Microfluidic-Assisted Fabrication of Microparticles for Drug Delivery Applications

Daniela Wollstén

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Supervisors:

Wei Li	Zhenyang Wei	Tapani Viitala
University of Helsinki	University of Helsinki	Åbo Akademi University

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Författare (förnamn efternamn) – Author (first name last name)

Daniela Wollstén

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Wei Li, University of Helsinki
Zhenyang Wei, University of Helsinki
Tapani Viitala, Åbo Akademi University

Abstrakt - Abstract

The number of poorly soluble drugs in development is growing, increasing the interest in developing new, improved pharmaceutical formulations and drug delivery systems. By utilising drug delivery systems, it is possible to broaden the range of administration routes, enhance treatment outcomes and decrease toxicity. Microfluidic-assisted fabrication of polymeric microparticles (MPs) is one potential candidate that promises both effective and controllable fabrication of drug delivery systems. When developing drug delivery systems, some critical parameters must be determined. The drug loading degree and encapsulation efficiency are of key interest in this thesis.

It is known that the drug loadability and encapsulation efficiency in polymeric MPs is highly dependent on drug-polymer compatibility. Hence, this master's thesis project aimed to fabricate MPS with high drug loading in an amorphous state using various drug-polymer combinations. Indomethacin and simvastatin were chosen as active pharmaceutical ingredients, and HPMCAS-MF, PLGA, PLA-PCL copolymer, PLA and PCL as polymers for this study. The drug loading degree, encapsulation efficiency, solid-state characteristics, and cell viability were investigated on the prepared MPs. The loading degree and encapsulation efficiency were determined with high-performance liquid chromatography. The solid-state characteristics were evaluated using Differential Scanning Calorimetry, Attenuated Total Reflection - Fourier-Transform Infrared spectroscopy and Scanning Electron Microscopy. The polymer solidification rate was determined with a light microscope, and the cell viability study was conducted with an ATP-based assay on RAW264.7 cells.

We prepared MPs with a high drug loading degree (>40%) with HPMCAS-MF, PLGA and PLA-PCL copolymer for both APIs. PLA and PCL could not reach a high loading degree and were determined incompatible with indomethacin and simvastatin. Although, PCL combined with simvastatin achieved a higher loading degree than indomethacin. The stabilisation of the API amorphous state was a challenge and could be achieved with only a few combinations. The polymorphic forms of indomethacin could be observed, suggesting that the formulation requires some adjustments for a completely amorphic state.

A fitting continuum to this study is determining the amorphous-solubility-advantage through the dissolution and stability studies of the best-performing combinations.

Nyckelord – Keywords

Microparticles, Microfluidics, Drug Delivery, Drug Development, Formulation, Amorphous Solid Dispersions

Övriga uppgifter – Additional information

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Wei Li, Helsingfors Universitet
Zhenyang Wei, Helsingfors Universitet
Tapani Viitala, Åbo Akademi

Abstrakt - Abstract

Antalet dåligt lösliga läkemedel inom läkemedelsutvecklingen växer, vilket ökar intresset för att utveckla nya, förbättrade läkemedelsformuleringar och läkemedelstillförselsystem. Genom utvecklingen av avancerade läkemedelstillförselsystem är det möjligt att utvidga utbudet av administreringsvägar, förbättra behandlingsresultaten och minska toxiciteten på läkemedlen. Mikrofluidistik-assisterad tillverkning av polymera mikropartiklar är en potentiell kandidat som lovar både effektiv och kontrollerbar tillverkning av läkemedelstillförelsesystem. Vid utvecklingen av system för läkemedelstillförsel finns det några kritiska parametrar som måste bestämmas. Laddningsgraden av läkemedel och inkapslingseffektiviteten är av intresse i denna avhandling.

Det är väl känt att läkemedelsladdningsgrad och inkapslingseffektivitet är starkt beroende av kompatibiliteten av läkemedlet och polymeren i frågan om polymera mikropartiklar. Därför var syftet med detta pro gradu -projekt att tillverka mikropartiklar med hög läkemedelsladdning i amorft tillstånd genom att kombinera olika läkemedel och polymerer. Indometasin och simvastatin valdes som aktiva farmaceutiska ingredienser och HPMCAS-MF, PLGA, PLA-PCL-sampolymer, PLA och PCL som polymerer för denna studie. Läkemedelsladdningsgrad, inkapslingseffektivitet, fasta tillståndsegenskaper och cellviabilitet undersöktes på de tillverkade mikropartiklarna. Laddningsgraden och inkapslingseffektiviteten bestämdes med hjälp av högpresterande vätskekromatografi. Fasta tillståndsegenskaperna utvärderades med olika metoder: differentiell svepkalorimetri, attenuerad totalreflektion - fourier-transform infraröd spektroskopi och svepelektronmikroskopi. Polymerens stelningshastighet bestämdes med hjälp av ett ljusmikroskop och cellviabilitetsstudierna utfördes med en ATP-baserad analys på RAW264.7-celler.

Vi förberedde mikropartiklar med hög läkemedelsladdningsgrad (> 40 %) med HPMCAS-MF, PLGA och PLA-PCL-sampolymer för både indometasin och simvastatin. En hög laddningsrad kunde inte nås med PLA och PCL, dessa bedömdes som inkompatibla med indometasin och simvastatin. Där emot kunde PCL i kombination med simvastatin uppnå en högre laddningsgrad än indometasin. Stabiliseringen av det amorfa tillståndet var en utmaning och lyckades endast med ett fåtal kombinationer. Polymorfa former av indometasin kunde observeras, vilket tyder på att formuleringen ännu kräver vissa justeringar för ett fullständigt amorft tillstånd.

Som fortsättning på denna studie kunde man studera vilken fördel man uppnått med en amorf struktur genom upplösnings- och stabilitetsstudier av de bäst presterade kombinationerna.

Nyckelord – Keywords

Mikropartiklar, Mikrofluidistik, Läkemedelsutveckling, Läkemedelsformulering, Amorfa Fasta Dispersioner

Övriga uppgifter – Additional information

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LIST OF ABBREVIATIONS

API	Active Pharmaceutical Ingredient
ASD	Amorphous Solid Dispersion
BCS	Biopharmaceutics Classification System
CP	Continuous phase
DDS	Drug Delivery System
DMSO	Dimethyl sulfoxide
DP	Dispersed phase
EA	Ethyl acetate
EE	Encapsulation efficiency
GIT	Gastrointestinal tract
HMG-CoA	3-hydroxy-3-methylglutaryl coenzyme A reductase
HPMC	Hydroxypropyl Methylcellulose
HPMCAS-MF	Hydroxypropyl Methylcellulose Acetate Succinate (MF grade)
IND	Indomethacin
LD	Loading degree
MESE	Microfluidic-assisted emulsion solvent evaporation
MP	Microparticle
NP	Nanoparticle
NSAID	Nonsteroidal Anti-inflammatory drugs
PCL	Poly(ε-caprolactone)
PLA	Poly(lactic acid)
PLA-PCL	Poly(lactic acid-co-ε-caprolactone)
PLGA	Poly(lactide-co-glycolide)
POLY	Polymer
SIM	Simvastatin
Tg	Glass transition temperature
Tm	Melting point

1. INTRODUCTION

Approximately 70% of active pharmaceutical ingredients (APIs) in development have poor aqueous solubility. As a result, most of these APIs fail to reach the market despite their desirable pharmacological properties. (Anane-Adjei et al., 2022) Furthermore, the trend of poorly soluble drugs in development is growing, increasing the interest in developing new, improved pharmaceutical formulations (Blagden *et al.*, 2007). The formulation development in the pharmaceutical industry is focused on enhancing limiting variables and diversifying the range of administration routes. In addition, improving treatment outcomes and decreasing toxicity through targeted and controlled drug delivery is also an incentive to develop new formulations and drug delivery systems (DDS) (Damiati *et al.*, 2018).

Formulation-related approaches such as amorphous solid dispersions (ASDs) have received much interest as a strategy to improve drug solubility and bioavailability. The utilisation of polymeric nanoparticles (NPs) or microparticles (MPs) as ASDs have been recently reported in an increasing manner (Wei *et al.*, 2022). MPs are defined as entities intended for drug delivery applications with a size ranging from 1 to 1000 micrometres, and NPs are entities smaller than 1000 nanometres (Medina *et al.*, 2007). Polymeric MPs can be utilised to address the four major issues in drug development: pharmacokinetic (e.g., low half-life), pharmacodynamic (e.g. low specificity), pharmaceutical (e.g. low solubility or stability) and pharmacotherapeutic (e.g. high dose, adverse events, or low patient compliance) (Tewabe *et al.*, 2021). These issues can be addressed by designing MPs with advanced functions, such as stimuli-responsive burst or synergetic release patterns (Wang, Zhang and Chu, 2014). Nonetheless, the combination of crystal engineering and nano- or microtechnology promises excellent opportunities for improving patient compliance, thanks to enabling more personalised drug delivery designs (Chen *et al.*, 2021; Wei *et al.*, 2022).

In a meta-analysis on oral bioavailability enhancement through supersaturation strategies, such as ASDs, it was found that supersaturating drug delivery systems are promising; however, the prediction of *in vivo* performance still presents challenges (Fong, Bauer-Brandl and Brandl, 2017). The development of these methods is currently based on trial and error, although more mechanistic insight and predictive tools would be needed. Parameters that would benefit from predictive tools would be the drug-loading degree (LD) and encapsulation efficiency (EE). LD and EE describe the amount of API in the MP compared to theoretical values. These depend

highly on polymer compatibility based on molecular interactivity (Pagels and Prud'homme, 2015). The LD and EE will be in the spotlight of this work.

In this project, we prepare drug-loaded MPs for drug delivery applications with a modern fabrication method, microfluidic-assisted emulsion solvent evaporation (MESE), which promises accuracy, controllability, and cost-efficiency (Liu *et al.*, 2017). The focus is on understanding the factors affecting the encapsulation of small molecular drugs into polymeric MPs. The reader will be guided through relevant theoretical basis, including bioavailability, drug delivery systems and microfluidics. These topics are covered in the literature review. Next, the experimental section is presented with an introduction to the project's aim. This is followed by the materials and methods used. The results are shortly presented before the discussion and interpretation of the findings.

2. LITERATURE REVIEW

The literature review is divided into three main themes. Firstly, a theoretical basis of the challenges in pharmaceutical development, specifically related to solubility-limited absorption and common strategies to enhance this, is discussed. The second theme covers microparticulate systems as an application for drug delivery. Lastly, microfluidics, a method for preparing MPs, is reviewed.

2.1. Challenges in modern drug development

Bioavailability is one of the critical properties of a substance that must be considered already in the early stages of drug development. Oral bioavailability is the fraction of the administered dose that reaches the systemic circulation and, thus, can reach therapeutic efficacy (Koch-Weser, 1974). Identifying the bioavailability and related properties guides the decision of which administration route and formulation is chosen for the final product. Oral administration is still one of the most desired routes, mainly due to the ease of commercialisation and high patient compliance. However, from a drug developers' point of view, this route is very complex compared to other administration routes. It includes many obstacles that must be solved for successful drug delivery (Viswanathan, Muralidaran and Ragavan, 2017).

When administered orally, a drug must dissolve in the gastrointestinal fluids before it can be absorbed into the systemic circulation. Absorption properties are, therefore, among the critical pharmaceutical properties that must be addressed. Dokoumetzidis and Macheras (2006) discussed that drug absorption is a complex process dependent primarily on substance-related factors. Inevitably, formulation-specific factors and physiological factors also play a role and affect bioavailability (Taylor and Zhang, 2016). Regarding the physiological and patient-related aspects, the human gastrointestinal tract (GIT) is a complex system consisting of numerous secreted fluids and enzymes affecting drug performance. In addition, the composition of the gastrointestinal fluid is dynamic and dependent on factors such as food intake. Although understanding the physiological aspects and complexity of the GIT are of utmost importance when developing orally administered drugs, it lies outside the scope of this thesis. (Zhou *et al.*, 2017) Formulation-related factors are in the spotlight and will be discussed thoroughly throughout this thesis. Although substance-related factors are partly outside the scope of this work, the topic is briefly discussed to lay the theoretical basis for further application.

The biopharmaceutics classification system (BCS) is a tool for categorising drugs based on properties affecting the absorption: aqueous solubility and intestinal permeability (Dokoumetzidis and Macheras, 2006; Samineni, Chimakurthy and Konidala, 2022). The framework, presented initially in 1995 by Gordon L. Amidon and colleagues, enables a systemic estimation of the drug absorption properties and limitations. The framework can guide the choice of a suitable strategy for enhancing the properties related to the limiting factor (Amidon *et al.*, 1995). The four BCS classes are,

I. High solubility and high permeability - well absorbed,
II. Low solubility, high permeability - solubility limited absorption,
III. High solubility, low permeability - permeability limited absorption,
IV. Low solubility and low permeability - poor absorption.

Solubility-related issues are more easily addressed than permeability-related issues, meaning that Class III drugs are seen as a more significant problem than Class II ones. Drugs belonging to the latter can be modified with various formulation strategies to reach a desired level of solubility and increased dissolution rate (Wong, Kellaway and Murdan, 2006). This topic earned its spotlight thanks to famous works and publications by Lipinski et al. (1997), who created an outline of desirable physicochemical properties of drug molecules for efficient

delivery via oral administration. These are commonly referred to as “Lipinski’s rule of 5” or “RO5” (Taylor and Zhang, 2016; Chen *et al.*, 2020). The RO5 states that a small molecular drug-like compound should have the following:

- a molecular weight of less than 500 g/mol,
- a log P value less than 5
- not more than five hydrogen bond donors
- not more than ten hydrogen bond acceptors. (Lipinski *et al.*, 1997)

After further research, two more conditions, a polar surface area of a max of 140 Å and no less than ten rotatable bonds, were added later (Chen *et al.*, 2020).

2.1.1. Solubility and dissolution rate

Dissolution is the process when two phases mix and create a new, homogenous solution through solvation. Solubility describes how the two phases are miscible to one another. Significantly, solubility may be affected by external factors such as temperature and pH. The opposite of solvation is called precipitation. A solute may be dissolved until its saturation point, where solvation and precipitation of the substance happen at the same rate (Shaikh *et al.*, 2018). This is when the dissolution has reached its equilibrium solubility. The dissolution rate describes how fast the substance dissolves in the solvent completely. (Shaikh *et al.*, 2018)

When a solution is saturated, the solute cannot further dissolve unless the equilibrium is disrupted, for example, by lowering the concentration of the dissolved substance. This can be done by increasing the solvent volume or removing the dissolved solute. The latter is referred to as a “sink condition”. Although equilibrium is rarely achieved in biological systems like the GIT due to sink conditions, it is still a parameter utilised in modern drug development. (Zhou *et al.*, 2017)

Central theories related to the dissolution of solids (i.e. crystals) are the Noyes-Whitney and Nernst-Brunner models (Taylor and Zhang, 2016). Both have created mathematical models for predicting how solid particles dissolve in bulk solvents. When the particle, the solute, meets the solvent, it will start to diffuse. Diffusion forms a saturated layer which the drug moves through. The layer will have a gradient in the concentration between the solid particle and the bulk liquid, this is called the diffusion (Seager *et al.*, 2018). The Noyes-Whitney equation (Equation 1) was developed to explain the dissolution process as a mathematical function.

Here, (dC/dT) is the change in concentration over time, A is the surface area of the particle, D is the diffusion coefficient, d is the thickness of the concentration gradient, Cb is the concentration in the bulk liquid, and lastly, the diffusion layer Cs (Dokoumetzidis and Macheras, 2006). The Noyes-Whitney parameters are presented in Figure 1.

Considering solubility enhancement, the surface area is the factor that can be easily modified to increase the dissolution rate. This strategy has been utilised in the pharmaceutical industry by decreasing particle size and, thus, increasing surface area. Interestingly, this strategy can also be used for sustained-release formulations by increasing particle size or reducing the particle surface area. (Gao *et al.*, 2021)

Equation 1. The Noyes-Whitney equation

$$\frac{dC}{dT} = A\frac{D}{d}(Cs - Cb) \quad \text{or} \quad \frac{dC}{dT} = k(Cs - Cb)$$

The Nernst-Brunner equation (Equation 2) is derived from the Noyes-Whitney equation and Fick's second law of diffusion, which predicts how diffusion affects the concentration change over time. Here, h is the thickness of the diffusion layer, and V is the volume of the dissolution medium (Gao *et al.*, 2021). This model describes the dissolution process considering the diffusion of solute molecules through the diffusion layer Cs of each particle when the solvent is unstirred. This model defines the dissolution rate as a dependency on the thickness of the diffusion layer when unstirred. Hixson and Crowell have derived a more dynamic model based on the earlier models. This "modified" Nernst-Brunner model will not be assessed in this thesis as the original Noyes-Whitney and Nernst-Brunner models have been more than sufficient for most applications. (Seager *et al.*, 2018)

Equation 2. The Nernst-Brunner equation

$$\frac{dC}{dT} = \frac{DS}{Vh}(Cs - Cb)$$

Substances with low aqueous solubility have a slow dissolution rate in the gastrointestinal fluids, leading to insufficient and inconsistent systemic exposure and, consequently, sub-optimal efficacy in patients (Blagden *et al.*, 2007). Several aspects affect the solubility of a substance. Firstly, hydro- or lipophilicity describes the dissolution properties of the drug in organic and inorganic solvents. This is also a problematic factor as high lipophilicity makes the substance more permeable, but it will not be able to dissolve in aqueous solutions making absorption impossible (Giménez *et al.*, 2010). This implies that only a specific range between

hydrophilic and lipophilic is ideal for permeability and dissolution, as seen from the RO5. Secondly, considering the dissolution rate, a substance's capacity to form intermolecular forces with surrounding molecules and solvents is vital for rapid dissolution (Kapustikova *et al.*, 2018).

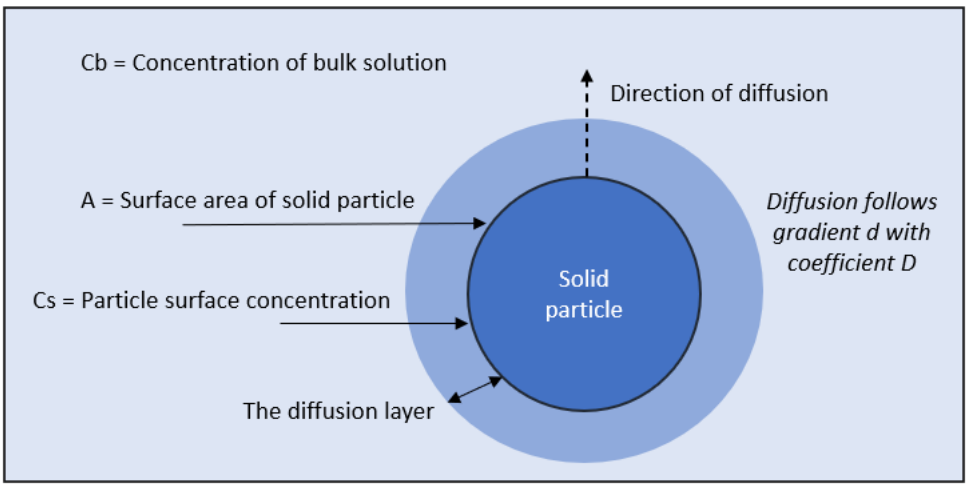


Figure 1. Schematic picture of the Noyes-Whitney parameters and dissolution of a solid particle as described by Noyes and Whitney.

Even if increasing the dissolution rate is seen as a potential strategy for increased bioavailability, it has been realised that more effort is needed for increased therapeutical efficiency. This is where supersaturated solutions get relevant. A supersaturated solution is formed when the concentration of the solute in the solution is higher than the concentration of the solute at equilibrium with the most thermodynamically stable form (Taylor and Zhang, 2016). Supersaturated solutions are discussed in more detail later in this literature review.

2.1.2. Crystallinity, polymorphism and amorphism

The physical form of the drug affects many properties of the substance. According to the European Pharmacopoeia, polymorphism is the ability of a compound in its solid state to exist in different crystalline forms having the same chemical composition. Respectively, amorphous substances exist in an entirely non-crystalline state. Theoretically, an API may have multiple physical forms such as crystal, polymorph, or an amorphous structure. These various physical forms have the same chemical behaviour, but their physicochemical and physical characteristics, including reactivity and bioavailability, may differ. (Ph. Eur., 50900:01/2008)

As a rule of thumb, the drug will resort to the solid state with the least free energy, i.e. the most thermodynamically stable form. The crystalline form holds the least free energy, whereas the amorphous form possesses a high free energy level (Yu *et al.*, 2022). Solubility-wise, the crystalline structure has the lowest solubility, whereas the amorphous state has the highest solubility due to the differences in free energy levels.

