanmoins, les mesures de libération *in vitro* doivent être considérées avec une grande prudence car les mécanismes sous-jacents de libération des substances actives sont complexes et peuvent être influencés de manière significative par les conditions expérimentales.

GENERAL CONCLUSION AND PERSPECTIVES

Our findings revealed that the initial drug physical state within the MPs does not impact release kinetics, as the amorphous drug becomes crystalline in contact with the release medium. Consequently, the observed transformation over time from amorphous to crystalline drug in the MPs has no bearing on the release process, suggesting the long-term stability of MPs when stored in appropriate conditions for several years.

The preparation method of MPs undeniably influences their physicochemical characteristics. Factors relating to each method, such as the concentration of the organic phase, the mechanism of droplet formation, and the precipitation process, exert a notable influence on the porosity and uniformity of the MPs concerning the size and distribution of the active ingredient. This, in turn, significantly affects the release kinetics.

Comparative analysis between ibuprofen and lidocaine MPs reveals that lidocaine MPs exhibited enhanced homogeneity in physical appearance and thus physical state, and a generally higher level of porosity compared to ibuprofen MPs. This difference can be attributed partially or entirely to the distinct process parameters required for lidocaine MPs. The greater porosity, and the more pronounced plasticizing effect of lidocaine MPs, contributed to a faster overall release of lidocaine compared to ibuprofen. However, the ionic interactions between PLGA and lidocaine and the neutralization of the release medium by the latter delayed its release, ultimately resulting in a release rate similar to that of ibuprofen.

Opting to study individual microparticles rather than groups offers the possibility of closely correlating specific microparticle characteristics with their release behavior. This focused methodology facilitated a more comprehensive exploration of polymer swelling's role. Notably, amongst the various mechanisms governing drug release from PLGA-based MPs, the swelling of the polymer matrix emerged as a determining factor. In addition, this methodological approach offers the possibility of improving the correlation between *in vitro* release setups and *in vivo* conditions.

Given the growing interest in PLGA-based drug delivery systems, understanding *in vitro* release mechanisms is crucial. However, care should be taken in interpreting such release measurements, given the complex nature of the underlying drug release mechanisms, which experimental variables can strongly influence.

This work could be continued in many interesting ways: Other preparation techniques, which involve different process parameters which may have an impact on the final characteristics of MPs, could be investigated; The release of other types of drugs (proteins, peptides, poorly soluble drugs,…), which may have different affinities for PLGA, could be studied; The characterization of the autocatalytic effect and its role might be deepened through pH measurements allowing for the precise assessment of low-volume samples, for example; Terahertz spectroscopy measurements could be performed in order to quantify the microparticles' porosity and pores' connections and determine their impact on drug release.

CONCLUSION GENERALE ET PERSPECTIVES

Nos recherches ont révélé que l'état physique initial de la substance active dans les MPs n'a pas d'impact sur les cinétiques de libération, car la substance active amorphe devient cristalline au contact du milieu de libération. Par conséquent, la transformation observée au fil du temps de la substance active amorphe en cristalline dans les MPs n'a aucune incidence sur le processus de libération, ce qui suggère la stabilité à long terme des MPs lorsqu'elles sont stockées dans des conditions appropriées pendant plusieurs années.

La méthode de préparation des MPs influence indéniablement leurs caractéristiques physico-chimiques. Les facteurs relatifs à chaque méthode, tels que la concentration de la phase organique, le mécanisme de formation des gouttelettes et le processus de précipitation, exercent une influence notable sur la porosité et l'uniformité des MPs en ce qui concerne la taille et la distribution de la substance active. Par conséquent, ceci affecte de manière significative les cinétiques de libération.

L'analyse comparative entre les MPs d'ibuprofène et de lidocaïne révèle que les MPs de lidocaïne présentent une plus grande homogénéité en termes d'apparence physique et donc d'état physique, ainsi qu'un niveau de porosité généralement plus élevé que les MPs d'ibuprofène. Cette différence peut être attribuée en partie ou en totalité aux paramètres de traitement distincts requis pour les MPs de lidocaïne. La plus grande porosité et l'effet plastifiant plus prononcé des MPs de lidocaïne ont contribué à une libération globalement plus rapide de la lidocaïne par rapport à l'ibuprofène. Cependant, les interactions ioniques entre le PLGA et la lidocaïne, et la neutralisation de l’acidité du milieu de libération par cette dernière, ont retardé sa libération, ce qui a finalement abouti à un taux de libération similaire à celui de l'ibuprofène.

Le choix d'étudier des microparticules individuelles plutôt que des groupes offre la possibilité d'établir une corrélation étroite entre les caractéristiques spécifiques des microparticules et leur comportement de libération. Cette méthodologie ciblée a facilité une exploration plus complète du rôle du gonflement du polymère. Notamment, parmi les divers mécanismes régissant la libération de substances actives à partir de MPs à base de PLGA, le gonflement de la matrice polymère est apparu comme un facteur déterminant. Par ailleurs, cette approche méthodologique offre la possibilité d'améliorer la corrélation entre les setups de libération *in vitro* et les conditions *in vivo*.

Compte tenu de l'intérêt croissant pour les systèmes d'administration de substances actives à base de PLGA, il est essentiel de comprendre les mécanismes de libération *in vitro*. Cependant, il convient d'être prudent dans l'interprétation de ces mesures de libération, étant donné la nature complexe des mécanismes de libération sous-jacents, que les variables expérimentales peuvent fortement influencer.

