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Optimizing Formulation Conditions of PLGA Microparticles to Enhance Indomethacin Encapsulation

*Martina Arabia, Eleonora Maretti, Armita Sedighidarijani, Cecilia Rustichelli, and Eliana Leo**

Drug delivery systems can avoid the drawbacks of Indomethacin (IND), a non-steroidal anti-inflammatory drug used to treat osteoarthritis and arthritis, which requires high doses to reach therapeutic plasma levels leading to significant systemic side effects. This study aims to optimize poly(lactic-co-glycolic acid) (PLGA) microparticles (MPs) for intra-articular IND administration. MPs are prepared by solvent evaporation and freeze-dried for stability. Initial formulations with Tween 80 yield rubbery samples with low drug loading (1%); replacement of Tween 80 with Gelatin produces a stable powder with syringable MPs (particles size: 7 µm), although, DL (3%) and EE (30%) remain suboptimal, due to IND polymorphic transformation. Differential Scanning Calorimetry and Fourier-Transform Infrared spectroscopy demonstrate a molecular dispersion of IND in PLGA. Adjusting the aqueous phase to pH 3 in the formulation process, i.e below IND pKa, significantly enhances EE (90%) due to the reduction of drug solubility in the external aqueous phase. In vitro release study shows prolonged IND release over several days, confirming an effective drug encapsulation. This study provides a foundational framework toward the optimization of the successful encapsulation of IND in PLGA MPs, potentially advancing future clinical applications of such drug delivery systems.

1. Introduction

Indomethacin (IND) is a nonsteroidal anti-inflammatory drug (NSAID) known for its antipyretic, analgesic, and anti-inflammatory effects. Chemically classified as an indole-acetic acid derivative, it is specifically identified as 1-(p-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid. IND inhibits cyclooxygenase (COX) isoforms, crucial enzymes in the synthesis of prostaglandins and thromboxane A2, making it effective for treating painful and inflammatory conditions such as arthritis and osteoarthritis. However, IND is a non-selective NSAID, inhibiting both COX-1 and COX-2, which reduces prostaglandin production but also results in gastrointestinal and cardiovascular side effects. IND belongs to Class II of the Biopharmaceutics Classification System (BCS) due to its low aqueous solubility and high permeability. Additionally, it has limited solubility in alcohol and a pKa of 4.5.[1] The polymorphism of IND significantly impacts its solubility. Diverse polymorphic forms have been observed: α, β, γ, δ, and an additional unnamed form. More recently, three more forms have been identified and named ϵ, ζ, and η as reported by Surwase et al.[2] The γ form is the commercially used and the most thermodynamically stable form, while the common β and α forms are metastable. Despite the higher solubility of the amorphous form, it tends to crystallize into the α or γ forms during storage, losing its solubility advantage. Crystallization into the γ form occurs at low humidity and temperatures below the glass transition (Tg), while the α form appears at high humidity and temperatures above the Tg in aqueous environments. The Tg is reported between 42 and 45 °C, with the α polymorph favored at 60 °C and the γ polymorph predominant at 35 °C.

These complex physicochemical aspects render IND a second-choice drug for inflammation treatment, highlighting the need for innovative pharmaceutical formulations to improve its efficacy and bioavailability while reducing side effects.

Poly(lactic-co-glycolic acid) (PLGA) is a promising biodegradable copolymer for drug delivery due to its biocompatibility, biodegradability, and FDA/EMA approval. The properties of PLGA, such as hydrophilicity and degradation rates, can be tailored by adjusting the lactate/glycolate ratio during polymerization.[3,4] A key property to consider is the Tg of PLGA, which ranges between 30 and 60 °C, affecting its glassy behavior. Depending on the copolymer composition, molecular weight, handling techniques, and type of loaded drug, the Tg value will

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shift to a lower (plasticizer effect) or higher (antiplasticizer effect) value.[5,6]