Aguiar et al. (1967) suggested that the significant free energy differences between polymorphs could be utilised in enhancing bioavailability. This realisation has highlighted crystal engineering as a new field in formulation development. Crystal engineering aims to create solubility-enhanced formulations with the API in better-dissolving solid forms, such as its' amorphous form. The first crystalline modification publications were published in the late 1960s when a metastable polymorph B of chloramphenicol palmitate showed better absorption than its other polymorph A (Aguiar *et al.*, 1967). The same study further suggested that significant free energy differences exist between polymorphs, which could be utilised in enhancing bioavailability.

2.1.3. Strategies to minimise bioavailability issues

Numerous strategies are available to increase the bioavailability through enhancing the dissolution rate or solubility, some of which are summarised in Table I. Still, some methods are available for increasing permeability, e.g. absorption enhancers that loosen the tight junctions between enterocytes in the GIT. However, these are relatively novel solutions, and an understanding of the mechanism of action may still be lacking. (Martins *et al.,* 2021). One common way to improve the dissolution is to minimise the particle size, thus, increasing the surface area as described by Noyes-Whitney (Cruz *et al.*, 2010). However, this strategy is only sometimes effective enough. This inefficiency has been explained by the fact that a reduction in particle size does not affect the equilibrium solubility of the API. The solution may be saturated during the dissolution process, which can lead to limited dissolution, even if the solubility of the particle itself is increased (Wong, Kellaway and Murdan, 2006).

DDSs are no new thing to the pharmaceutical industry; ever since proteins, peptides, and monoclonal antibodies have entered the market, so have the need for various DDS (Gao *et al.*, 2023). DDS can be used not only for enhancing desirable properties such as bioavailability but also to design the system to target specific tissues and cells better or to release it at a specific

rate, to name a few. As the personalisation of treatments increases to fit certain patient groups better, e.g. related to gender or age, more advanced DDSs are needed.

ASDs have been presented as a potential solution to solubility-limited BCS Class II substances. ASDs are a product of crystal engineering. This approach stabilises the drug in its amorphous form into a solid formulation. The crystallisation is either inhibited or retarded by creating a mixture of the drug with a stabilising excipient, e.g. a polymer. The excipient will hinder or slow the crystal growth by inhibiting the formation of the crystal lattice and/or by decreasing the molecular mobility (Sahoo, Suryanarayanan and Siegel, 2020). Polymers have shown great potential and ideal properties for ASD development. The "amorphous solubility advantage" is an evaluation based on the ratio of amorphous-to-crystalline solubility. It describes the possible improvement of bioavailability when using the amorphous form of the drug compared to its crystalline structure. (Taylor and Zhang, 2016)

When administered, ASDs often exhibit fast dissolution that results in supersaturated solutions. The physical chemistry of these supersaturated solutions has yet to be understood. Although, it is well known that the dissolution and crystallisation mechanisms define the formation of supersaturated solutions. The supersaturated system is often in a metastable equilibrium, meaning that conversion from the amorphous form to a crystalline form may occur quickly. (Taylor and Zhang, 2016; Moseson *et al.*, 2023) This conversion is not desired, as it would decrease the overall dissolution due to increased crystalline form. Therefore, maintaining the supersaturated state is preferred and is one of the more significant challenges when developing ASDs (Schittny, Huwyler and Puchkov, 2020). On the other side, it has been shown that some excipients, mainly polymers such as polyethylene glycol (PEG) and hydroxypropyl methylcellulose (HPMC), can inhibit the solid-state conversion and, thus, open up an exciting opportunity for further development (Tian *et al.*, 2007).

Table I. Strategies for bioavailability enhancement based on solubility-limited absorption

Strategy	Approach	Challenges	Reference
Micronisation (e.g., milling)	Increased surface area enables increased dissolution. Easy to establish.	Achieved increase in dissolution rate may not be sufficient for drugs with exceptionally low aqueous solubility due to limitations set by equilibrium solubility. Great mechanical stress on API	(Wong, Kellaway and Murdan, 2006)

Strategy	Approach	Challenges	Reference
De-novo drug design	Designing novel chemical entities with desirable properties.	Can impact development timelines and negatively affect the structure-activity relationship.	(Anane-Adjei *et al.*, 2022)
Salt formation	Enhances dissolution profiles by utilising counterions	Often prepared from organic solvents that may not behave as expected in aqueous solutions. Limitations related to counterion compatibility that can delay development.	(Serajuddin, 2007)
Crystal engineering (e.g., ASDs)	The utilisation of solid-state structures with higher levels of free energy and solubility.	Concerns related to stability during processing and storage. Solubility differences between polymorphs may be too small for sufficient benefit.	(Blagden *et al.*, 2007; Yu *et al.*, 2022)

2.2. Microparticles as drug delivery systems

Drug delivery systems are intended to deliver and release the drug to its target site. By targeting and controllability, one can reduce unwanted effects or enhance the drug desired properties (Simonazzi *et al.*, 2018). ASDs and MPs have been discussed earlier in this thesis through the solubility and bioavailability points of view. In the following sections, the focus will be on the structure and fabrication of these entities.

Polymeric MPs can be utilised as efficient formulations for drug delivery purposes. In fact, polymers have already been widely used in various formulations as excipients in the pharmaceutical industry. The possibilities seem endless, as polymers have been reported to be utilised in encapsulating small molecular drugs and biologics such as proteins, genes, and whole cells (Gong *et al.*, 2012; Lu *et al.*, 2015; Yu *et al.*, 2019). MPs come in all shapes, structures, and sizes, some presented in Figure 2. MPs with entrapped API (A in Figure 2) were prepared and studied in the experimental part of this project.

Polymer particles can be divided into various categories depending on their properties, e.g. based on the polymer's hydrophilic or -phobic character. Hydrophilic polymers draw water to them, creating hydrogels, which are ideal for encapsulating sensitive materials such as proteins or cells (Pagels and Prud'homme, 2015). Alginate is an example of a natural hydrophilic polymer with excellent biocompatibility and biodegradability properties (Yu *et al.*, 2019). Hydrophobic polymers form an impenetrable barrier towards aqueous solutions. This type is better for controlled or sustained release than its hydrophilic counterparts. (Pagels and Prud'homme, 2015) Other ways to categorise polymers are based on their biodegradation,

biocompatibility, and molecular properties, e.g. functional groups. This thesis will discuss the role of the functional groups later in this literature review.

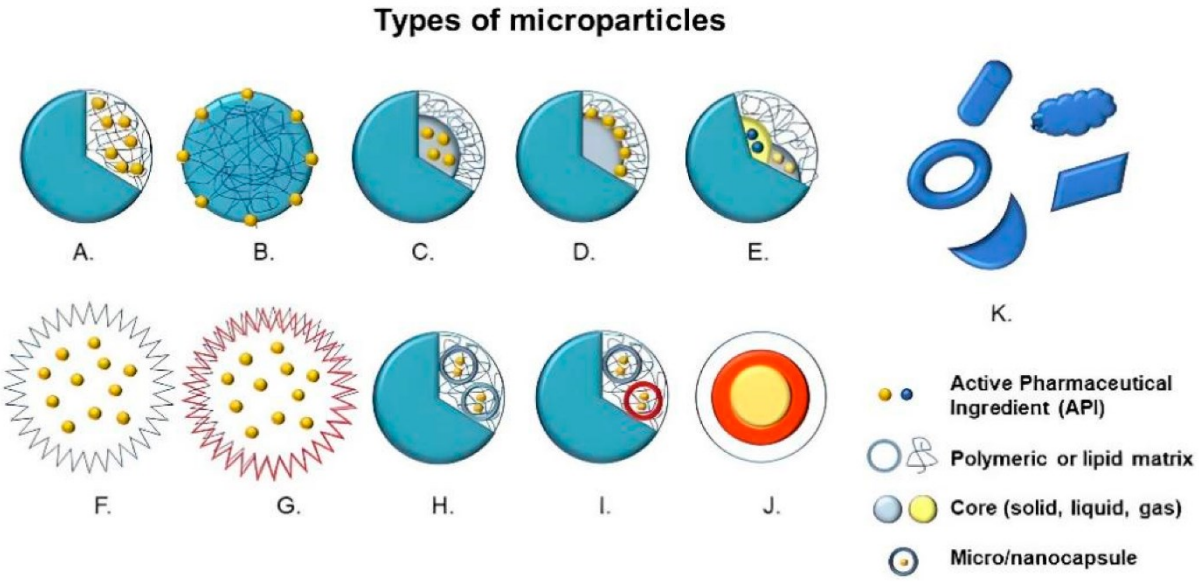


Figure 2. Da Silva et al. (2023) illustrate different types of MPs. (A) MP with entrapped API, (B) MP with adsorbed API, (C) Microcapsule (MC) with entrapped API, (D) MC with adsorbed API, (E) Multinucleated MC, (F) Hollow MP, (G) Hollow MP with several layers, (H) MP containing MCs, (I) MP containing multinucleated MCs, (J) Multilayer MPs and (K) MP with irregular shapes. Illustration by Da Silva et al. (2023).

2.2.1. Key parameters of drug delivery systems

Drug-loading degree (LD) is one of the critical parameters when designing DDSs. It describes the fraction of the encapsulated API's weight compared to the total weight of the MP, as presented in Equation 3. This information is essential in formulation development as it will affect the dose burden and release of the API, which could affect patient compliance (Damiati and Damiati, 2021). The LD depends on multiple factors, ranging from fabrication methods to the compatibility of the materials. Thus, the physicochemical properties of the API and polymers are once again highlighted to achieve desired properties.

Equation 3.

$$LD\ (\%) = \frac{Encapsulated\ API\ weight}{MP\ weight} * 100$$

Equation 4 describes how the encapsulation efficiency (EE) is calculated. EE describes how much of the total theoretical weight of API is encapsulated within the MPs (Khan *et al.*, 2013).

The theoretical weight means the total input of API in fabrication. This is an important parameter specifically for developing the fabrication process, as it can determine how much of the API is efficiently entrapped and how much is wasted due to poor encapsulation properties (Khan *et al.*, 2013).

Equation 4.

$$EE\ (\%) = \frac{encapsulated\ API\ weight}{theoretical\ API\ weight} * 100$$

In one study, IND was encapsulated in PLGA MPs and reached an LD of 7.79% and an encapsulation efficiency of 62.35% (Damiati and Damiati, 2021). These values were presented as "acceptable" by the researchers. Simvastatin has been encapsulated in PLGA NPs prepared by spray drying; in this study, the obtained EE was 63.07% and LD at approximately 8,5% (Anzar *et al.*, 2018). Higher LDs up to 64% with fenofibrate encapsulated in poly(ethylene glycol) diacrylate have been demonstrated by Bora *et al.* (2022), which can be considered a high LD. These examples illustrate a considerable variation in LD and EE based on the combination of raw materials and fabrication methods. It further proves that the current knowledge is limited and scattered, as no frameworks for compatible materials or methods have been established.

It has been proven that a high LD results from a high grade of intermolecular interactions between the polymer and the API. An increased amount of intermolecular interactions decreases molecular mobility, which has been raised as one of the primary mechanisms behind delaying crystallisation (Mistry *et al.*, 2015). Intermolecular hydrogen bonds (H-bonds) have been shown to be efficient stabilisers of the amorphous structure in ASDs (Damiati and Damiati, 2021). H-bonds are a specific dipole-dipole interaction between a hydrogen atom and an electronegative atom, such as O, N and F (Bruice, 2017). Hydrophobic and hydrophilic interactions based on the same-dissolves-same principle may stabilise the amorphous structure of API-loaded MPs (Damiati and Damiati, 2021). Thus, H-bonds and hydrophilic/hydrophobic interactions could be related to achieving a higher LD (Pagels and Prud'homme, 2015; R. Liu *et al.*, 2022).

Both LD and EE are affected by numerous factors in the fabrication process, not only the intermolecular interactions. The solidification rate and its effect on EE have been discussed, especially in the late 1990s and early 2000s. As a general observation, it has been believed that a faster solidification rate will result in a higher EE (Jyothi *et al.*, 2010). However, this theory

is challenged by an earlier finding from Yang, Chia and Chung (2000), where they discussed that a higher temperature increased the drug diffusion into the continuous phase leading to a lower EE. Again, the rules determining the LD and EE may be more complex than estimated, as new affecting factors are observed by increased attention from researchers.

2.2.2. Challenges with polymeric microparticles

Both physical and chemical stability are of concern when formulating ASDs. The high energy state is susceptible to microenvironmental pH, temperature, and humidity variation. These conditions must be considered to ensure that quality and safety are sustained even when MPs are stored for the long term (Badawy and Hussain, 2007; Enxian *et al.*, 2009).

Appropriate temperature and humidity combined with sufficient molecular mobility may cause the crystallisation of the amorphous form. The transformation depends on the degree of molecular interactions between API and excipient and the glass transition temperature (Tg) of the API. Glass transitioning is the phase where a material transitions from a brittle crystalline state into its viscous amorphous state. The glass transition may be gradual and reversible. The Tg of ASDs may be increased with inert carriers or additives with higher Tg (Yu, 2001).

In a study of ASDs prepared by hot-melt extrusion, it was shown that chemical stability could be increased by combining with excipients such as HPMC derivatives (Alshahrani *et al.*, 2015). A high polymer concentration, high polymer molecular weight, and good ability to form strong intermolecular interactions between the drug and polymer have been reported to create physically stable ASDs (Yu *et al.*, 2022). However, increasing the amount of polymer is not always desired, as it can cause a dose burden during administration. Therefore, a polymer that can form robust interactions with the API even at relatively low polymer content is desired (Mistry *et al.*, 2015; Yu *et al.*, 2019).

Wong and colleagues (2006) also raised a valid concern; they reported that the obtained increase in particle surface area might not be sufficient to improve the absorption and bioavailability of the API. In their experiment, an attempt to enhance the dissolution rate by decreasing particle size did not perform as expected in the *in vivo* studies. Although the *in vitro* studies presented an improvement, they reported no increase in bioavailability *in vivo* could be observed. This issue could be explained by the works of Moseson et al. (2023), where they noted that the amorphous state of bicalutamide combined with polyvinylpyrrolidone vinyl acetate copolymer (PVPVA) could not be maintained for a long enough period and caused the

bicalutamide to crystallise. The crystallisation of the API would then lead to reduced solubility, as explained prior.

Hydrogen bonds have been presented as a factor affecting drug loading and maintenance of the amorphous structure in the MPs. The transformation from amorphous to crystalline is sought to be reduced due to the reduced molecular mobility caused by the increased H-bonds. The more interactive bonds there are, the more molecular mobility is restricted, and crystallisation is delayed. On the other hand, hydrogen bonds are disrupted when exposed to water, leaving the amorphous drug unprotected and susceptible to matrix crystallisation. (Moseson *et al.*, 2023) Ionic bonds are another type of molecular interaction believed to delay crystallisation thanks to the robust and rigid bonds they create (Mistry *et al.*, 2015).

Although polymer technology and MPs are no new topics in pharmaceutical development per se, there still is a great need for gathering and unifying existing knowledge (Pagels and Prud'homme, 2015; Schittny, Huwyler and Puchkov, 2020). The fabrication methods available lack understanding, possibilities for scalability and, thus, low investment from pharmaceutical companies (Pagels and Prud'homme, 2015). Furthermore, a lack of consensus on the above makes the classification of research data inconsistent (Schittny, Huwyler and Puchkov, 2020).

2.3. Fabrication of microparticles

MPs can be fabricated via numerous methods (Moghadam et al., 2008). The more traditional and commercialised methods include spray drying, emulsion solvent evaporation, coacervation, freeze-drying, and extrusion methods (Blagden *et al.*, 2007). The schematics of emulsion solvent evaporation and spray drying methods are illustrated in Figure 3.

Although these have been successfully implemented for bulk manufacturing, they have disadvantages, such as low yield and cost-efficiency. Furthermore, spray drying is unsuitable for products susceptible to thermal degradation, as the process requires high temperatures. The solvent evaporation method, which relies on droplet formation by mixing the two immiscible liquids, can reach a satisfactory particle size distribution. However, extensive parameter adjustments are required to obtain an emulsion with low particle size distribution. (Rao and Geckeler, 2011)

Some issues must be considered when working with an emulsion that is not monodisperse enough. Coalescence is a minor issue but might affect the final particle size distribution.

Coalescence is the process where the surface of two particles comes in contact and merges together, resulting in one larger particle. Secondly, Ostwald ripening is a phenomenon related to the dissolution of smaller particles and the growth, or "ripening", of larger particles in a solution. Here, the growth of larger particles is based on the lesser solubility compared to smaller particles. The smaller particles "prefer" to dissolve and merge with larger particles due to greater thermodynamic stability. Both coalescence and Ostwald ripening are related to the distribution kinetics of the emulsion, including particle size distribution. Thus, these can be addressed by adjusting the particle size distribution.

As a relatively modern fabrication method, the MESE method has gained increasing attention thanks to its tunability, controllability, versatility, and robustness (Liu *et al.*, 2017). The MESE method is presented in Figure 4. It is very similar to the bulk emulsion solvent evaporation method, although a microfluidic device replaces the droplet formation method in this process. The microfluidic device can form extremely monodisperse emulsions at a high rate (Kang *et al.*, 2008). This eliminates effects such as coalescence and Ostwald ripening.

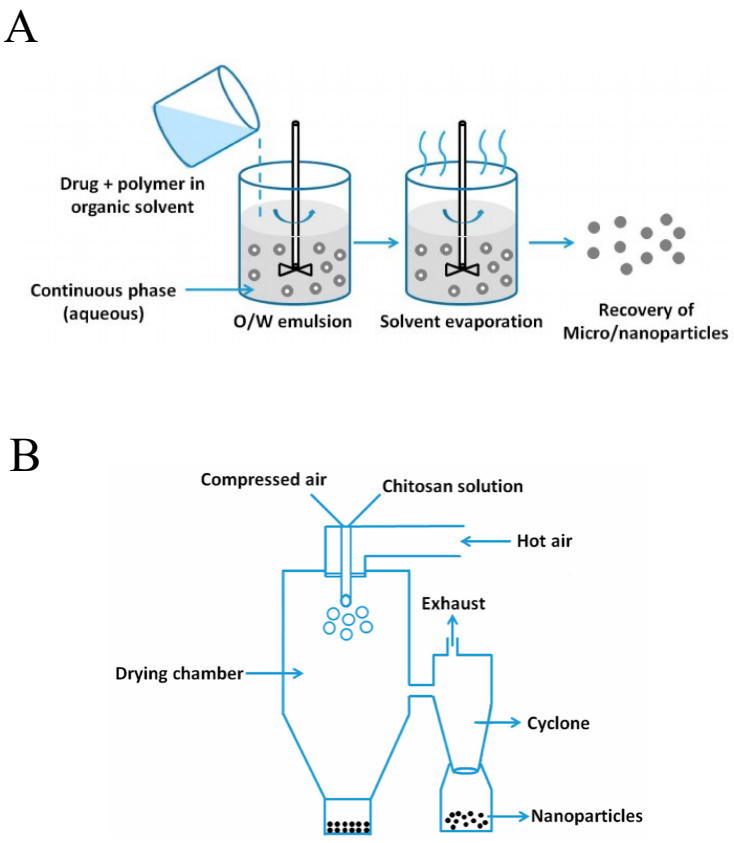


Figure 3. Illustrations of common MP & NP fabrication methods illustrated by Wang *et al.* (2016). (A) illustrates the emulsion solvent evaporation method, closely related to the MESE method, and (B) illustrates a typical spray drying method. The chitosan solution works as an example and can be replaced with another solution depending on the fabricated product.

When discussing polymer technology and microfluidics, there are some significant advantages. Firstly, microfluidics can be used to develop highly monodisperse emulsions. This enables more accurate implementation of dissolution models due to the sample's low variation in particle size, thus enabling a more precise prediction of real-time dissolution properties (Taylor and Zhang, 2016; Moseson *et al.*, 2023). Secondly, an extremely regular shape, such as a sphere, complies in a higher grade with dissolution models. The diffusion layer and its thickness are the same over the entire particle surface. This is not the case with conventional crystalline particles, which employ highly irregular geometries (Gao *et al.*, 2021). Lastly, the reduced particle size allows for faster diffusion, as previously described in this work. The fabrication method will be presented in greater detail in the next section, which is focused on.