Ces travaux pourraient être poursuivis de nombreuses manières intéressantes : d'autres techniques de préparation, qui impliquent différents paramètres de processus pouvant avoir un impact sur les caractéristiques finales des MPs, pourraient être étudiées ; la libération d'autres types de substances actives (protéines, substances actives peu solubles,...), qui peuvent avoir des affinités différentes pour le PLGA pourrait être étudiée ; la caractérisation de l'effet autocatalytique et de son rôle pourrait être approfondie, grâce à des mesures de pH permettant l'évaluation précise d'échantillons de faible volume, par exemple ; des mesures par spectroscopie térahertz pourraient être effectuées afin de quantifier la porosité des MPs ainsi que la connexion des pores, et de déterminer leur impact sur la libération de la substance active.

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ABSTRACT

Over the past few decades, poly(lactic-co-glycolic acid) (PLGA) has attracted growing interest thanks to its great potential for the controlled release of drugs. Its biocompatibility and biodegradability make it a material of choice for the development of new drug delivery systems (DDS). Nevertheless, the marketing of PLGA-based products is hampered by a lack of understanding of PLGA release mechanisms. There is therefore a major interest in investigating the mechanisms involved in drug release.

This investigation encompassed multiple factors that may influence the drug release kinetics and behaviors of PLGA-based microparticles (MPs). These MPs were produced by an oil-in-water emulsion-solvent extraction/evaporation technique. Surprisingly, MPs with different appearances were obtained: Some appeared transparent while others were opaque. This difference was found to be due to the physical state of the encapsulated drug within the MP. Interestingly, the initial physical state of the drug did not influence release kinetics, as the amorphous drug transformed to a crystalline state upon interaction with the release medium. An emerging method for preparing MPs was tested: Microfluidics, and the resulting drug release profiles showed that the preparation method significantly influenced MPs' physicochemical characteristics. Method-specific parameters, substantially impacted MPs' porosity, size uniformity, and distribution of the active ingredient, thereby affecting release kinetics. A comparative analysis between ibuprofen and lidocaine MPs revealed differences in release mechanisms. These disparities were attributed to the fact that lidocaine MPs exhibited a more pronounced plasticizing effect and higher porosity. However, these parameters, which suggested a more rapid release of lidocaine, were counterbalanced by ionic interactions between the PLGA and lidocaine molecules, which slowed down the release process. By comparing the drug release in a traditional bulk fluid setup with an agarose gel setup where the MPs' swelling is hindered, we noticed that the drug release was slowed down. It has thus emerged that swelling, although often neglected in the literature, is a mechanism playing a fundamental role in drug release.

Additionally, the study emphasized the significance of studying individual MPs for better correlating MPs' characteristics with their release behavior. Indeed, release studies are usually carried out on ensembles of MPs. However, each MP is unique in its structure and composition, and will therefore have its own release behavior.

Keywords: PLGA, single microparticles, release mechanisms, controlled release, microfluidics, swelling

RESUME

Au cours des dernières décennies, l'acide poly(lactique-co-glycolique) (PLGA) a suscité un intérêt croissant en raison de son grand potentiel pour la libération contrôlée de substances actives. Sa biocompatibilité et sa biodégradabilité en font un matériau de choix pour le développement de nouveaux systèmes d'administration de médicaments. Néanmoins, la commercialisation de produits à base de PLGA est freinée par un manque de compréhension des mécanismes de libération du PLGA. Il y a donc un intérêt majeur à étudier les mécanismes impliqués dans la libération des substances actives.

Cette étude a porté sur de multiples facteurs susceptibles d'influencer la cinétique de libération de la substance active et le comportement des microparticules (MPs) à base de PLGA. Ces MP ont été produites par une technique d'émulsion huile dans eau puis extraction/évaporation de solvant. De manière surprenante, des MP d'apparences différentes ont été obtenues : certaines semblaient transparentes tandis que d'autres étaient opaques. Cette différence s'explique par l'état physique de la substance active encapsulée à l'intérieur de la MP. Il est intéressant de noter que l'état physique initial de la substance active n'a pas influencé la cinétique de libération, car la substance active amorphe s'est transformée en un état cristallin lors de l'interaction avec le milieu de libération. Une nouvelle méthode de préparation des MP a été testée : la microfluidique, et les profils de libération de la substance active qui en résultent ont montré que la méthode de préparation influençait de manière significative les caractéristiques physicochimiques des MPs. Des paramètres spécifiques à la méthode ont eu un impact important sur la porosité, l'uniformité de la taille et de la distribution de la substance active, affectant ainsi la cinétique de libération. Une analyse comparative entre les MPs d'ibuprofène et de lidocaïne a révélé des différences dans les mécanismes de libération. Ces disparités ont été attribuées au fait que les MPs de lidocaïne présentaient un effet plastifiant plus prononcé et une porosité plus élevée. Cependant, ces paramètres, qui suggéraient une libération plus rapide de la lidocaïne, ont été contrebalancés par des interactions ioniques entre les molécules de PLGA et de lidocaïne, qui ont ralenti le processus de libération. En comparant la libération de la substance active dans un traditionnel fluide en vrac avec celle dans un environnement composé de gel d'agarose où le gonflement des MPs est entravé, nous avons remarqué que la libération de la substance active était ralentie. Il est donc apparu que le gonflement, bien que souvent négligé dans la littérature, était un mécanisme jouant un rôle fondamental dans la libération des substances actives.

De plus, l'étude a souligné l'importance d'étudier les MPs de manière individuelle pour mieux corréler les caractéristiques des MPs avec leur comportement de libération. En effet, les études de libération sont généralement réalisées sur des ensembles de MPs. Cependant, chaque MP est unique dans sa structure et sa composition, et aura donc son propre comportement de libération.

Mots clés : PLGA, microparticules individuelles, mécanismes de libération, libération contrôlée, microfluidique, gonflement