PLGA-based drug delivery systems, particularly microparticles, provide controlled drug release, prolonged therapeutic effects, and enhanced patient compliance.[7] Various preparation methods, including emulsion-based and precipitation-based techniques, offer distinct advantages according to the drug's characteristics and desired release profiles.[8–10] In musculoskeletal diseases such as osteoarthritis and rheumatoid arthritis, where symptomatic relief is critical, intra-articular drug delivery presents a promising opportunity. PLGA-based intra-articular particles offer targeted drug delivery to intra-joint components, enabling sustained therapeutic effects and minimizing systemic side effects.[11,12] Moreover, clinical studies have shown the efficacy of PLGA-based controlled drug delivery systems in treating osteoarthritis and other joint diseases.[4,13]

For these reasons, a combination of IND and PLGA represents a valuable therapeutic approach for treating musculoskeletal diseases. IND offers rapid symptom relief, while PLGA provides a versatile platform for targeted drug delivery. Integrating these two compounds could lead to significant improvements in managing joint diseases, enhancing patients' quality of life, and reducing the side effects associated with conventional treatments. Despite the potential benefits, there is limited literature on the combined use of IND and PLGA for musculoskeletal diseases.[14,15] This gap underscores the need for more comprehensive studies to optimize the formulation parameters, such as the drug-to-polymer ratio, particle size, and release kinetics, to maximize therapeutic outcomes.

Therefore, this work focused on optimizing microparticle preparation and characterization, including drug loading efficiency, morphology, diameter, and drug release kinetics. Further investigations by thermal and infrared analysis will explore the interaction between PLGA and different polymorphic forms of IND, aiming to refine formulation parameters and optimize drug loading and release profiles.

2. Experimental Section

2.1. Materials

Indomethacin (IND, γ form) from ThermoFisher Scientific (Milan, Italy), Resomer RG 504 from Evonik Degussa GmbH (Essen, Germany), Gelatin Type A from Acros Organics (Geel, Belgium), and Tween 80 from Sigma–Aldrich (Milan, Italy) were purchased. Sodium hydroxide and potassium dihydrogen phosphate salts from Sigma–Aldrich were used to prepare Phosphate buffer pH 7.4. High purity water was obtained from the MilliQ system (Millipore, MA, USA). All chemicals and solvents were of analytical grade.

2.2. Methods

2.2.1. Preparation of Indomethacin-Loaded Microparticles

Polymeric microparticles (MPs) were prepared using the solvent evaporation method. Briefly, PLGA (100 mg) and INDγ (10 mg) were dissolved in dichloromethane (DCM, 4 mL) and then emulsified by an Ultra Turrax (T-25 basic Ika Labortechnik, Staufen, Germany) at 8000 rpm for 10 min with 50 mL of aqueous solution containing a surfactant, as reported in **Table 1**. The solvent was evaporated first under agitation at room temperature (RW 20 DZM, Ika Labortechnik, Staufen, Germany) at 1900 rpm for 1 h and then by rotary evaporator (R-100, Buchi, Milan, Italy) for 3 min. The MPs suspension was recovered by centrifugation at 8000 rpm for 10 min at 25 °C (Rotina 380R, Hettich, Kirchlengern, Germany) and washed two times with 50 mL of MilliQ water. Samples (Table 1) were frozen and then freeze-dried (Lio 5P, Cinquepascal, Milan, Italy) for at least 24 h without the use of cryoprotectants.

When mentioned, the empty MPs were prepared by the same technique without IND.

Table 1. Formulation conditions of each sample.

Sample name	Type and concentration of surfactant	pH of aqueous phase[a)]
MPs_T	Tween 80 (1%, w/v)	7
MPs_G	Gelatin type A (0.5%, w/v)	7
MPs_G_1	Gelatin type A (0.5%, w/v)	1
MPs_G_3	Gelatin type A (0.5%, w/v)	3
MPs_G_5.5	Gelatin type A (0.5%, w/v)	5.5
MPs_G_9	Gelatin type A (0.5%, w/v)	9

[a)] For MPs_G_*samples the pH of the aqueous phase was adjusted using HCl or NaOH.