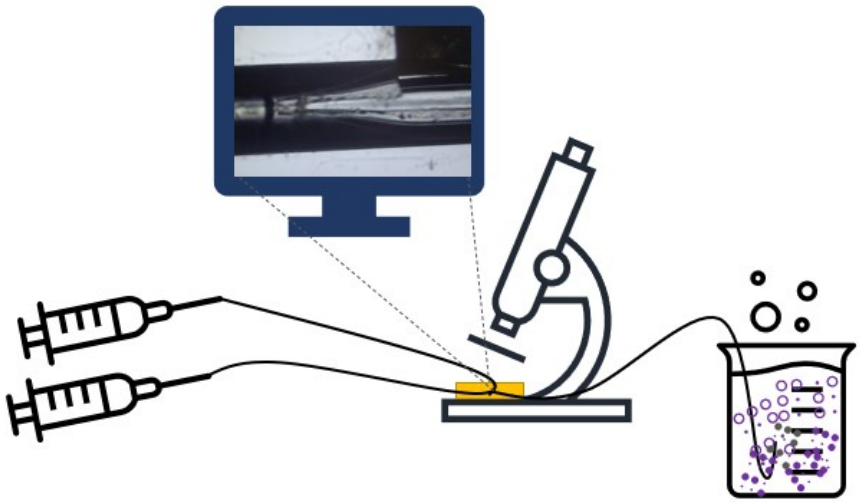


Figure 4. Schematic of a simple microfluidics-assisted fabrication set-up. In this schematic, the syringe on top and the beaker contain the outer fluid/continuous phase. The lower syringe contains the inner fluid/dispersed phase. The microscope or a high-speed camera is used to observe the fluids' flow and monitor the droplet formation. The yellow cube resembles the microfluidic device, i.e. the microchip. The purple droplets inside the beaker portray the droplet solidification to MPs.

2.3.1. Microfluidic-assisted emulsion solvent-evaporation method

According to Maeki (2019), microfluidics is generally used when controlling or manipulating a small volume of fluids on a micrometre scale. Microfluidic devices, also called microchips, are portable and have low consumption of samples or reagents, making them adaptable and suitable candidates for DDS development. Additionally, microchips can be easily built with a

vast range of materials, such as silicon, glass, ceramics, or polymer-based materials. (Wei *et al.*, 2022).

The microchip is used to produce a microemulsion. The devices come in many structures; the simpler ones can be self-built and often consist of a simple tubular system attached to a plate. The microchip creates droplets in uniform shape and size which will solidify into MPs. The concept is based on immiscible fluids, an injection tube, and a collection tube. One of the tubes usually has a tapered end resembling a funnel shape. The inner fluid, known as the dispersed phase (DP), contains the polymer and/or API will work as the building block for the MPs. The DP is injected into the outer fluid, also known as the continuous phase (CP). The liquids are immiscible or partly immiscible, resulting in a homogenous emulsion (Wei *et al.*, 2022).

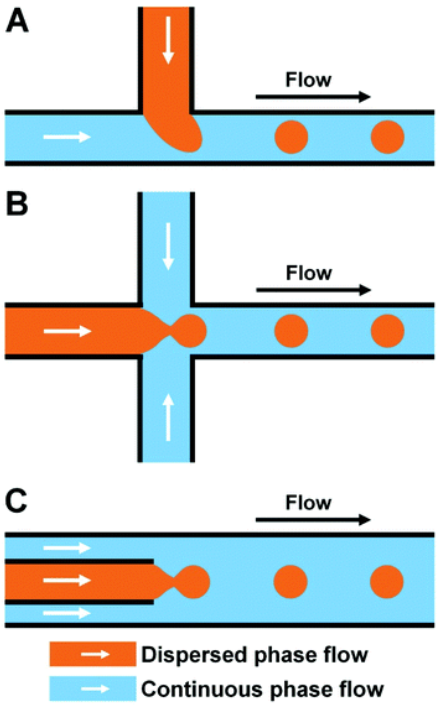
There are many geometries of microfluidic devices. The most frequently used flow-focusing, co-flow, and T-junction devices are used to fabricate spherical droplets and are presented in Figure 5. The shape and size of the droplets depend primarily on the device geometry and orifice, fluid flow rate, and fluid properties (e.g. viscosity) (Lewis *et al.*, 2005). The device's geometry describes the fluid flow direction and how the DP is "pinched" into droplets. The fluid flows into and out of the microchip are depicted in Figure 6. The shearing force between the surface tensions of the two liquids is the base for droplet formation (Zhu and Wang, 2017).

Some parameters must be adjusted before and monitored during fabrication for optimal droplet formation. For example, the flow rates of the fluids, and the ratio between these, will affect the droplet size and size distribution (Lewis *et al.*, 2005). Additionally, the fluid viscosities may also affect the droplet formation and should be considered when determining polymer, surfactant and API concentrations (as well as other excipients (Li *et al.*, 1999).

Each microfluidic method has its advantages and disadvantages. The flow-focusing approach has become popular due to its efficiency; it generates stable monodisperse MPs within the smaller size range of 1-10 micrometres (Wei *et al.*, 2022). In addition, the flow-focusing method allows precise control over the droplet size, velocity, and frequency. However, extensive parameter optimisation is required. This optimisation is currently mainly based on trial and error. Some attempts at utilising machine learning and AI in parameter optimisation have been presented (Damiati *et al.*, 2018).

Bora and colleagues (2022) successfully fabricated drug-loaded MPs in high yield with flow-focusing microfluidic technology. The fenofibrate-loaded MPs were rather large, but the size differences were achieved by tuning the DP and CP flow ratio. This resulted in particles in

sizes varying from 200 to 1200 micrometres and LDs of 64%, 41% and 25%, respectively. Interestingly, the smallest particle sizes resulted in the highest LD.



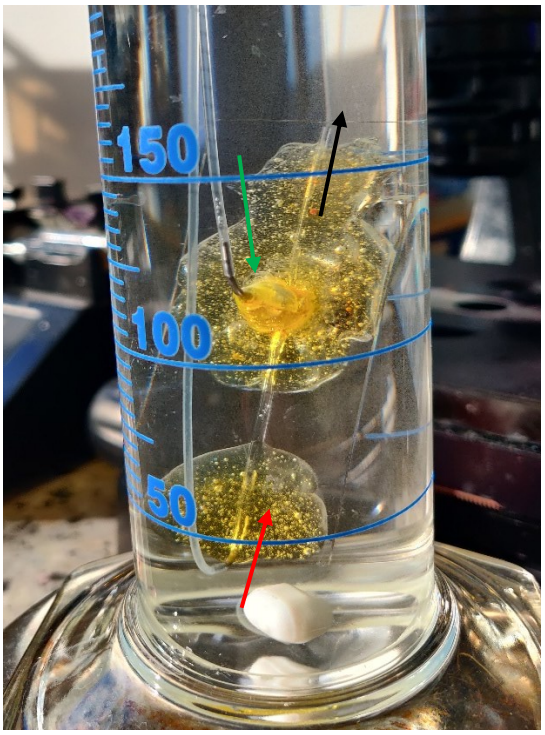


Figure 5. (Left) The three primary geometries used for microfluidic devices (i.e. microchips) for the preparation of MPs: T-junction (A), the flow-focusing (B) and co-flow (C) (D. Liu *et al.*, 2022)

Figure 6. (Right) Flow-Focusing set-up of a microchip for MP fabrication. In this example, the microchip is submerged into the continuous phase in the collection column, with its outlet facing upwards (black arrow). This way, the exiting particles are directed toward the surface. The dispersed phase is put in through a tube from the end of the microchip (red arrow), and the continuous phase from the middle of the microchip (green arrow).

Particle solidification can be established through various strategies. Notably, It has been understood that the solvent removal rate during the solidification has a profound impact on the internal structure of the MPs (Otte and Park, 2022). The solvent evaporation methods mainly on passive diffusion of the DP out of the MP followed by a polymerisation-inducing process, e.g. via UV radiation (Kang *et al.*, 2008). A lower solidification rate is believed to result in a lower EE, as the API is continuously diffused into the CP during solidification. On the contrary, a fast solidification rate would entrap the API within the rigid polymer skin resulting in higher EE (Pagels and Prud'homme, 2015). Solvent evaporation can be induced by increasing the temperature during the fabrication cycle. However, this strategy may have drawbacks, such as

lower LD and EE, as well as irregular MP matrices (Kang *et al.*, 2008; Pagels and Prud'homme, 2015).

2.3.2. Raw materials required for fabrication of polymeric microparticles

This section covers the basic principles regarding choosing raw materials to fabricate MPs through MESE. The materials make the product; thus, they play a significant role in determining the safety and quality of the formulation. The choice of materials with justifications for this specific project is described in section 4. As already discussed, microfluidic-assisted fabrication is based on the emulgation of two or more immiscible fluids into water-in-oil (W/O) emulsions or, vice versa, oil-in-water (O/W) emulsions. Hence, at least two solvents are required, of which one will work as the continuous phase and the other as the dispersed phase. This thesis oversees singular O/W emulsions; thus, this will be the standard for the rest of this work, and more complex systems will not be covered further.

The choice of the polymer depends on what type of API will be encapsulated and the desired characteristics of the MPs (Mistry *et al.*, 2015). Not only the material but the concentrations of them must be considered. As has been described, it is not always feasible to increase the polymer concentration to achieve the desired drug stability. Unfortunately, this field is still developing, and a tool for adequately choosing materials has not yet been established. Thus, the choice of materials and compatibility is based on trial and error (Mistry *et al.*, 2015).

The choice of polymer can be based on its biochemical properties, such as biocompatibility and biodegradability. Biodegradability is vital to consider, especially when developing sustained or controlled-release MPs or implants. Delayed biodegradation of the MP might, in some situations be desired, but generally the polymer must be biodegradable for safe and easy excretion from the body. This also raises the biocompatibility and toxicity into the spotlight, as the degradation products should not comprise the safety or efficacy of the product (Ting *et al.*, 2015).

Moseson et al. (2023) found that the drug loading and homogeneity of it may impact the amorphous-to-crystalline phase transformation. They suggested that an MP with a congruent release pattern can maintain a homogenous matrix longer; thus, phase transformation is less likely. They summarise their findings into one recommendation: selection of drug loading should be done so that the polymer releases the API congruently. HPMCAS has recently been

identified as a suitable polymer with evidence of maintaining a supersaturated solution in the GIT (Li *et al.*, 2019).

The first thing to assess when choosing the solvents is whether the API and polymer can be dissolved. Distilled or purified water is commonly selected as the inorganic solventTypicalon organic solvents used in MP fabrication are dichloromethane and chloroform. However, these have shown some toxicity and have started to be replaced by less-toxic ethyl acetate (EA) (Rao and Geckeler, 2011; Pagels and Prud'homme, 2015). It is important to note that EA has greater miscibility with inorganic solvents (i.e. water) than dichloromethane and chloroform, which must be considered when choosing the rest of the materials for the fabrication.

Surfactants act as stabilisers during the manufacturing process. These are commonly surface-active agents and tend to form micelles. Micelles are molecular aggregates of polar molecules, where the hydrophobic regions form a core and the hydrophilic "heads" face towards the outer surface of the micelle. These micelles can increase the solubility of lipophilic drugs. Surfactants stabilise the polymeric particle while the polymerisation, i.e. solidification of the MP, occurs. Surfactants have also been argued to increase the encapsulation efficiency of hydrophobic drugs thanks to having a greater tendency to dissolve hydrophobic substances (Italia *et al.*, 2007).

Like organic solvents, surfactants are also hazardous in biomedical and environmental applications and should be removed entirely during fabrication (Rao and Geckeler, 2011). Thus, although surfactants are important in bulk emulsion solvent evaporation and MESE, concerns about increased production times and energy concerns have been raised (Rao and Geckeler, 2011). It has been argued that a lower surfactant concentration is desired to minimise the drawbacks (Italia *et al.*, 2007). Interestingly, MPs have been prepared by microfluidic technology without surfactants, where the polymerisation of ethyl acrylate was induced with UV light (Khan *et al.*, 2013).

To briefly summarise this literature review, bioavailability remains one of the main obstacles to the market release of new drugs. However, significant input is seen from academia and industry toward solving this issue. Micro- and nanosized particles have increased interest thanks to their outstanding ability to work as drug delivery systems and form ASDs. Drug-loaded delivery systems can be fabricated by various methods, of which the microfluidic solvent-evaporation method shows great potential due to its excellent tunability and high

throughput. However, many questions concerning microfluidic-assisted fabrication are yet to be answered, leaving a great area that needs to be covered before broader implementation in the pharmaceutical industry.

3. AIM

This project aimed to understand the encapsulation properties of polymeric MPs better. This was done by combining various polymers and APIs into MPs through the MESE method. This aim is divided into two main themes with respective research questions. Firstly, we will determine the drug loading degree (LD) and encapsulation efficiency (EE) of MPs of various combinations of polymers and APIs. Secondly, we will study the solid-state characteristics of the fabricated MPs.

This work excludes experiments and analysis related to the MP's disintegration or release profiles, short-term or long-term stability, and particle size distribution. However, these are fundamental parameters in further process development and have been covered in academic literature for curious minds. The focus of this thesis is thus on fabrication-related issues rather than product-specific topics.

3.1. Determination of drug loading degree and encapsulation efficiency

The drug loading degree and encapsulation efficiency properties have been extensively researched concerning more traditional fabrication methods, such as the emulsion-solvent-evaporation method, as demonstrated by works such as El-Say (2016). However, the academic consensus concerning drug loading and encapsulation efficiency still needs to be completed through further research, especially considering modern fabrication methods such as microfluidics. Another topic that needs more agreement is what amount of loading degree is considered high for this type of MP. In this project, the goal was to achieve drug loading of over 40%. The following questions were raised:

- Which API and POLY combinations can achieve a high loading degree and encapsulation efficiency?

3.2. Evaluation of solid-state characteristics of drug-loaded microparticles

To further interpret the mechanism and reasoning behind drug loading and encapsulation efficiency, we will study the solid-state characteristics of the fabricated particles. The goal is to fabricate particles that have an entirely amorphous structure. Essentially, we aim to achieve MPs with a regular spherical shape and low porosity or channelling of the inner matrix. Again, the following questions are raised:

- Is the solid-state of the API amorphous when encapsulated in the MP?
- How does the drug loading degree relate to the solid-state characteristics?
- What is the solidification rate of the polymer, and how does it relate to LD and EE?

4. MATERIALS

The polymers used in this project were hydroxypropyl methylcellulose acetate succinate (MF grade) (HPMCAS-MF), poly(ε-caprolactone) (PCL), poly(lactic acid) (PLA), poly(lactic acid-co-ε-caprolactone) (PLA-PCL, 70:30) and poly(lactide-co-glycolide, 50:50) (PLGA). Indomethacin and simvastatin were chosen as the APIs for this project. Each API is listed with its' key chemical and pharmaceutical properties in Table II, and respective information concerning the polymers are listed in Table III. The molecular structures of the APIs are presented in Figure 7, and the polymers in Figure 8. The following sections will include short presentations of the materials.

The polymers PCL, PLA, PLGA (50:50) and PLA-PCL (70:30) were gifted by Corbion (Amsterdam, NL); HPMCAS-MF was bought from Shin-Etsu Chemical (Tokyo, JPN). IND and SIM were purchased from Sigma-Aldrich (US). Other materials used in fabrication and assays were of analytical or HPLC grade.

4.1. Active Pharmaceutical Ingredients

SIM and IND are prime examples of APIs with poor bioavailability and other factors limiting efficient use. Simvastatin, which is an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA) and is used as a cholesterol-lowering drug, possesses a very poor

bioavailability of only 5% and a high protein binding capacity of 95%. Furthermore, simvastatin has a high log P (octanol/water) of 4.68, making it a lipophilic drug (Corsini *et al.*, 1999).

Indomethacin is an NSAID commonly used for inflammatory pain such as rheumatoid arthritis. The mechanism of action is based on nonspecific and reversible inhibition of cyclo-oxygenases that are a part of prostaglandin synthesis (National Center for Biotechnology Information (NCBI), 2023a). Like SIM, IND also has poor water solubility and bioavailability. Furthermore, like other NSAIDs, IND tends to easily cause ulceration and haemorrhage in the GIT due to its narrow therapeutic window. A formulation that allows the controlled release of IND is desirable to minimise adverse events.

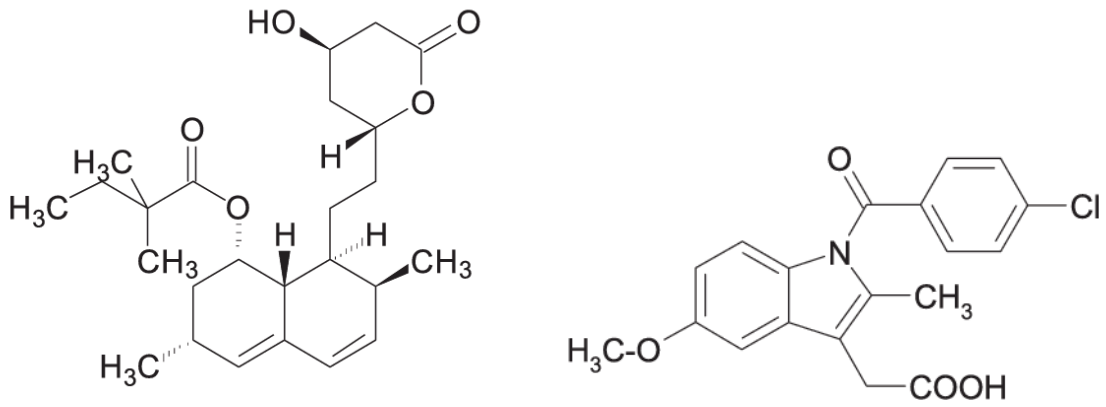


Figure 7. Molecular structures of simvastatin (left) and indomethacin (right). (Murtaza, 2012; Ruidiaz Martinez, Delgado and Martínez, 2023)

Table II. Physicochemical and biopharmaceutical properties of indomethacin and simvastatin

	INDOMETHACIN	**SIMVASTATIN**	**References**
Molecular Weight	357,8 g/mol	418.6 g/mol	*(Ph. Eur. 11.2. 04/2019:0092; 04/2019:1563)*
Molecular Formula	$C_{19}H_{16}ClNO_4$	$C_{25}H_{38}O_5$	*(Ph. Eur. 11.2. 04/2019:0092; 04/2019:1563)*
LogP	4.3	4.68	(NCBI 2023a, NCBI 2023b)
Log K_{ow}	0.91 (at pH 7.4)	4.68	(NCBI 2023a, NCBI 2023b)
Solubility (25 °C)	Practically insoluble in water, sparingly insoluble in ethanol (96%)	Practically insoluble in water, very soluble in methylene chloride, freely soluble in ethanol (96%)	*(Ph. Eur. 11.2. 04/2019:0092; 04/2019:1563)*
Hydrophilic/-phobic	Hydrophobic	Hydrophobic	*based on LogP and RO5*
H-bond donors	1	1	(NCBI 2023a, NCBI 2023b)

	INDOMETHACIN	SIMVASTATIN	References
H-bond acceptors	4	5	(NCBI 2023a, NCBI 2023b)
Rotatable bonds	4	7	(NCBI 2023a, NCBI 2023b)
Polar surface area	68.5 Å	72.8 Å	(NCBI 2023a, NCBI 2023b)
Melting Point	158-162 °C	135-138 °C	*(Ph. Eur. 11.2. 04/2019:0092; 04/2019:1563)*
BCS Class	II	II	(Surwase *et al.*, 2013; Simões *et al.*, 2018)
Polymorphism	Eight different forms	Three different forms	(Surwase *et al.*, 2013; Simões *et al.*, 2018)

4.2. Polymers

The selected polymers are briefly presented in this section. The fundamental physicochemical properties are listed in Table III, and the molecular structures are shown in Figure 7.

HPMCAS is a cellulose-derived polymer available in various grades (L, M and H). The grade is determined by the content of acetyl, succinyl, methoxy- and hydroxypropyl groups. HPMCAS contains carboxylic acid groups that act as proton donors, which can form strong intermolecular interactions through ionic interaction and hydrogen bonds. Thus, HPMCAS has received much attention due to its great stabilising effect. (Ting *et al.*, 2015; Yu *et al.*, 2022)

PLGA is another polymer that has gained increased interest over the last decade. It is a biodegradable and biocompatible polymer, of which the drug release is reported to be two-phased. First, the API will be released from the polymer matrix through diffusion, after which the degradation of PLGA will launch an accelerated release pattern (Italia *et al.*, 2007). The first products containing PLGA were launched in 1989, after which approximately 20 new PLGA-based products have been released (Otte and Park, 2022).

Table III. Physicochemical properties of polymers used in the fabrication of MPs in this project

	HPMCAS-MF	PLGA (50:50)	PLA-PCL (70:30)	PLA	PCL
Molecular weight (g/mol)	17 000	17 000	80 000	15 000	80 000
H-bond donors	Yes	No	No	No	No
H-bond acceptors	Yes	Yes	Yes	Yes	Yes
Tm	Amorphous	Amorphous	168 °C	172 °C	59-64 °C
Tg	ca 120 °C	45-50 °C	ca 60 °C	62,5°C	60 °C
Hydro-/lipophilic	Amphiphilic	Hydrophobic	Hydrophobic	Hydrophobic	Hydrophobic
Reference	(NCBI, 2023c)	(Friesen *et al.*, 2008)	(Matta *et al.*, 2014)	(Zhou *et al.*, 2007; Matta *et al.*, 2014)	(Azimi *et al.*, 2014)

Copolymers such as PLA-PCL and PLGA are useful when the properties of two different molecules are desired. By variating the ratios of the monomers in each polymer blend, one can tune the polymer's properties, such as degradation rates and release rates (Pagels and Prud'homme, 2015). Furthermore, when it has been established how different functional groups impact the API-polymer interactions, new sophisticated polymers/copolymers can be designed to answer those requirements (Ting *et al.*, 2015).

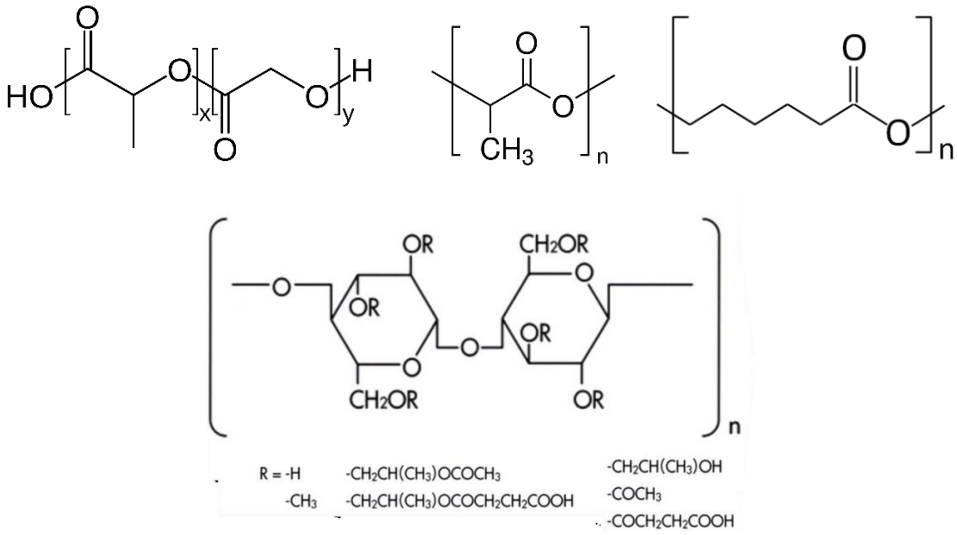


Figure 7. Molecular structures of polymers chosen for this project, from upper left to right: PLGA, PLA, PCL and HPMCAS-MF on the lower row. (Fvasconcellos, 2008; Polimerek, 2008; Sbyrnes321, 2012; Nasereddin, 2020)

5. METHODS

The MESE method was used for the fabrication of MPs. The sample groups, the naming of samples, and which samples were included in each assay are presented in Annex 1. A total of four different sample groups were fabricated and used in the assays,

1. Control 1 (C1): Pure polymer MP
2. Control 2 (C2.1 and C2.2): Pure API powder
3. Control 3 (C3.1 and C3.2): Physical mixture of pure polymer MPs with pure API powder (API+POLY)

4. Sample (S): API-loaded polymer MPs (API@POLY)

The fabrication method is explained in detail in the next section, after which the corresponding assays and techniques will be presented.

5.1. Fabrication of microparticles

The fabrication process begins with preparing the solutions used in microfluidic fabrication. The MPs are then produced. The fabrication process's final steps include washing and collecting prepared particles and lyophilising before the particles can be used in designated assays. The fabrication setup and process flow are presented in Figures 7 and 8, respectively.

5.1.1. Preparation of dispersed and continuous phases

The dispersed phase (DP) was prepared by dissolving the materials into ethyl acetate (EA). In the preparation of the pure polymer MPs (C1), only the polymer is dissolved; for the drug-loaded MPs (S), both the polymer and API are dissolved. The polymer concentration was always kept at 10 mg/ml, regardless of the type of particles prepared. Indomethacin samples were prepared at a concentration of 15 mg/ml and simvastatin at a concentration of 10 mg/ml.

The preparation of the DP was performed in a 15 ml falcon tube and was transferred into a 5 ml syringe after complete dissolution (Terumo, JPN). In this project, 5 ml of DP was prepared for each run at a flow rate of 1 ml/h. A 1% solution of Kolliphor P407 (Sigma-Aldrich, US) with pH adjusted to 5 was used as the continuous phase (CP). The pH adjustment was made after the dissolution of Kolliphor P407 into Milli-Q water by adding hydrochloric acid (HCl) or sodium hydroxide (NaOH). The acid or base was added until the desired pH of 4.90-5.10 was reached. The CP was drawn into a 50 ml syringe (Terumo, JPN) in addition to a collection column containing 420-450 ml of CP for the fabrication of drug-loaded MPs (S) and 60-80 ml of CP for fabrication of pure polymer MPs (C1). A magnetic stirrer was used with PLGA particles to reduce the aggregation of the MPs.

5.1.2. Fabrication of microparticles

Handmade glass capillary microchips with an inner orifice of 100 μm were used to fabricate the MPs. Flow-focusing (FF) geometry was used due to excellent properties such as fine-

tuneability and high throughput. The DP and CP were drawn into syringes, set up in respective pumps (Harvard Apparatus, US), and connected to the microchip with plastic tubes. A light microscope (Leica Microsystems, DE or Thermo Fisher Scientific, US) was used to monitor the stability of the flow and desired droplet formation. When preparing the drug-loaded MPs, the microchip was carefully sunk into the collection column with the outlet facing upwards. This step was done only after a steady droplet formation and flow were achieved. The fabrication process of drug-loaded MPs is captured in Figure 8, and the fabrication set-up of instruments and materials is pictured in Figure 9.

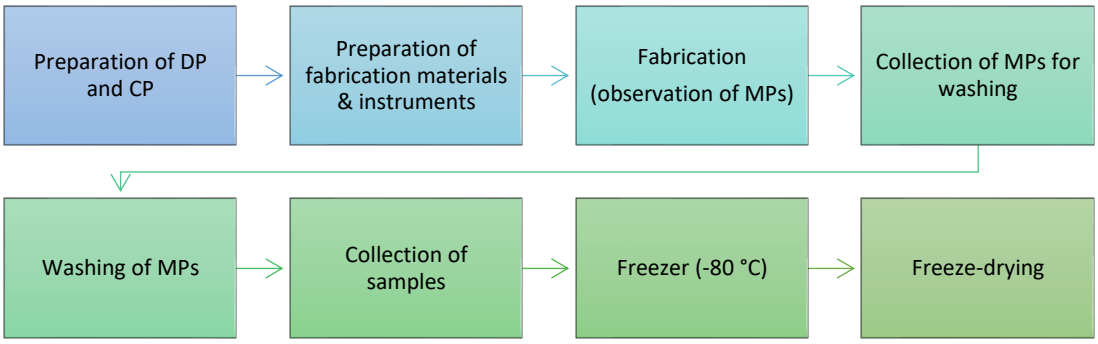


Figure 8. The process flow of the fabrication of MPs in this project.

When the particles exit the microchip into the continuous phase, the low density of the DP will cause the droplets to rise toward the surface of the CP. As described in section 2.2, the dispersed fluid will start evaporating out of the formed droplets, leaving behind a solidified MP with the API entrapped inside the particle matrix. After solidification, the particle will sink to the bottom of the column. Once fabrication was complete, the column was covered and set aside to let all particles sink to the bottom for more accessible collection. After the CP had turned transparent and the particles could be observed in the bottom of the vessel, the particles could be collected. The particles are observed under a light microscope during the fabrication process to detect any crystal formation (Figure 10).

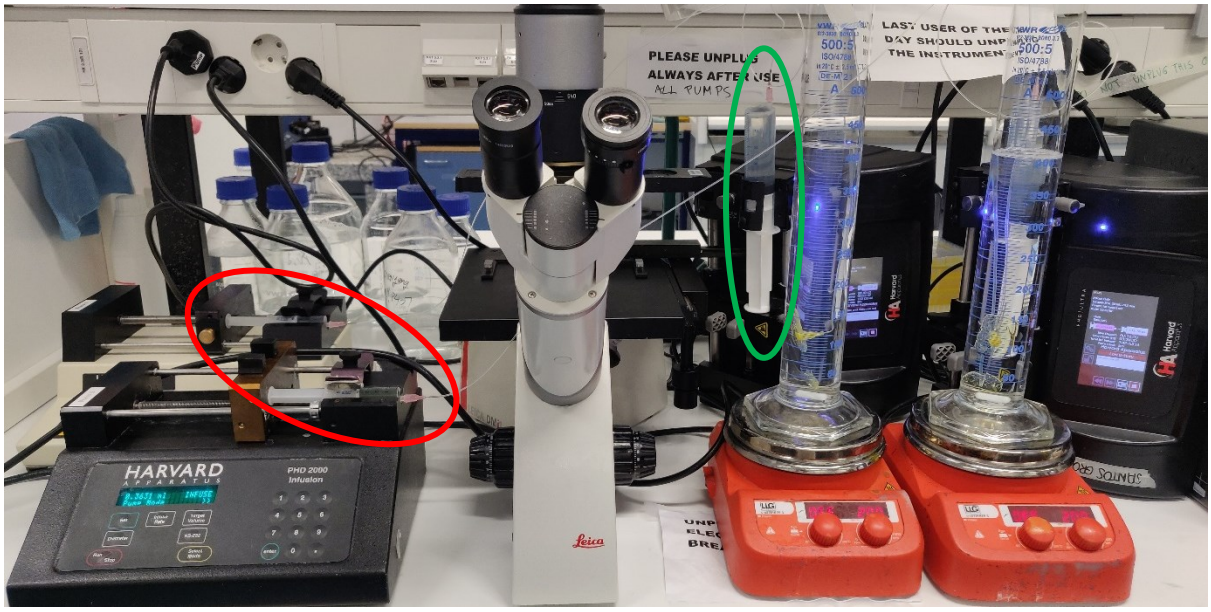


Figure 9. Two fabrication setups are pictured. One setup consists of a light microscope in the middle, the dispersed phase syringes attached to syringe pumps (red) and the continuous phase syringes attached to the respective pumps (green) (the second syringe is covered by one of the collection columns). The microchips are submerged in the collection columns on the right-hand side of the picture.

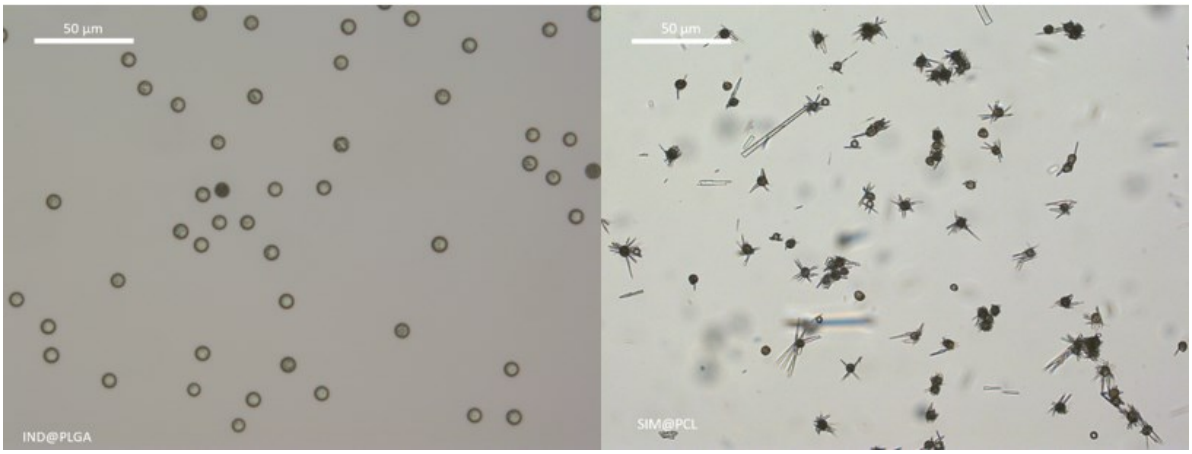


Figure 10. Light microscope images of MPs during fabrication. IND@PLGA MPs with precise spherical shape and no crystals (left) and SIM@PCL particles with crystals (right). Photos were taken during fabrication with a light microscope (Leica Microsystems, DE).

5.1.3. Collection and washing of microparticles

Careful and extensive washing is hydrophobic residual solvents or surfactants from the prepared sample (Rao and Geckeler, 2011). The collection and washing of particles occur by carefully discarding the CP and ensuring that the MPs remain at the bottom of the column. The

particles could then be transferred and rinsed with Milli-Q water into a 50 ml Falcon tube. For the washing, the falcon tube was filled with 35-50 ml of Milli-Q water, and the particles were dispersed by carefully shaking the tube, mixing with a pipette or by sonication if needed, whereafter, the particles sank to the bottom of the tube. The washing was repeated a total of three times. Centrifugation was avoided when washing drug-loaded MPs to minimise the risk of API escaping the MP. Alas, pure polymer MPs were centrifuged instead of sinking. After the last washing, the particles were collected by letting them fall to the bottom, and the excess water was discarded so that approximately 5 ml remained. The particles were then collected into 2 ml Eppendorf tubes. The collection process varied depending on which assay the fabricated MPs were used for.

5.1.4. Lyophilisation

For the determination of drug loading degree and encapsulation efficiency, three 1 ml samples are collected. Before sampling, the weights of the empty Eppendorf tubes are recorded. This is done so that the weight of the MPs can be calculated after lyophilisation. Before collection, the MPs are dispersed into the small remainder of water (ca 5 ml) to ensure homogenous samples, after which 1 ml of the fluid is collected into each tube. The MP collection for all other assays is different from what has been described, as the exact weight of the sample is not needed, and we aim to gather as much as possible of the MPs. In this case, the MPs are sunk to the bottom of the small remaining amount of water, after which the particles can be collected with a pipette and transferred into one Eppendorf tube. When all samples are collected, the tubes are frozen at -80 °C for at least one hour. When the samples are completely frozen, they are lyophilised overnight. The dried MPs are stored in a closed Eppendorf tube inside a Falcon tube in a dark and dry place.

Some things to address with lyophilisation or other fast solvent removal methods: particles may be trapped in a non-equilibrium structure that may affect the release profile over time when the structure relaxes. Another thing to address is that a polymer shell may form, which in turn might trap the solvent inside of the particle. This needs to be considered to ensure the proper safety of the product. (Pagels and Prud'homme, 2015)

5.2. Determination of drug loading degree and encapsulation efficiency

The loading degree and encapsulation efficiency were determined with reversed-phase high-performance liquid chromatography (RP-HPLC) on drug-loaded MPs. The RP-HPLC creates a standard curve that can be utilised to define the API concentration (µg/ml) in the samples. The obtained data helps calculate the fabricated particles' drug loading degree and encapsulation efficiency. The method parameters are presented in Table IV. The RP-HPLC (Agilent Technologies, US) was used to determine the API concentration in each sample. Each assay included three samples so that a reliable average could be achieved. The data were integrated and analysed with Agilent Technologies HPLC software.

As explained in the previous section, three Eppendorf tubes are weighed, and 1 ml of MPs dispersed in Milli-Q water is transferred to each tube. After lyophilisation, the tubes are weighed a second time so that the weight of the MPs can be calculated by subtracting the weight of the empty tube from the weight of the tube containing the MPs. The MPs are then dissolved into 1 ml of dimethyl sulfoxide (DMSO), followed by generous mixing and sonication to ensure complete dissolution. Then, the samples are diluted 10-folded with the RP-HPLC mobile phase. Dilution was done by combining the DMSO solution and mobile phase in a 1:9 ratio to a total volume of 1 ml.

The RP-HPLC was used to establish a standard curve and determine each sample's API concentration. Each assay included three samples to achieve a reliable trend on average concentration. Data were integrated and analysed with the Agilent Technologies HPLC software.

Table IV. Method parameters for RP-HPLC for determination of drug loading degree and encapsulation efficiency

	Indomethacin	**Simvastatin**
Mobile phase & diluent (ACN:AA, pH 3.0)	65:35	85:15
Retention time (approx.)	5.5 min	3.7 min
Total run time	6 min	5 min
Detection wavelength	240 nm	238 nm
Injection volume	20 µl	
Dilution factor	10	
Column	Supelco analytical, Discovery C18 (15 cm x 4.6 mm, 5µm).	
Temperature	Room temperature (21 °C)	
Flow rate	1.0 ml/min	

5.3. Determination of solid-state characteristics

The solid-state characteristics are determined by Differential Scanning Calorimetry (DSC), Attenuated Total Reflection-Fourier-Transform Infrared Spectroscopy (ATR-FTIR), and Scanning Electron Microscope (SEM) imaging. Initially, MPs were supposed to be analysed with X-Ray Powder Diffraction (XRPD) in addition to the other assays, but unfortunately, the instrument was out of order and could not be repaired before the end of the experimental phase of this project.

5.3.1. Differential Scanning Calorimetry

A true crystal has a specific melting point, which can be identified by its sharp peak in the DSC thermogram. An amorphous structure with no regular structure in its solid state will not have any specific melting point and will, therefore, not present sharp peaks or other signs of melting (Yu et al., 2022). DSC is especially useful in determining the solid-state structure and the stability or metastability of polymorphs. Combined with XRPD, this technique is a very robust method for identifying polymorphic forms.

DSC was used to study the samples' melting points, or lack thereof, upon heating at a rate of 10 °C/min from 25 °C to 275 °C under a nitrogen gas flow of 50 ml/min. The parameters for DSC are presented below in Table V. Approximately 1-3 mg of each sample was sealed in hermetical aluminium pans with an empty pan as a reference. The thermograms were plotted using OriginPro 2023 (OriginLab Corporation, US).

Table V. DSC method parameters for all sample groups

Sequence	Dynamic
Crucible	Aluminium, hermetical seal, two pinholes
Crucible volume	40 µl
T_{start}	25 °C
T_{target}	200 °C
Heating rate	10 °C/min
Purge gas	N_2
Purge gas flow rate	50 ml/min

5.3.2. Attenuated Total Reflection – Fourier-Transform Infrared Spectroscopy

The intermolecular interactions were examined with ATR-FTIR spectroscopy with Thermo Scientific's iS50 FTIR (US), and the obtained spectra were observed with OPUS software (US). The drug-loaded MPs (S1 & S2) and pure API powder (C2.1 & C2.2) were used for this experiment. The spectra were obtained in absorbance mode at room temperature. The scanning range was from 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1} and 64 scans for each sample. The peaks were plotted using OriginPro 2023 (OriginLab Corporation, US).

5.3.3. Scanning Electron Microscope

The particles were cross-sectioned with cryo-microtome to investigate MPs' surface morphology and inner structure. The cross-sectioned MPs were mounted onto metallic stubs with double-sided adhesive. Then, the stubs and MPs were coated with a 5 nm thick platinum coat. The MPs were then examined with a scanning electron microscope (Philips, NL).

5.4. Determination of microparticle solidification rate

Determining the solidification rate measures the time required for one polymer MP to solidify from droplet formation to a solid particle. This phenomenon is monitored by observing the solidification of a single pure polymer particle with a light microscope and measuring the MP diameter. A picture is taken, and the diameter is measured until the particle has completely solidified. For the first 20 minutes, the size is monitored every two minutes, and for the exceeding time, every 5 minutes. The solidification is complete when the following factors can be observed:

- the MP diameter is unchanged,
- the MP has lost its transparency,
- the MP has sunk to the bottom of the capillary and, thus, fallen out of focus.

Unlike the other tests and particle preparations, we used an elongated glass capillary microchip with a co-flow geometry to produce the singular MP for this experiment, pictured in Figure 11. The co-flow geometry is a better option for this purpose, as it enables the fabrication of only one particle with a finely tuned size. The solidification rate study of pure PCL MPs is demonstrated in Figure 12.

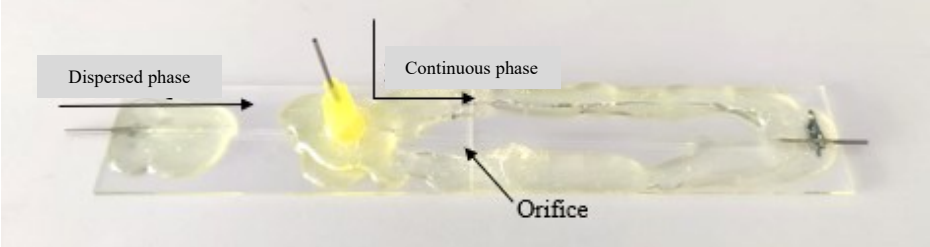


Figure 11. The elongated co-flow microchip was used to prepare particles for the solidification rate study.

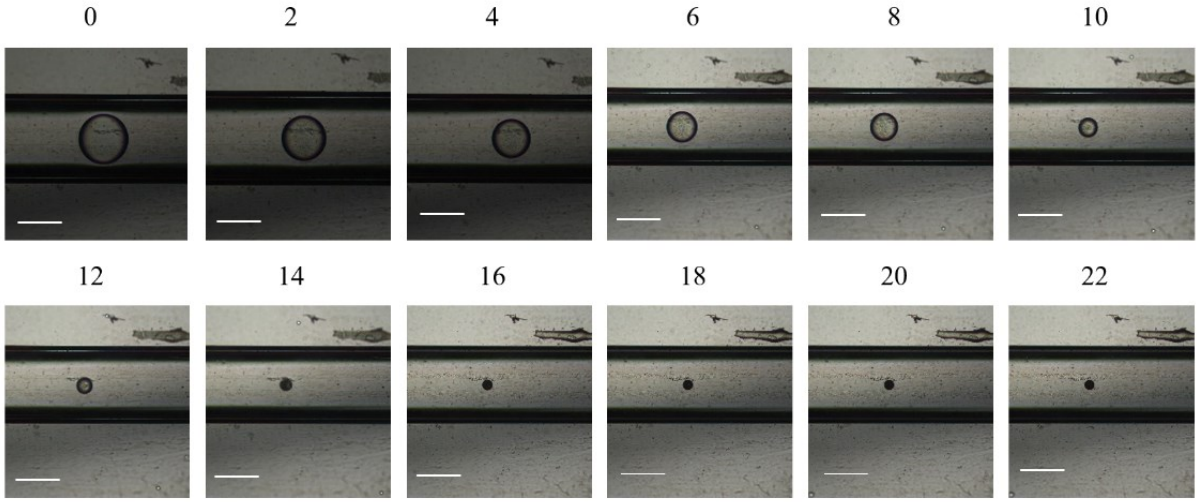


Figure 12. The determination of solidification rate was conducted with visual observation through a light microscope; numbers represent the minutes passed since droplet formation. The white scale bars are 50 µm in all pictures.

5.5. Cell viability studies

Cell viability measures the number of healthy cells in a sample, and these assays are mainly used to screen the response of cells against a drug (Kamiloglu *et al.*, 2020). Although all the polymers used in this project are widely utilised in biomedical applications and have FDA approval, we decided to perform an *in vitro* cell viability assay to ensure that the fabrication does not leave any toxic residuals in the final product.

RAW264.7 cells were seeded into a 96-well plate (100 µl, $1x10^4$ cells/well) for this experiment and cultured overnight. Then, the culture medium was discarded, and the cells were exposed for 24 hours to solutions of pure polymer MPs dispersed in the culture medium. The experiment was performed with two different MP concentrations: 10 µg/ml and 50 µg/ml. The blank group consisted of triptan, and the negative group consisted of only cells with only culture medium

(no manipulation). All experiments were performed six times, and the cells were observed under a light microscope before the assay, as presented in Figure 12. CellTiter Glo (Promega Corporation, US) was used for the assay, and the cell viability results were analysed with a Varioskan microplate reader (Thermo Fisher Scientific, US).

6. RESULTS

6.1. Loading degree and encapsulation efficiency

The drug LD was determined with data obtained from the RP-HPLC experiment. The LD was calculated according to Equation 3, which states that the encapsulated API weight is divided by the total weight of the MPs. The EE was calculated according to Equation 4, where the actual weight of encapsulated API is divided by the theoretical drug weight. The results are presented in Table VI and Figure 13 for each API@POLY-MP.

Overall, higher LDs could be obtained for the IND-MPs. Of the indomethacin group, the PLA-PCL, PLGA, and HPMCAS-MF exceeded the aimed LD of 40%, and PLA was just below with an LD of 36.3%. However, IND@PCL did not reach a higher average than 2.3%; the lowest LD obtained overall. The SIM-MPs would not reach as high LDs as the IND-MPs but had two groups exceeding the limit of 40%, PLGA and PLA-PCL. Interestingly, SIM@PCL showed a higher LD than its IND counterpart, with LD exceeding 10%.

The encapsulation of SIM in PLGA and PLA-PCL was higher compared to the IND counterparts. This was expected as the polymer-to-API-ratio was higher when fabricating the SIM-MPs. Overall the EE results were higher compared to LD, and relatively linear compared to the LD. IND@PCL stands significantly out with the low result of 1.56%.

Table VI. Loading degrees and encapsulation efficiencies with standard deviations. The aim was to reach LD > 40%, which has been marked with a dotted line

	Loading Degree (%)	Standard dev. (LD)	Encapsulation Efficiency (%)	Standard dev. (EE)
IND@PLA-PCL	49.99	0.24	66.63	0.65
IND@PLGA	48.79	1.35	63.58	3.39
IND@MF	44.83	0.64	54.18	1.41
SIM@PLGA	43.12	4.8	76.67	15.35
SIM@PLA-PCL	40.99	1.67	69.55	4.75
IND@PLA	36.32	10.47	40.12	19.22

	Loading Degree (%)	Standard dev. (LD)	Encapsulation Efficiency (%)	Standard dev. (EE)
SIM@MF	32.75	8.14	50.25	19.1
SIM@PLA	25.06	4.17	33.72	7.68
SIM@PCL	12.27	5.74	14.32	7.74
IND@PCL	2.25	2.11	1.56	1.49

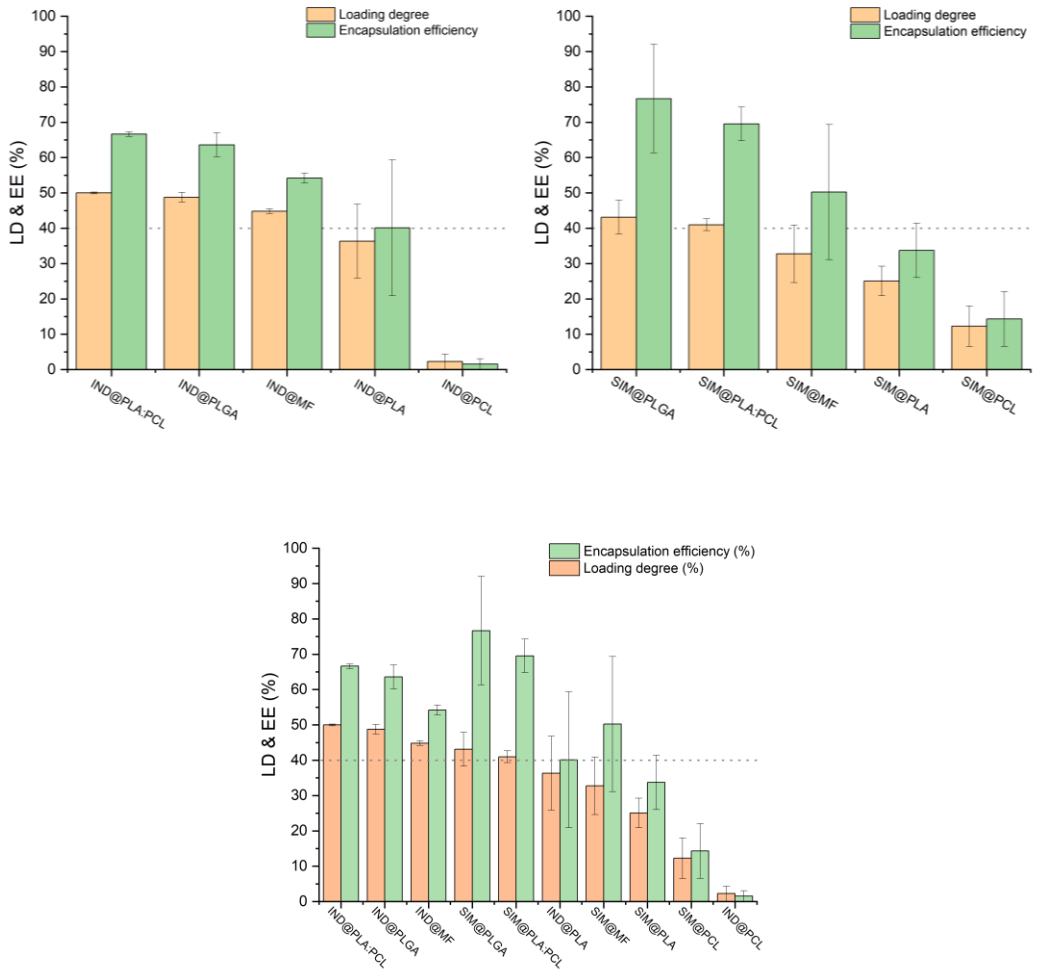


Figure 13. LD and EE results as column charts sorted from highest LDs to lowest for IND-MPs (top left) SIM-MPs (top right), and all samples (bottom).

6.2. Solid-state characteristics

6.2.1. Differential Scanning Calorimetry

The thermal analysis results are presented as the graphs of the API@POLY samples and control groups of pure polymer MPs, pure API and the physical mixture in Figures 14 and 15. The

thermal specifications of the APIs are listed in Table II, and the polymers in Table III. The pure API graphs present the typical thermal characteristics of simvastatin and indomethacin and portray their distinctive melting points, proving they exhibit a crystalline structure as a standard.

The pure polymers HPMCAS-MF, PLGA, PLA and PLA-PCL possess amorphous structures; this is proven by the fact that they present only the Tg as thermal activity in the DSC thermogram, which is characteristic of amorphous forms (Yu, 2001). As expected, based on its specifications, PCL presented a sharp peak near 60 °C, indicating its crystalline structure. This could be one explaining factor as to why the LD of API-loaded PCL MPs remains relatively low compared to other API-polymer combinations.

It was found that IND@HPMCAS-MF, IND@PCL, SIM@PLGA and SIM@PCL were the only combinations that presented an amorphous API structure. However, in both PCL cases, the polymer seems to be crystallised as they show a small peak forming in the region of its Tm. Especially the IND MPs portrayed signs of polymorphs in the samples; this can be observed by the endothermic peaks close to the Tm of crystalline IND. This could be a sign of instability within the MP matrix. If the polymer cannot stabilise the API in its amorphous state, the API will start to transform to its more stable states, such as a polymorph or crystalline. These curves also show exothermic activity in the 90-100 °C region. This exothermic activity is macroscopic crystallisation, typical for polymorphous indomethacin (Svoboda *et al.*, 2022).

Some errors in the process that could explain some deviations include low sample amount and too long or wrong storage conditions before conducting the experiment. A low sample amount may not give a strong enough sign to be detected or interpreted. The SIM@HPMCAS-MF MPs could be an example of this issue. This is indicated by the very tiny curve in the same region as the melting point of SIM. However, it is so tiny that it is hard to determine if it is a melting point or, e.g. glass transitioning.

Another plausible explanation for why so many of the MPs show signs of crystallinity or polymorphs is that the samples were not analysed right after lyophilisation. Due to time limitations, some samples had to be stored for a while before being analysed. While waiting to be analysed, the MPs were stored at room temperature, protected from light and humidity. This storage period could be the reason why some of the samples portrayed polymorphism or crystallinity. Even if this were the case, the obtained information is beneficial and raises some follow-up research topics.

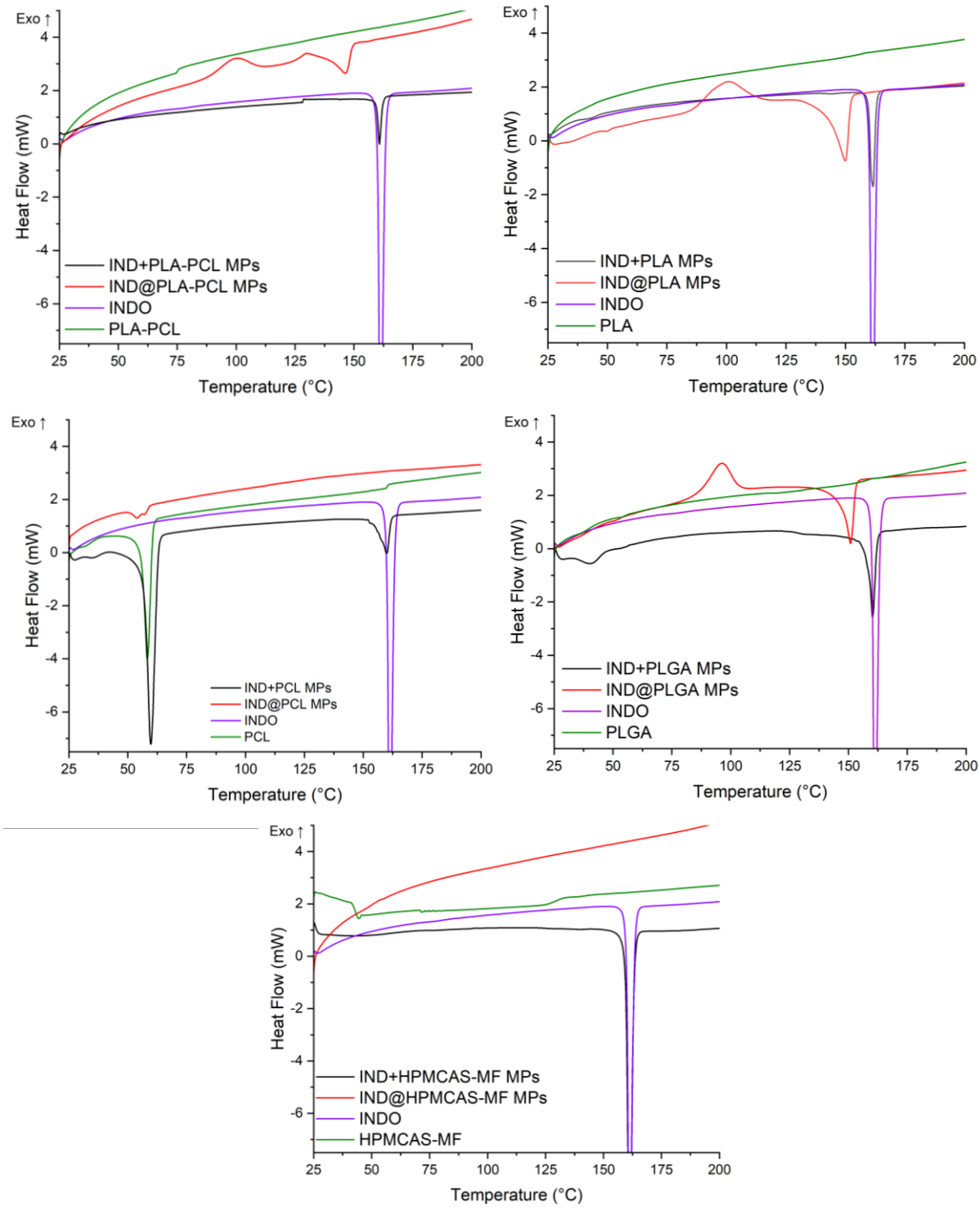


Figure 14. Obtained DSC curves for the indomethacin group**.** The aim was to achieve a fully amorphous structure for the drug-loaded MPs, which can be observed when no sharp peaks or signs of melting are shown.

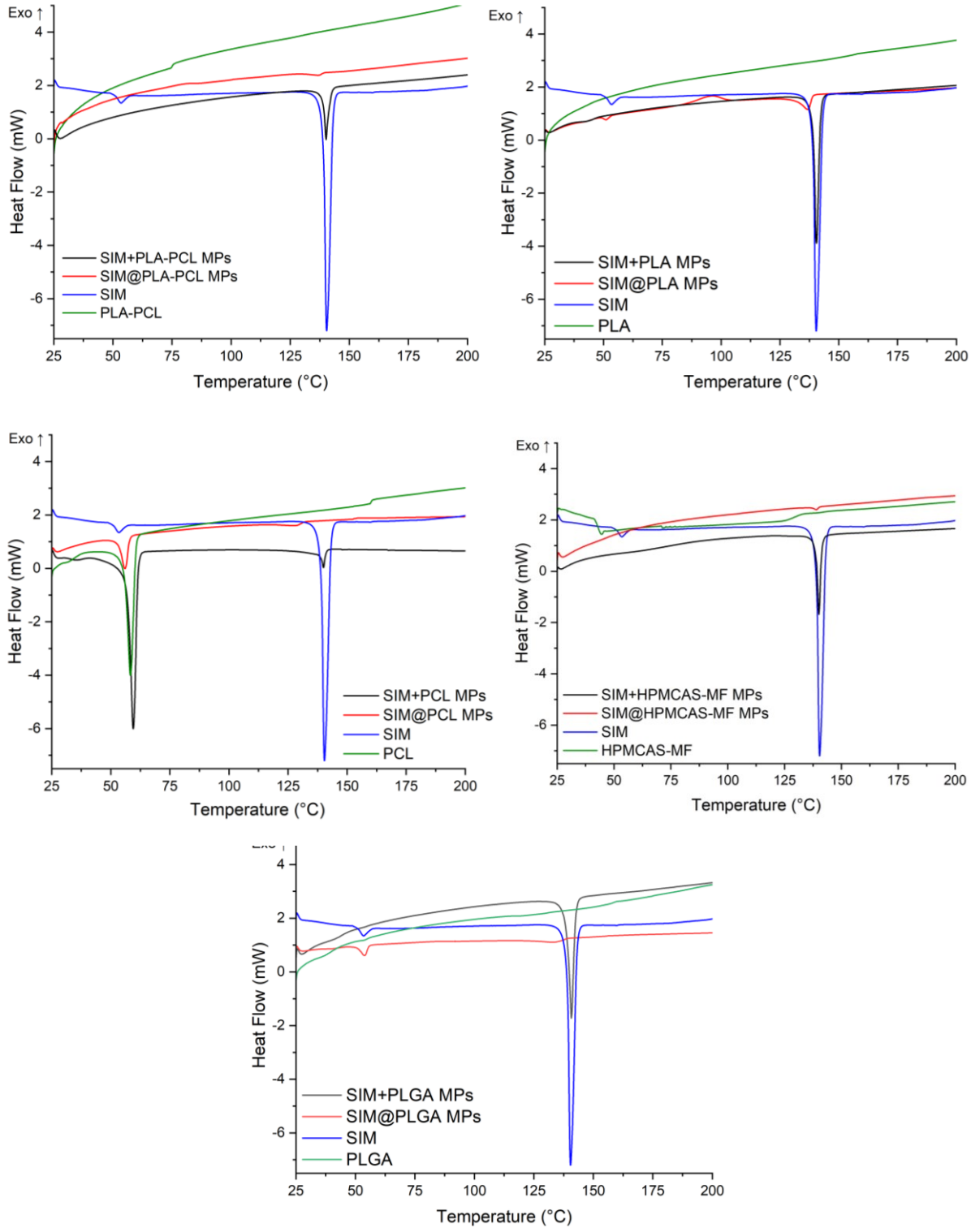


Figure 15. Obtained DSC curves for the simvastatin group**.** The aim was to achieve a fully amorphous structure for the drug-loaded MPs, which can be observed when no sharp peaks or signs of melting are shown.

6.2.2. Attenuated total reflection – Fourier-transform infrared spectroscopy

ATR-FTIR spectroscopy was used as a second method to determine the solid state of the samples. In addition, ATR-FTIR could be used to determine whether there are molecular interactions between the API and polymer. Regarding the solid-state characteristic analysis, each spectrum was focused on the wavenumbers 4000-2000 cm^{-1} for more straightforward interpretation. The spectra between 2000-400 cm^{-1} are presented in Figure 16 for IND and Figure 17 for SIM and are included as these show the busiest area of the IR spectrum. The spectra in full sizes are included in Annex II. Additionally, some of the characteristic bands for the APIs were chosen and focused on for the interpretation of molecular interactions.

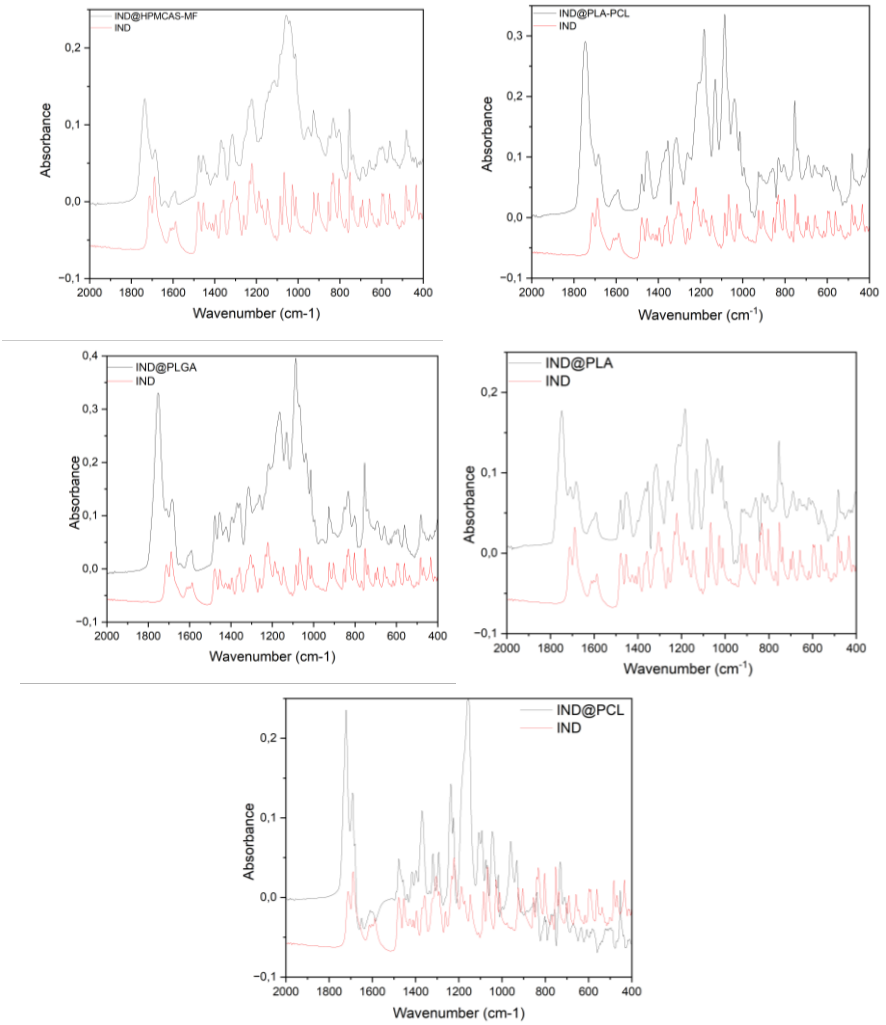


Figure 16. IND-loaded MP and pure IND spectra focused on the 2000-400cm^{-1} region.

The solid-state characterisation is done by comparing the pure API spectrum to the API-loaded MP spectrum. An amorphous structure will not result in as sharp or distinguished peaks as the crystalline but can be broader or more muted. IND@HPMCAS-MF, IND@PLGA, and IND@PLA-PCL show fewer peaks than pure IND; some peaks seem to have merged to a broader peak. This indicates that the MP is in an amorphous state. However, as HPMCAS-MF is naturally amorphous, it would be good to ensure that the IND@HPMCAS-MF spectrum is not only a summation of the API and polymer spectra. Both IND@PLA and IND@PCL samples show a higher number of peaks compared to the other API-polymer combinations. However, some differences between the MPs and API can be seen, for example, around wavenumbers 1800-1400 cm^{-1} for IND@PCL and throughout the IND@PLA spectrum.

As IND is known for having multiple polymorphic forms, this raises the question of whether these samples portray a polymorphic form rather than a completely amorphous or crystalline nature (Surwase *et al.*, 2013). Retrospectively, an analysis of the other control groups, such as pure polymer MPs (C1) and physical mixture (C3.1. & C3.2.), would greatly help determine the solid-state characteristics. It is challenging to decide which bands could be caused by the present polymer and which are relevant changes in the API's vibration and bending behaviour.

The spectra of SIM-loaded MPs are analysed to assess potential molecular interactions between the API and polymer. SIM portrays characteristic bands that can be found at wavenumbers 3550 cm^{-1} (free –OH), 1043 cm^{-1} (C−O−C) and 1011 cm^{-1} (C=O) (Bonthagarala *et al.*, 2013). These spectra are presented in Figures 18 and 19, respectively. A shifted and broadened curve in the O–H stretching region (3550 cm^{-1}) indicates hydrogen bonding, as the O–H group of SIM acts as a hydrogen bond donor (Ting *et al.*, 2015). This phenomenon can be observed in SIM@PLA-PCL, SIM@HPMCAS-MF and SIM@PLA. The SIM@PCL MPs present a slightly lower peak compared to pure API. Still, it does not show any significant difference in peak shape (broadening or shoulder) characteristic of hydrogen bonding (Singh, Philip and Pathak, 2012). The SIM@PLGA presents a minimal peak forming at 3550 cm^{-1}. However, it isn't easy to distinguish the root cause for this. The sample concentration might be so low that it won't translate a strong enough signal, or another explanation might be that some other type of molecular interaction is taking place at this site.

The changes in stretching of C−O−C (1043 cm^{-1}) and C=O (1011cm^{-1}) functional groups were also investigated. Both groups act as hydrogen bond acceptors. SIM@HPMCAS-MF, SIM@PLA and SIM@PLA-PCL However, as the hydrogen bonding between this site and the

polymer would be interesting to investigate, another analysis method should be applied, e.g. nuclear magnetic resonance (NMR) or Raman spectroscopy (Bruice, 2017).

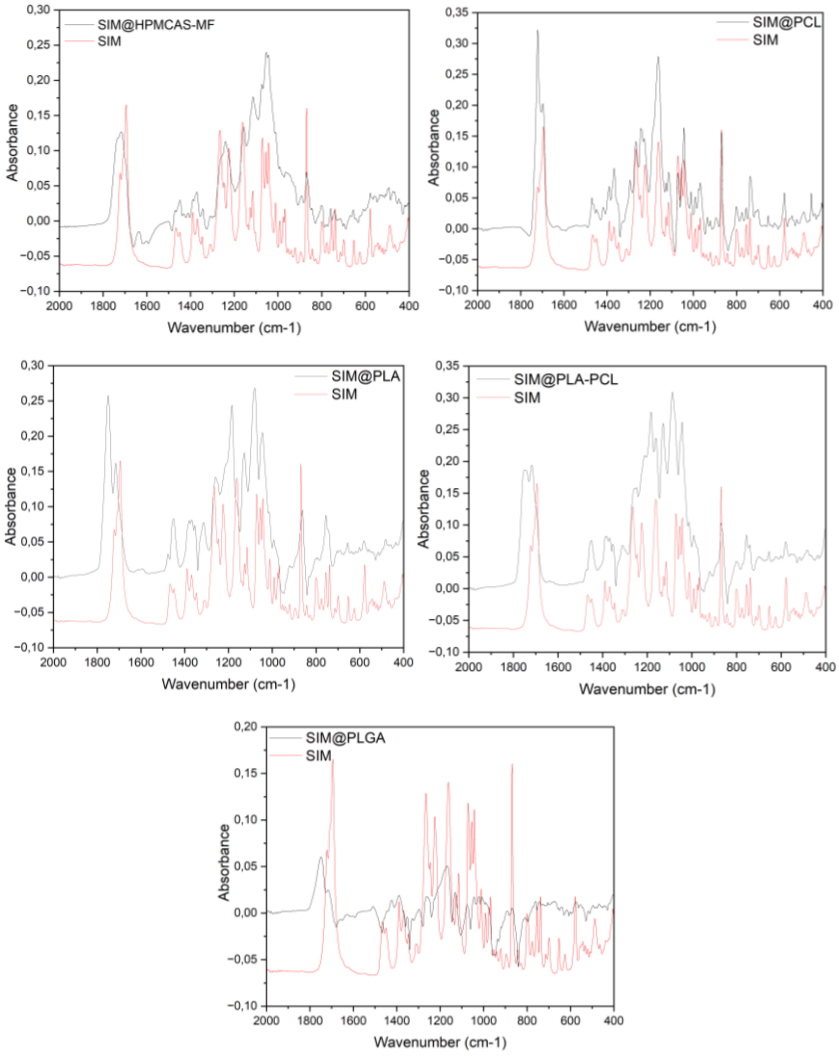


Figure 17. SIM-loaded MP and pure SIM spectra focused on the 2000-400cm^{-1} region.

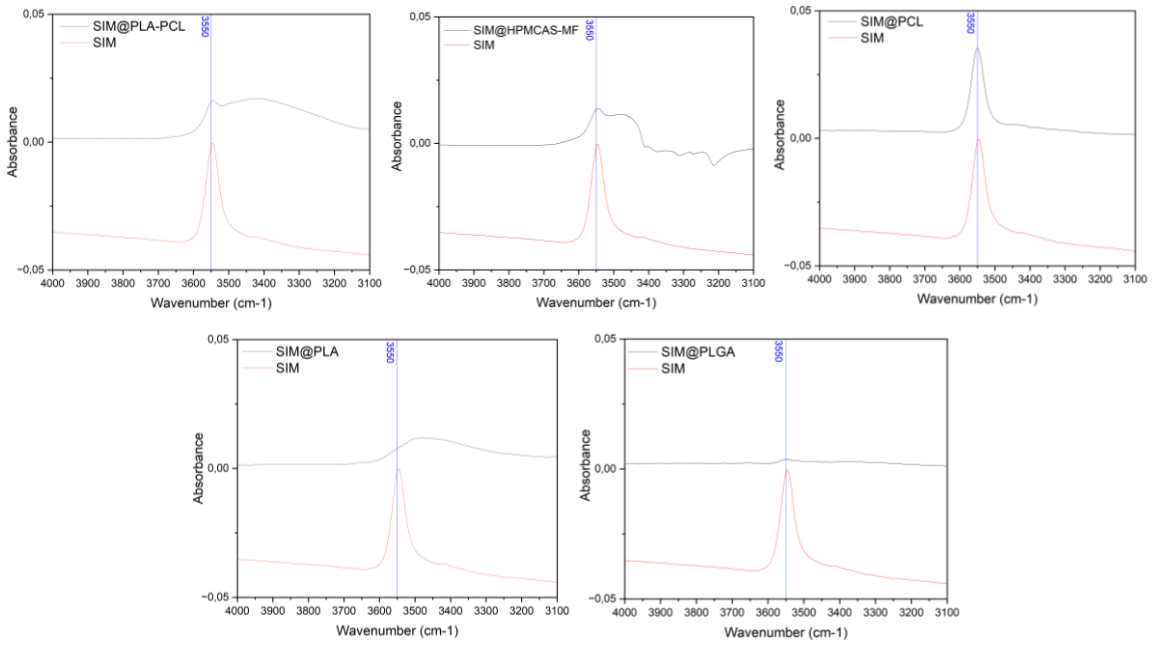


Figure 18. Obtained FTIR spectra of simvastatin samples portraying peak at 3550 cm^{-1}.

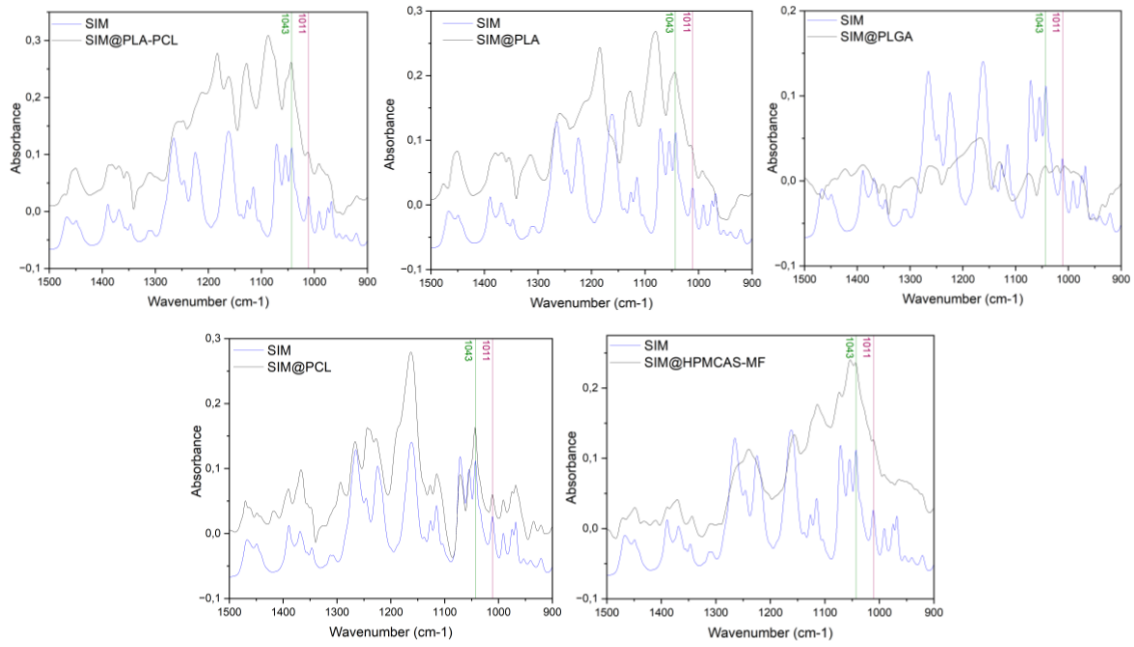


Figure 19. Obtained FTIR spectra of simvastatin samples portraying peaks at 1043 cm^{-1} and 1011 cm^{-1}.

6.2.3. Scanning Electron Microscopy

The IND-loaded MPs were observed with an SEM to get an insight into the particle's inner structure and shell. Selected pictures obtained with the SEM are presented in Figure 20 and Figure 21. The MPs were expected to have an amorphous structure and not show signs of irregular crystals or crystal growth. Desirably, the MPs have a regular spherical shape, a smooth and coherent shell and matrix. This means that the MPs should not present channelling of the matrix or porosity on the surface. Furthermore, the MPs should be individual entities, not aggregated or fused into each other.

The PLGA, PLA-PCL and HPMCAS-MF MPs presented the best results, considering that no crystallinity could be observed; however, both PLGA and HPMCAS-MF present channelling of the matrix and shell porosity. Channelling creates a diffusion pathway inside the MP. This could affect the degradation of the MP, thus, affecting the release profile of the final product. As the channel and pore formation is hard to control, it may result in inconsistent product quality. MPs might degrade more rapidly than others (Saltzman and Langer, 1989; Pagels and Prud'homme, 2015).

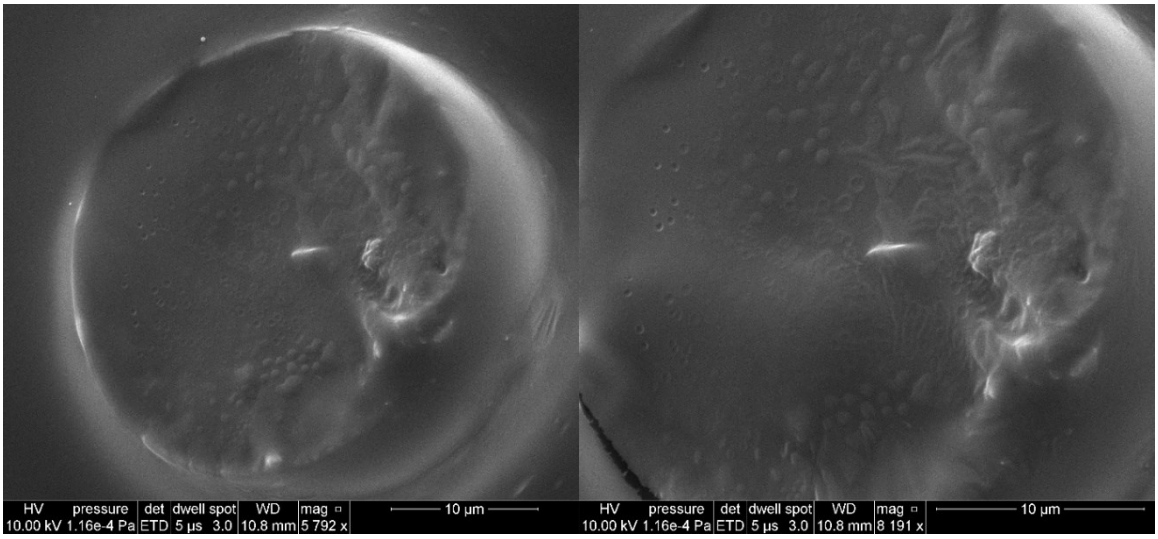


Figure 20. Image of IND@PLA-PCL MP cross-section taken with SEM. The small "bubbles" in the MP matrix could be caused by burning from the SEM electron beam.

It has been reported that polymers with low molecular weight are capable of closing out pores due to their higher chain mobility, implicating that polymers with higher molecular weight might result in a higher degree of channels and pores (Kang and Schwendeman, 2007). This theory could explain why the HPMCAS-MF samples show great channelling and porosity, as

the molecular weight of one monomer is higher than the other polymers. HPMCAS-MF also possesses a larger molecular structure consisting of cyclic and chain structures. Thus, HPMCAS-MF may be less mobile than the other polymers used in this project.

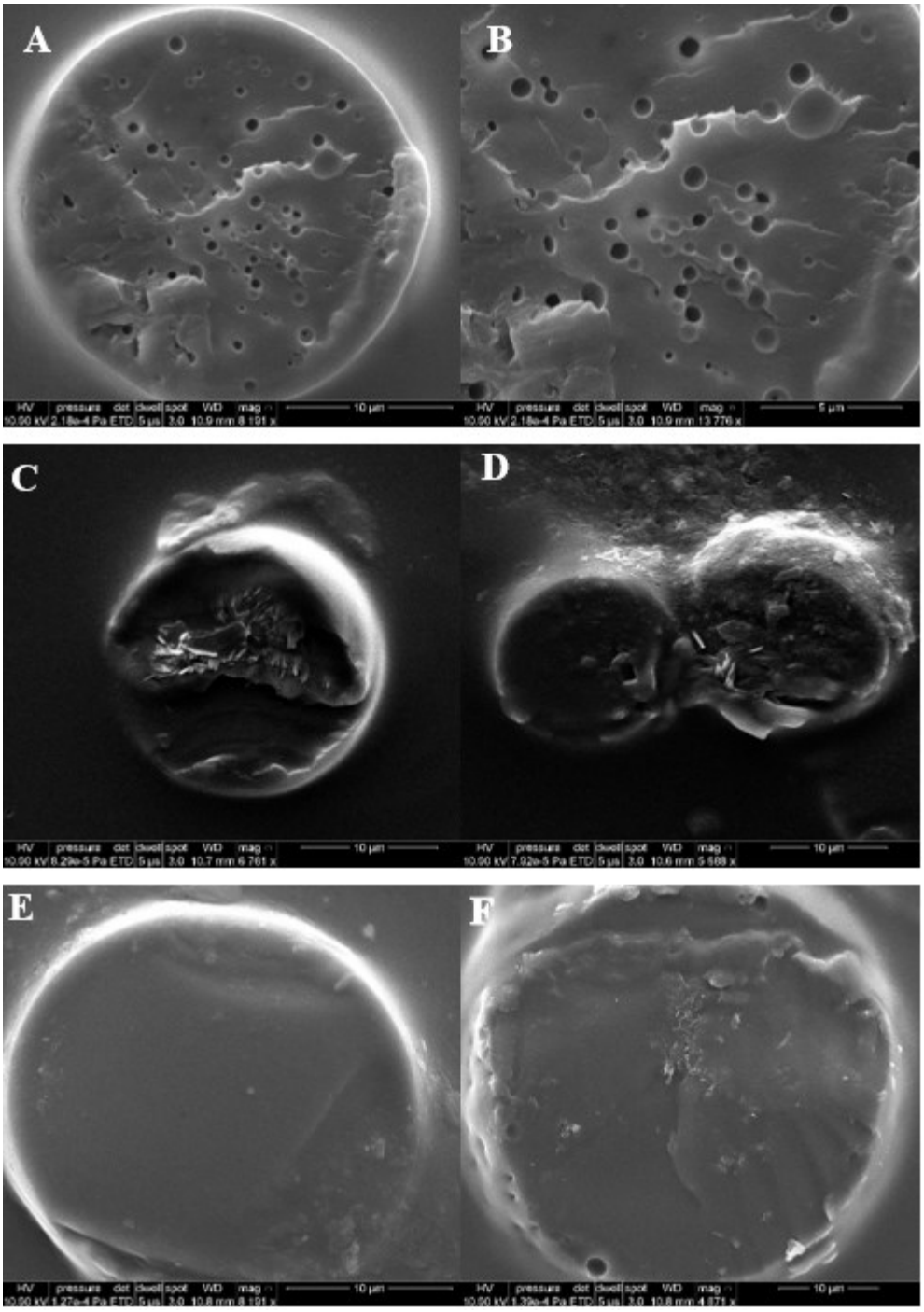


Figure 21. SEM images of cross-sectioned IND MPs. IND@HPMCAS-MF (A and B) showing channelling of the MP matrix. IND@PLA cross-sectioned MP presenting crystal growth in the centre of the particle (C) and two conjoined IND@PLA MPs (D). Image of the surface of IND@PLGA MPs without pores (E) and a cross-sectioned IND@PLGA MP with pores in the outer shell (F). The scale bars are all 10 µm except for (B), which is 5 µm.

6.3. Determination of microparticle solidification rate

The polymer solidification rate was determined to understand if there could be a correlation between drug encapsulation and the solidification of the particle. The results are presented in Figure 22. In this experiment, HPMCAS-MF and PLGA were the slowest to solidify at approximately 50-55 minutes. PCL was the fastest, with a solidification rate of roughly 18 minutes. The PLA-PCL copolymer was slightly after PCL at about 25 minutes. Surprisingly, the solidification of PLA was closer to HPMCAS-MF and PLGA at approximately 45 minutes.

The differences between the PLA, PCL and PLA-PCL copolymer are surprising. As the polymer ratio was 70% PLA and 30% PCL, one would estimate the solidification rate to be closer to the pure PLA result. However, in this case, the solidification at the beginning seemed right between PLA and PCL, but then the rate was more aligned with the PCL for the rest of the solidification.

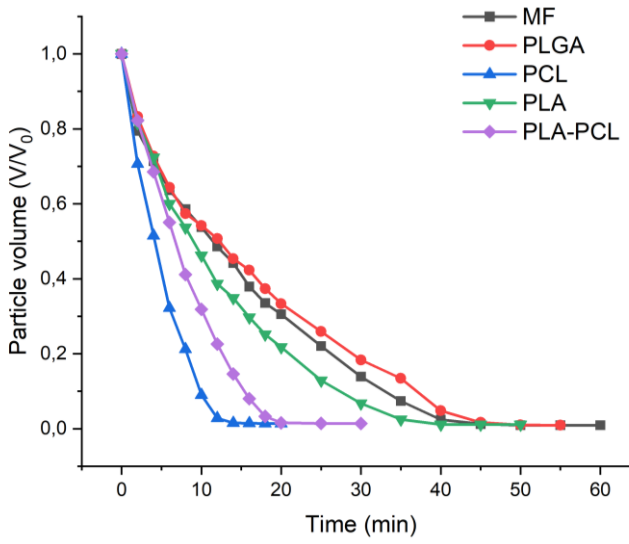


Figure 22. Obtained solidification rate of pure polymer MPs as a function of change in particle volume and time. Diagram created with OriginPro 2023 (OriginLab Corporation, US).

6.4. Cell viability

The cell viability was calculated using Equation 5, and the obtained results are presented in Figure 23. As hypothesised, none of the samples resulted in significant cell death, and all groups had an average cell viability of over 80%. On the contrary, PCL at a concentration of 50 µg/ appeared to have some proliferating effect. To validate this result, a second assay with another method would be recommended (Kamiloglu *et al.*, 2020).

Equation 5.

$$Cell\ viability\ (\%) = \frac{(intensity\ of\ test\ sample - intensity\ of\ blank\ group)}{(intensity\ of\ negative\ group - intensity\ of\ blank\ group)}\ x100$$

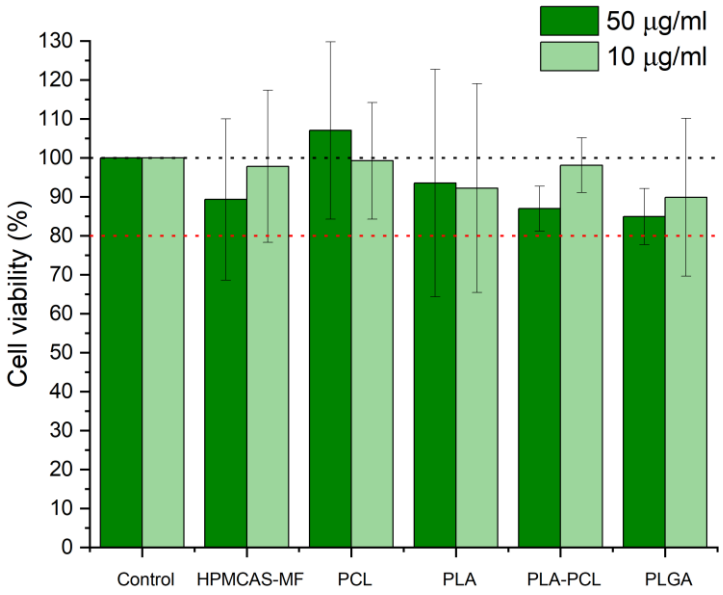


Figure 23. Chart presenting obtained cell viability (%) results and standard deviations for tested MP concentrations. As seen from the chart, all groups exceeded 80% (red dotted line), with one group even having proliferating effect exceeding 100% (black dotted line). Bar chart created with OriginPro 2023 (OriginLab Corporation, US).

7. DISCUSSION

A summary of all the relevant findings is presented in Table VII. Based on the results, a conclusion about API-polymer compatibility was divided into three categories: *compatible, potential*, and *incompatible*. A *compatible combination* has proven to result in high LD and EE and an amorphous structure. Slight adjustments might still be needed to achieve a perfect result. *Potential combinations* are those that have either reached a high LD and EE or an amorphous structure, but necessarily not both. These are considered potential combinations, as process adjustments could improve the result. These adjustments could be, e.g. an increase in polymer or API concentration. *Incompatible combinations* are those which have not resulted in a

sufficient LD or EE, or amorphous structure. Concerning *incompatible combinations*, other polymers are suggested for better properties.

It was found that HPMCAS-MF, PLGA and PLA-PCL show the best compatibility with both indomethacin and simvastatin. HPMCAS-MF showed excellent properties encapsulating both IND and SIM, as expected based on earlier reports showcasing the great potential of the polymer (Friesen *et al.*, 2008; Ting *et al.*, 2015). The most compatible polymer for IND was HPMCAS-MF, which resulted in both high LD and an amorphous structure. PLGA was shown to be the most compatible polymer for the encapsulation of SIM. However, some issues remain unresolved. Firstly, the channelling of the particle matrix remains a concern and requires further attention to achieve MPs with a homogenous structure. The problem with matrix channelling was mainly observed with IND@HPMCAS-MF MPs. The SIM@POLY MPs were not observed under SEM, meaning no conclusions on SIM MP morphology can be drawn.

The matrix channelling is an interesting finding, as it contradicts what has been understood earlier. According to Pagels and Prud'homme (2015), A slow solidification will lead to an MP with a uniform structure throughout. This is believed to be thanks to the solvent concentration being in equilibrium during the entire solidification process, thus enabling a homogenous polymer solidification process. HPMCAS-MF was one of the slowest solidifiers in our project but still presented some channelling. PLGA was another slow solidifier, but the matrix structure was significantly more uniform. The difference between the performance of HPMCAS-MF and PLGA related to the MP morphology could be explained by the differences in polymer structure and mobility, as discussed earlier in section 6.2.3 (Kang and Schwendeman, 2007).

Interestingly, both drugs have their issues related to encapsulation. The greatest challenge with IND was to achieve a fully amorphous structure. Regarding the encapsulation of SIM, a high LD was harder to achieve. Especially the stability of the amorphous structure during storage and dissolution is a known issue with ASDs (Schittny, Huwyler and Puchkov, 2020). Therefore, it might be that the MPs were stored incorrectly (ambient room temperature) before analysis. This issue must be assessed as the phase transformation to a more stable polymorph will affect the drug dissolution and, consequently, bioavailability. Related to the low LD, this could be solved with minor process adjustments.

Smaller particle size has been shown to result in higher EE. This is thought to be related to the solidification rate: smaller droplets solidify faster than bigger ones. This means there is a shorter period for the API to escape into the continuous phase during solidification. (Khan *et*

al., 2013) In this study, particle size was not determined for the prepared MP batches, which opens a gap for future research studies: is there a significant correlation between LD and EE, particle size and solidification rate?

Another interesting finding from this project relates to the solidification rate and LD. In Figure 24, the solidification rate, LDs and EEs are plotted into the same graph with Excel (Microsoft, US). This finding suggests that the LD and solidification rate of the polymer might have some correlation. The only samples deviating from this rule are the PLA-PCL copolymer MPs. This is interesting because earlier reports have shown that a higher LD can be achieved when the solidification is fast, as the drug is quickly trapped inside the particle shell (Damiati and Damiati, 2021). This "leaking" can be reduced by choosing an API and POLY combination with similar hydrophobic properties, making the API more drawn to the POLY.

This project has shown that MP structure, LD, EE and the polymer solidification rate are connected in a complex way. Not only has it shown contradicting results compared to published literature, but it also suggests a complex function that must be balanced to achieve a satisfactory product. According to this study, a longer solidification rate might help achieve a high LD. However, if a uniform MP structure is sought after, the polymer must be chosen carefully, as it might be the determining factor behind matrix channelling and shell porosity.

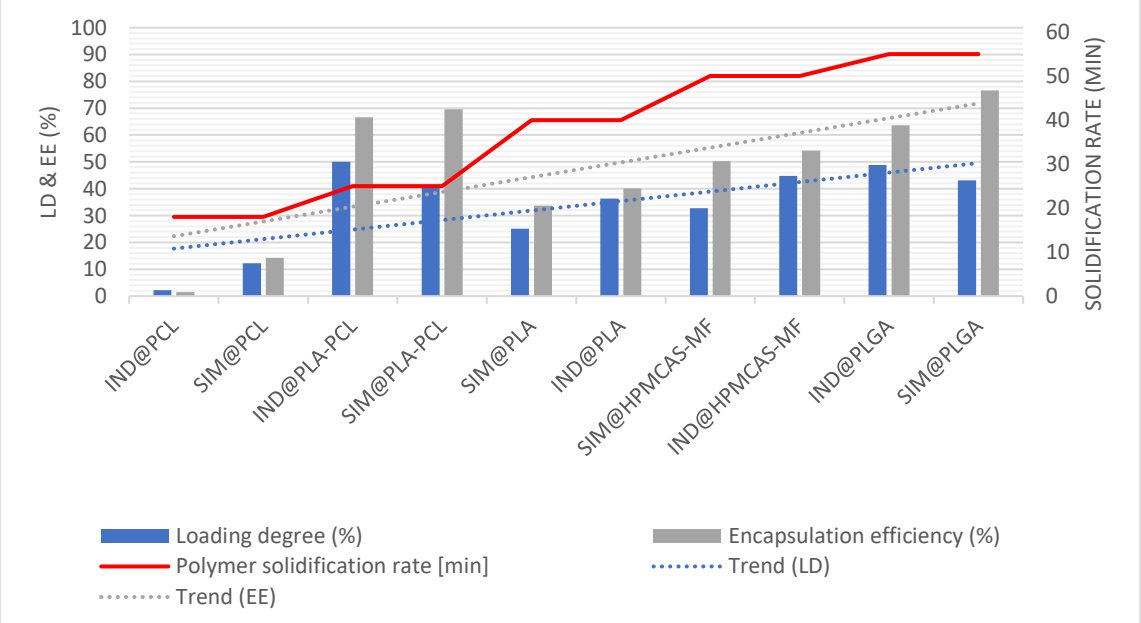


Figure 24. Graph presenting the loading degree, encapsulation efficiency and solidification rate. This presentation suggests that the solidification rate could also affect the drug loading degree and encapsulation efficiency.

The cell viability results show that the pure polymer MPs did not induce cell death. This would suggest that no toxic surfactant or organic solvent was present in the sample. However, validation and advanced safety studies are required. Careful washing and lyophilisation, although time-consuming, may be sufficient to remove harmful residues.

As this project was conducted over a limited period, all tests and adjustments could not be repeated to achieve desired properties. Although, some potential could be recognised in multiple API-POLY combinations. Especially the beginning of the project was prone to technical difficulties, which most of were fixed along the journey.

Table VII. Summary of results from this project. x= substantiated, - = unsubstantiated, ? = could not be determined, repetition recommended, A = amorphous, P= Polymorph, NA = not applicable/not analysed

	HPMCAS-MF		**PLGA**		**PLA-PCL**		**PLA**		**PCL**	
	IND	*SIM*	*IND*	*SIM*	*IND*	*SIM*	*IND*	*SIM*	*IND*	*SIM*
LD (>40%)	x	-	x	x	x	x	-	-	-	-
EE (>50%)	x	x	x	x	x	x	-	-	-	-
Solid-state	A	A?	P	A	P	A?	P	P	A	A
H-bonds	NA	x	NA	?	NA	x	NA	x	NA	-
Solidification rate	50		55		25		40		18	
Cell viability (>80%)	x	x	x	x	x	x	x	x	x	x
Compatibility	**Compatible**	Potential	Potential	**Compatible**	Potential	Potential	Incompatible	Incompatible	Incompatible	Incompatible
Comment	Process adjustments may be needed to achieve uniform MP morphology	Requires process adjustments to reach high LD	Requires process adjustments to reach amorphous structure	MP morphology was not analysed; thus, studies focused on MP structure are recommended.	Requires process adjustments to reach amorphous structure	Require process adjustments to reach high LD	Better matches available	Better matches available	Better matches available	Better matches available

8. CONCLUSIONS

Formulation-related approaches, such as drug-loaded polymeric MPs, promise great opportunities to drug solubility and bioavailability. In addition, unwanted properties such as bad taste or odour can be masked by utilising microtechnology. (Wei *et al.*, 2022) In this project, we prepared drug-loaded MPs with the MESE method, a modern fabrication method which promises accuracy, controllability, and cost-efficiency. As the drug loading properties highly depend on the intermolecular interactions between the API and polymer, there is a great need for data concerning API-polymer compatibility. In this study, several polymer-API combinations were prepared and analysed.

We concluded that HPMCAS-MF, PLGA and PLA-PCL present the best properties for encapsulating indomethacin and simvastatin. It was found that indomethacin could reach a higher loading degree, but the stability of ASDs remains unresolved. This could be solved with minor process adjustments, such as increasing the polymer concentration. Simvastatin could not be encapsulated with as a high loading degree as indomethacin but could be easier to stabilise in its amorphous form. The fabrication process is prone to human errors, so repetition of the selected combinations is advised.

As the stability of the amorphous forms remains an issue, further research in this field is encouraged. An interesting continuum on this project would be to study the dissolution profile of the MPs and determine the ASD stability both in storage and during dissolution. Dissolution is one of the most detrimental root causes of failure (Moseson *et al.*, 2023). To understand the mechanisms behind drug loading and stability, computational models could be utilised to understand the interactions between the materials. Exemplary publications have been demonstrated recently (Li *et al.*, 2019; Damiati and Damiati, 2021). As the interest towards more cost-efficient and precise manufacturing methods has increased, the upscalability of the microfluidic-assisted fabrication process must be validated. The process parameters and in-line process analytical technologies still require development (Otte and Park, 2022).

Beyond formulation aspects, manufacturing methods are still developing, and new techniques with promising efficiency and accuracy are sought after (Cook and Clemons, 2022). Continuous manufacturing has emerged in the pharmaceutical industry, raising the need and interest towards developing suitable production methods (Burcham, Florence and Johnson, 2018). Several approaches are available for particle engineering; however, these tend to be

inefficient both cost and yield-wise. Concerning MESE, the only potential in process control to monitor the formulation is monitoring the MP droplet size. Therefore, development should also focus on developing the manufacturing method itself. (Otte and Park, 2022)

9. SUMMARY IN SWEDISH – SVENSK SAMMANFATTNING

Mikrofluidistikassisterad tillverkning av polymermikropartiklar för läkemedelsadministrering

9.1. Inledning

Biotillgängligheten är en av de mest kritiska egenskaperna hos ett läkemedel som måste beaktas redan i tidiga stadier av läkemedelsutvecklingen. Majoriteten av de läkemedel som utvecklas har låg biotillgänglighet, i vissa fall så pass låg att vidare utveckling inte anses lönsamt, oavsett om den farmakologiska effekten varit eftersträvad. Oral biotillgänglighet definieras som mängden av den administrerade dosen som når den systemiska cirkulationen och därmed kan ge terapeutisk effekt. (Anane-Adjei et al., 2022)

Beslut om administreringsväg och formuleringstyp för läkemedlet baserar sig i stor utsträckning på dess biotillgänglighet. Doseringsformer för oral administrering är de mest eftersträvade, främst på grund av att de är lätta och kostnadseffektiva att tillverka och har en hög patientföljsamhet. Utmaningen är dock det komplexa matsmältningssystemet där läkemedlet måste upplösas i mag-tarmkanalens vätskor för att absorberas i blodcirkulationen. Dessutom måste substansen även motstå kemiska och biologiska störningar. (Viswanathan et al., 2017)

Biotillgängligheten begränsas oftast av en låg absorptionsnivå i mag-tarmkanalen. Absorptionen i sin tur är oftast begränsat av ämnets låga löslighet i vatten eller dess låga permeabilitet igenom tarmväggen. Även om permeabilitetsfaktorer är möjliga att påverka är det betydligt mycket lättare att öka på vattenlösligheten med hjälp av diverse formuleringsrelaterade strategier. En av dessa strategier är att tillverka amorfa fasta dispersioner (ASD), en typ av kristallteknik som baserar sig på att öka vattenlösligheten av ett ämne genom att fånga och stabilisera substansen i dess amorfa tillstånd. Den ökade lösligheten hos det amorfa tillståndet beror på en hög grad fri energi jämfört med det mer stabila kristallina tillståndet med låg fri energi (Mistry et al., 2015; Schittny et al., 2020).

I ASD:n hämmas eller fördröjs kristallisationen genom bildningen av en stabiliserande matris med hjälp av ett hjälpämne, till exempel en polymer. Hjälpämnet minskar kristalltillväxten

genom att hämma bildningen av kristallgittret och/eller genom att minska den molekylära rörligheten hos läkemedlet (Sahoo et al., 2020). Stabila ASD:n har tillverkats med hjälp av en hög polymerkoncentration, hög molekylvikt hos polymeren och god förmåga att bilda intermolekylära interaktioner mellan läkemedlet och polymeren (Yu et al., 2022). ASDn kan tillverkas genom formulering av polymera mikropartiklar.

Mikropartiklars fördel är förutom att lösligheten förbättras även att de kan maskera dålig smak eller lukt av läkemedlet och därmed öka patientföljsamheten. Dessutom kan partiklarna modifieras med egenskaper som till exempel fördröjd eller kontrollerad frisättning (Gao et al., 2021). En regelbunden sfärisk form gör även matematiska modeller mer tillämpningsbara eftersom hos oregelbundna kristallformer måste även avvikelser i partikelform tas i beaktande (Murtaza, 2012). Polymera mikropartiklar kan tillverkas genom flera olika metoder varav mikrofluidistikassisterad indunstningsmetod är i fokus i detta projekt.

Enligt Maeki (2019) innebär mikrofluidistik sådana processer där en liten volym av vätskor manipuleras i mikrometerskala med hög kontrollerbarhet. Mikrofluidistisk tillverkning av mikropartiklar baserar sig på att tillverka emulsioner av två eller flera icke-blandbara vätskor med hjälp av högt kontrollerad emulgeringsprocess. För själva tillverkningen av mikropartiklarna används små enheter, även kallade mikrochipp som styr vätskorna igenom ett kapillärsystem. (Wei et al., 2022) Mikrochippen kan vara handgjorda och flera olika material kan tillämpas. De enklaste versionerna består ofta av ett enkelt kapillärsystem i glas fäst på en platta och kan ha en av många olika geometrier: flödesfokuserande, samflödes- och T-korsningsgeometrier är de vanligaste typerna och presenteras i Figur 5.

Tillverkningen av mikropartiklarna baserar sig på emulgering av icke-blandbara vätskor. Dessa kallas kontinuerlig fas (KF) och dispergerad fas (DF). Vilken fas som är organisk eller oorganisk beror på vilka egenskaper råvarorna har och vad som förväntas av den slutliga produkten. I det här projektet bestod DF av polymer och/eller läkemedel utlösta i organisk fas. Det vill säga att DF fungerade som byggstenen för mikropartiklarna. Eftersom vätskorna inte löser sig i varandra, bildas en monodispers emulsion av dropparna med hjälp av mikrochipset (Wei et al., 2022). Formen och storleken på droppen beror främst på mikrochippets geometri, vätskeflödeshastigheten och vätskornas egenskaper, till exempel viskositet (Zhu och Wang, 2017). Droppen kan antingen sedan gå igenom en gelnings- eller torkningsprocess för ytterligare tillämpning.

9.2. Syfte och ämnesmotivering

Syftet med det här slutarbetet var att tillverka mikropartiklar av olika polymer-läkemedel-kombinationer med målet att uppnå gynnsamma egenskaper, så som hög laddningsgrad (LD), inkapslingseffektivitet (EE) samt amorf struktur. De tillverkade mikropartiklarna analyserades med diverse farmaceutiska analysmetoder. Först analyserades LD- och EE-egenskaperna varefter de fasta tillståndsegenskaperna studerades. De tillverkade mikropartiklarnas egenskaper jämfördes med läkemedel utan modifikationer (dvs. rent kristallint pulver) samt blanka, icke-läkemedelsladdade polymerpartiklar. Alla kontroll- och sampelgrupper samt respektive analysmetoder är listade i Bilaga I.

I detta projekt kombinerades indometacin och simvastatin med fem olika polymerer. Målet var att uppnå LD över 40 % för de läkemedelsladdade mikropartiklarna. För att ytterligare tolka mekanismen bakom LD och EE studerades de fasta tillståndsegenskaperna hos mikropartiklarna. Målet var att åstadkomma partiklar med amorf struktur och en jämn partikelmorfologi och -topologi. Förutom det ovannämnda, förväntades in-vitro cellviabiliteten inte resultera i onormal eller tydlig celldöd. Studier gällande partiklarnas upplösning, absorption och *in-vivo* säkerhets- eller effektivitetsexperiment uteslöts ur projektet.

9.3. Material och metoder

9.3.1. Material

Polymerer som användes i projektet var hydroxipropylmetylcellulosa-acetat-succinat av MF-kvalitet (HPMCAS-MF), poly-ε-kaprolakton (PCL), polylaktid (PLA), polylaktid-ε-kaprolakton (PLA-PCL) och polylaktid-co-glykolidsyra (PLGA). Indometacin och simvastatin valdes som läkemedel för detta projekt och deras nyckelvärden listas i Tabell II. Alla polymerer som användes i studien med respektive nyckelegenskaper listas i Tabell III. Simvastatin, som används som kolesterolsänkande läkemedel, har en mycket låg biotillgänglighet på endast 5 % med en mycket hydrofob struktur (Corsini et al., 1999). Indometacin hör till de icke-steroida anti-inflammatoriska läkemedel som vanligtvis används som inflammationsdämpande smärtmedel mot inflammatoriska sjukdomar som till exempel artrit. Även indometacin är ett hydrofobt läkemedel men skiljer sig för övrigt från simvastatin, t.ex. med en mindre molekylstruktur och olika funktionella grupper.

9.3.2. Tillverkning av mikropartiklar

Mikropartiklarna tillverkades som olja-i-vattenemulsioner med hjälp av mikrofluidistikassisterad indunstningsmetod. Indunstning innebär att det organiska lösningsmedlet i DF kommer att extraheras ur dropparna och avdunsta ut ur KF naturligt under tillverkningens lopp. Då DF fungerar som byggstenen för partikeln, får indunstningen av det organiska lösningsmedlet polymeren att stelna. Denna process resulterar i en fast mikropartikel med läkemedlet inkapslat innanför sig. Etylacetat användes som DF och Milli-Q-vatten med surfaktant som KF.

Tillverkningsprocessen av mikropartiklarna börjar med förberedning av KF och DF. Därefter sätts tillverkningsprocessen i gång. Innan partiklarna kan användas tvättas de och frystorkas de. Tillverkningsuppsättningen och hela processflödet presenteras i Figurerna 8 och 9.

9.3.3. Analys av läkemedlets laddningsgrad samt inkapslingseffektivitet

Laddningsgraden och inkapslingseffektiviteten av de läkemedelsladdade mikropartiklarna analyserades med högupplösande vätskekromatografi (HPLC). HPLC användes för att bestämma läkemedelskoncentrationen (µg/ml) i proverna. LD och EE beräknas med hjälp av data och enligt ekvationerna 3 och 4. Metodparametrarna för HPLC presenteras i Tabell IV. Varje experiment upprepades med tre prover så att ett tillförlitligt medelvärde på LD och EE kunde uppnås.

9.3.4. Analys av mikropartiklars fasta tillståndsegenskaper och stelningshastighet

De fasta tillståndsegenskaperna bestämdes med hjälp av differentiell svepkalorimetri (DSC), Fouriertransform-infraröd spektroskopi (FTIR) för att utvärdera ifall läkemedlet befinner sig i amorft tillstånd eller inte och ifall det skett tydliga förändringar i molekylbindningar som följd av inkapslingen. Partiklarnas morfologi och topologi analyserades med hjälp av svepelektronmikroskop (SEM).

Med stelningshastighet menas den tid som krävs för polymeren att fullständigt stelna från droppbildning till en fast mikropartikel. Stelningen övervakades genom att observera en enstaka polymerpartikel (icke-laddat) med ljusmikroskop. Partikelns diameter mättes och följdes regelbundet tills partikeln stelnat helt. Under de första 20 minuterna övervakas storleken

varannan minut och för den överskridande tiden var femte minut. Polymeren har stelnat när följande faktorer kan observeras: mikropartikelns diameter är oförändrad, partikeln är inte längre transparent och partikeln har sjunkit till bottnen av kapillären och därmed fallit ur mikroskopets fokus.

9.3.5. Polymerers effekt på cellers viabilitet

Även om alla polymerer som användes är accepterade för biomedicinska tillämpningar, beslöt vi att utföra en *in vitro* cellviabilitetsanalys. Detta gjordes för att säkerställa att tillverkningsprocessen inte lämnar några toxiska rester i slutprodukten. För detta experiment såddes RAW264.7-celler i en 96-brunns platta (100 µl, 1x104 celler/brunn) och odlades över natten. Sedan ersattes odlingsmediet med lösningar bestående av icke-laddade mikropartiklar dispergerade i odlingsmedium. Experimentet utfördes med två olika koncentrationer: 10 µg/ml och 50 µg/ml av mikropartiklar i odlingsmedium. Cellerna exponerades för mikropartikellösningen i 24 timmar. Den blanka gruppen bestod av triptan och den negativa gruppen bestod av celler med endast odlingsmedium (ingen manipulation). Alla experiment utfördes 6 gånger och cellerna observerades under ljusmikroskop före analys. CellTiter Glo (Promega Corporation, USA) användes för analysen och resultaten lästes med Varioskanmikroplattläsare (Thermo Fisher Scientific, USA).

9.4. Undersökningens resultat

9.4.1. Läkemedlets laddningsgrad och inkapslingseffektivitet

LD beräknades enligt ekvation 3 med erhållna data från HPLC-experimentet. EE beräknades enligt ekvation 4. Resultaten presenteras i Tabell VI och Figur 13 för respektive kombination. Översiktligt kunde högre LD-resultat erhållas för indometacin-mikropartiklarna. I denna grupp översteg PLA-PCL, PLGA och HPMCAS-MF det eftersträvade LD på 40 % och PLA låg strax under med 36,3 %. IND@PCL nådde dock inte LD över 2,3 %, vilket var det lägsta LD resultatet överlag. Simvastatin-mikropartiklarna uppnådde inte lika höga LD-resultat som de motsvarande för indometacin. Två partikeltyper överskred gränsen på 40 %. Intressant nog, visade SIM@PCL högre LD jämfört med dess IND@PCL-motsvarighet.

9.4.2. Fasta tillståndsegenskaper och polymerens stelningshastighet

Den amorfa strukturen kan observeras i DSC-graferna av att de inte presenterar tecken av smältning. Alla grafer är presenterade i Figurerna 14 och 15. Smältning kan observeras som starka och långa toppar i termogrammet. För indometacin-gruppen lyckades endast PCL och HPMCAS-MF stabilisera läkemedlet i dess amorfa struktur. I de övriga proven kan en tydlig smältningspunkt för en av indometacinets flera polymorfer observeras. För simvastatin kunde endast PCL och PLGA stabilisera läkemedlet i dess amorfa tillstånd. Resultaten för PLA-PCL och HPMCAS-MF är svåra att tyda. En väldigt liten topp kan ses vid smältpunkten för simvastatin, vilket kunde tyda på att provet har haft en väldigt liten koncentration av kristallint simvastatin i sig.

För FTIR-analysen analyserades fulla spektra men för att tolka möjliga förändringar i spektrum mellan mikropartiklarna och kristallint läkemedel. FTIR-spektra presenteras i Figur 16 och Figur 17. För simvastatin analyserades potentiella förändringar vid våglängderna 1011 cm-1, 1043 cm-1 och 3550cm-1. Stora skillnader mellan alla prover kunde observeras, tydligast som att en topp för det rena läkemedlet antingen blivit mjukare eller lägre i mikropartiklarnas spektrum. Detta kunde tyda på intermolekylära bindningar mellan polymeren samt läkemedlet som resulterar i mindre/lägre atomvibrationer, vilket i sig kan avläsas som mindre och lägre toppar i spektrumet.

Ur SEM-bilderna i Figurerna 20 och 21 kan man observera typiska problem som stöts på vid formuleringen av mikropartiklar. En stor del av partiklarna hade en fast matris med mindre och större hål och kanaler. Detta är inte önskvärt eftersom kanaler i matrisen gör partiklarna sköra och påverkar löslighetsprofilen. Likaså kunde porer i partikelskalet observeras, vilket även det påverkar löslighetsprofilen av partikeln och gör löslighetskinetiken svårare att förutspå. Vissa partiklar, speciellt IND@PLA visar tydliga tecken på kristallisering i mitten av partikelmatrisen, vilket kan bero på att polymeren inte klarar av att stabilisera den amorfa strukturen för längre tider. Faktorer som dessa bör tas i beaktande vid utvecklingen av formuleringens stabilitet.

Resultaten för analys av stelningshastighet presenteras i figur 14. PLGA och HPMCAS-MF var de polymerer som stelnades långsammast med ett resultat på ca 55 minuter får vardera partikel. PCL och PLA-PCL var de snabbaste med totala tider på ca 20 minuter.

9.4.3. Effekt på cellviabilitet

Cellviabiliteten beräknades med hjälp av ekvation 5 och de erhållna resultaten presenteras i Figur 23. Som förväntat, resulterade inget prov i signifikant celldöd och alla grupper hade en genomsnittlig cellviabilitet över 80 %. Intressant nog verkar PCL (50 μg/ml) till och med ha en prolifererande effekt då dess viabilitetsgrad överskridit 100 %. En till analys med en annan analysmetod vore rekommenderat för att validera det erhållna resultatet (Kamiloglu et al., 2020).

9.5. Diskussion och slutsats

Läkemedelsutvecklingen kräver nya innovationer och lösningar för att kunna utveckla läkemedel för oral administrering. Behovet har markerats speciellt då det visat sig att majoriteten av de läkemedel som utvecklas drabbas av antingen låg vattenlöslighet eller permeabilitet, vilket i sin tur leder till en låg biotillgänglighet. En låg biotillgänglighet innebär ofta att själva läkemedelsmängden i formuleringen blir hög, vilket i sig kan leda till risker med ökad toxicitet eller totalt nedlagda utvecklingsprojekt.

Som en lösning har amorfa fasta dispersioner presenterats för att öka på vattenlösligheten av läkemedel med låg vattenlöslighet. Dessa kan beredas till exempel som polymera mikropartiklar med läkemedlet inkapslat i polymermatriset. Mikropartikeln löser sig lättare i vatten, inte endast på grund av dess ökade ytliga area utan även för att de möjliggör övermättade lösningar. Förutom det ovannämnda, är den regelbundna formen av sfäriska partiklar till nytta vid utvecklingsskedet av formuleringen då de tillgängliga löslighetsmodellerna (t.ex. Noyes-Whitney) är mera exakta jämför med kristallina partiklar med oregelbundna partikelformer. Polymera mikropartiklar kan tillverkas till exempel med hjälp av mikrofluidistik-assisterad metod. Utförligare uppfattning om olika polymerers samt läkemedels kompatibilitet behövs dock fortfarande.

Syftet med det här slutarbetet var att tillverka diverse mikropartiklar av olika polymer-läkemedel kombinationer med gynnsamma egenskaper, så som hög laddningsgrad samt en amorf struktur. Genom att utforska dessa egenskaper anses man få en djupare inblick i vilka typers kombinationer som är gynnsamma för vidare tillämpning. Som det bevisades, kan det vara stora skillnader i laddningsgrad och inkapslingseffektivitet beroende på vilken kombination av polymer och läkemedel man har.

I detta projekt kombinerades indometacin och simvastatin med fem olika polymerer. Under projektet tillverkades mikropartiklar med hög laddningsgrad (>40 %) och med amorf struktur. HPMCAS-MF och PLGA visade sig vara de mest kompatibla polymererna för båda läkemedlen både gällande inkapslingsegenskaper och fasta tillståndsegenskaper. Även sampolymeren av PLA och PCL lyckades överraska med sina goda resultat gällande inkapslingseffektivitet och laddningsgrad, även om de som enskilda polymerer inte lyckades uppnå alla kriterier.

Detta projekt bygger på de teorier och modeller som utvecklats i samband med utvecklingen av mikropartiklar och mikrofluidistik för tillämpning som läkemedelsformuleringar för läkemedel med låg biotillgänglighet orsakat av substansens låga vattenlöslighet. En utredning av partiklarnas prestationsrelaterade egenskaper (t.ex. löslighetsprofil i mag-tarmkanalen) kunde främjas som fortsättning till den här studien.

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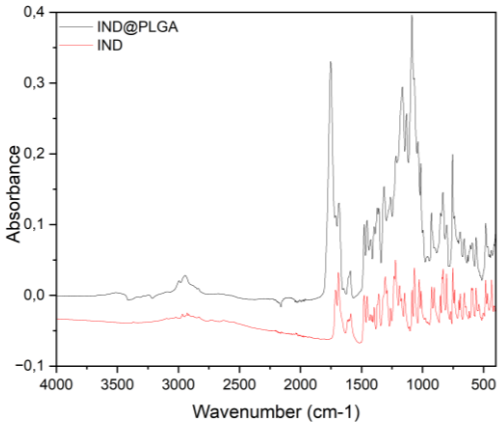
11. ANNEX

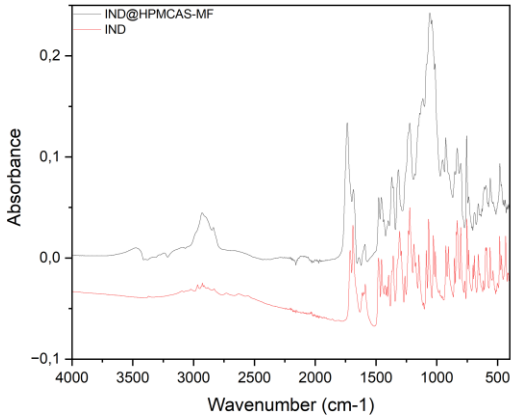
11.1. ANNEX 1. Sample and control groups with respective assays

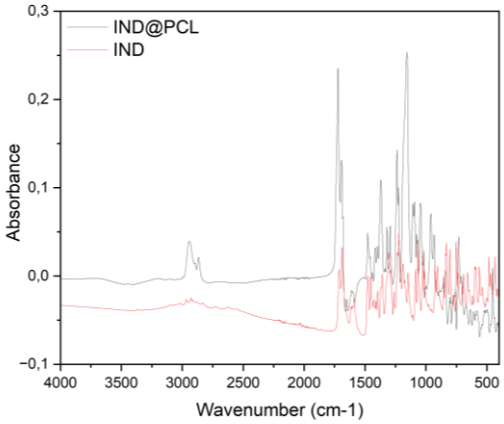
<table>
<tr><td></td><td>Pure polymer MPs</td><td colspan="2">Pure API[1]</td><td colspan="2">Physical mixture[2]</td><td colspan="2">Drug loaded MPs</td></tr>
<tr><td>Sample name</td><td>Polymer-MP</td><td colspan="2">API</td><td colspan="2">API + Polymer MP</td><td colspan="2">API@Polymer MP</td></tr>
<tr><td>API</td><td>-</td><td>IND</td><td>SIM</td><td>IND</td><td>SIM</td><td>IND</td><td>SIM</td></tr>
<tr><td>No polymer</td><td>-</td><td>C2.1</td><td>C2.2</td><td>-</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td>HPMCAS-MF</td><td rowspan="5">C1</td><td>-</td><td>-</td><td rowspan="5">C3.1</td><td rowspan="5">C3.2</td><td rowspan="5">S1</td><td rowspan="5">S2</td></tr>
<tr><td>PLGA</td><td>-</td><td>-</td></tr>
<tr><td>PLA</td><td>-</td><td>-</td></tr>
<tr><td>PCL</td><td>-</td><td>-</td></tr>
<tr><td>PLA-PCL</td><td>-</td><td>-</td></tr>
<tr><td colspan="8">Assays</td></tr>
<tr><td>DSC</td><td>x</td><td>x</td><td>x</td><td>x</td><td>x</td><td>x</td><td>x</td></tr>
<tr><td>LD & EE</td><td colspan="5"></td><td>x</td><td>x</td></tr>
<tr><td>ATR-FTIR</td><td colspan="2">x</td><td>x</td><td colspan="2"></td><td>x</td><td>x</td></tr>
<tr><td>SEM</td><td colspan="5"></td><td>x</td><td></td></tr>
<tr><td>Cell viability</td><td>x</td><td colspan="6"></td></tr>
<tr><td>Solidification rate</td><td>x</td><td colspan="6"></td></tr>
</table>

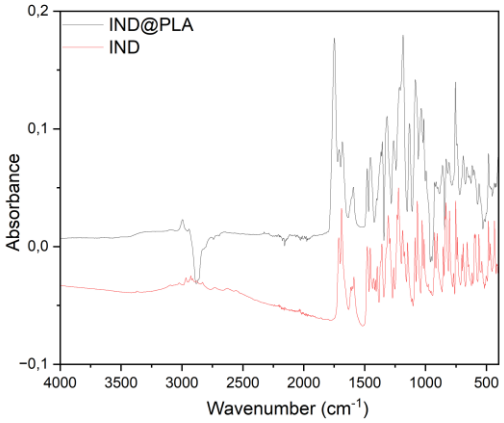
[1] as a crystalline powder
[2] consisting of crystalline API powder and pure polymer MPs

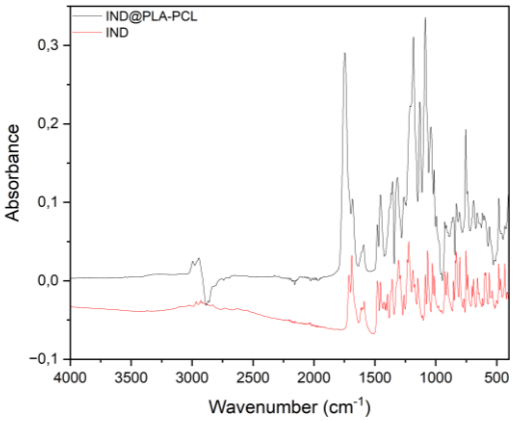
11.2. ANNEX 2. Complete ATR-FTIR spectra

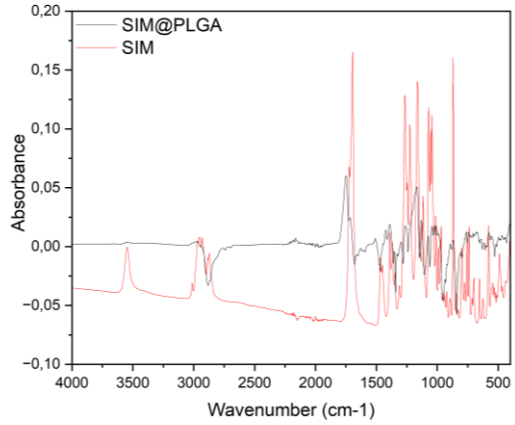


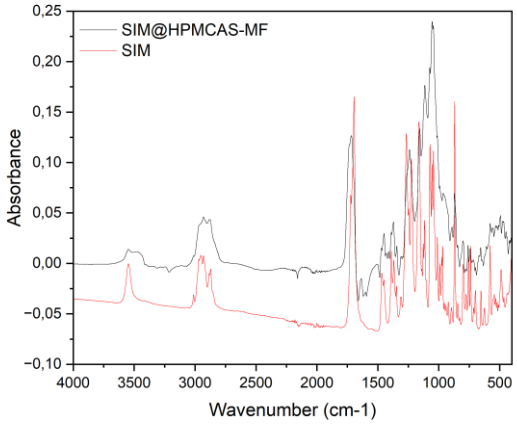


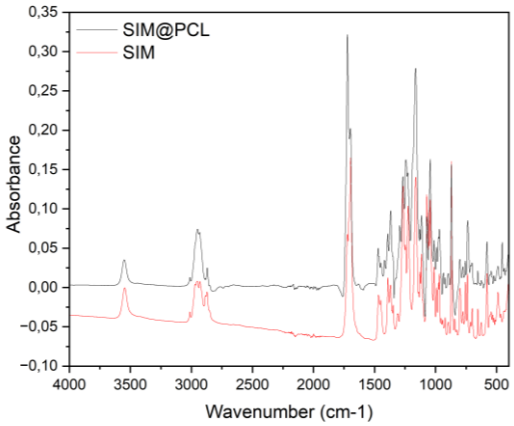


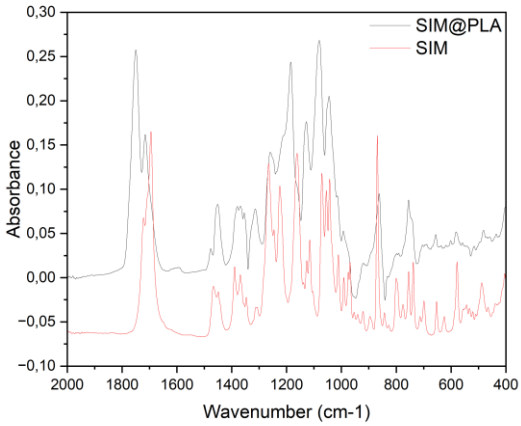


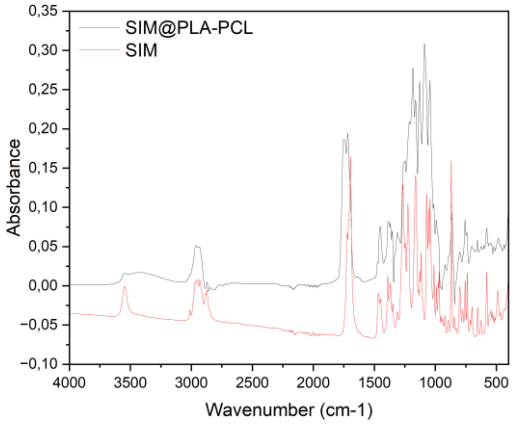



SIM@PLGA
SIM
Absorbance
Wavenumber (cm-1)


SIM@HPMCAS-MF
SIM
Absorbance
Wavenumber (cm-1)


SIM@PCL
SIM
Absorbance
Wavenumber (cm-1)


SIM@PLA
SIM
Absorbance
Wavenumber (cm-1)


SIM@PLA-PCL
SIM
Absorbance
Wavenumber (cm-1)