2.2.2. Morphology and Size Distribution

Morphological characteristics of the MPs (i.e. shape, size, and size distribution) were studied by Environmental Scanning Electron Microscope (ESEM) (Quanta 200, Fei, Hillsboro, Oregon, USA). For each sample, a few drops of re-suspended freeze-dried MPs were placed on an aluminum stub (TAAB Laboratories Equipment Ltd, Aldermaston, Berks, UK) covered by a glass dish (TAAB Laboratories Equipment) and observed after drying

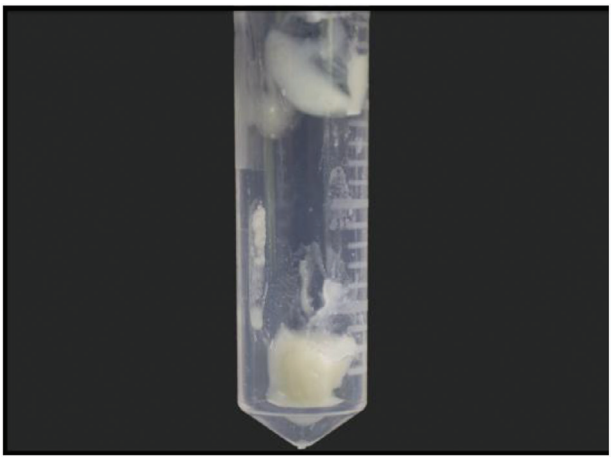

Figure 1. A picture representing sample MPs_T recovered after freeze-drying.

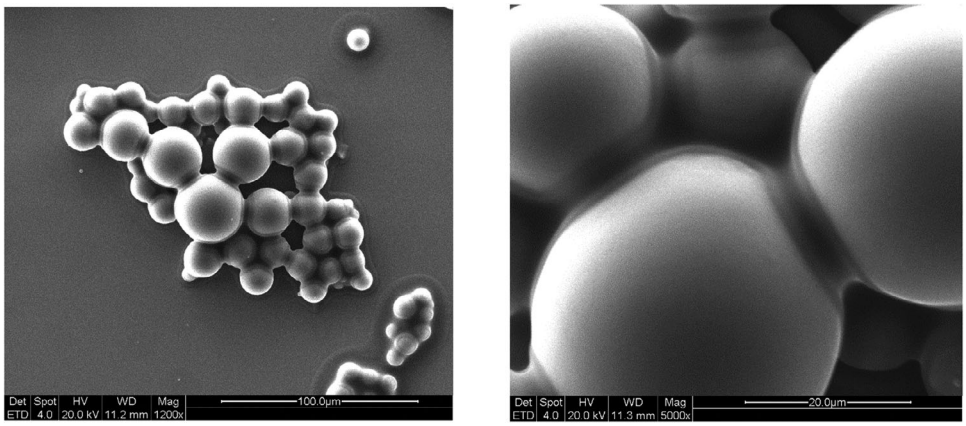

Figure 2. ESEM images of sample MPs_T at two different magnifications (1200x and 5000x).

and vacuum coating with gold-palladium in argon atmosphere for 60 s (Sputter Coater Emitech K550, Emitech Ltd, Ashford, Kent, UK).

The mean size of particles was obtained by measuring almost one hundred particles for each sample from ESEM images. The data are processed using ImageJ (version 1.53k) and Prism GraphPad (version 9.0.0).

The particle size distribution was calculated using the *Span* value as follows:

$$\text{Span} = \frac{D_{V,90\%} - D_{V,\,10\%}}{D_{V,50\%}} \quad (1)$$

where $D_{v,90\%}$, $D_{v,50\%}$, and $D_{v,10\%}$ are the size diameters at 90%, 50%, and 10% cumulative volumes, respectively. A smaller *Span* value indicates a closer size distribution.[16]

2.2.3. Drug Loading and Encapsulation Efficiency

Drug content was determined by dissolving a weighed amount of freeze-dried MPs (≈10 mg) in 0.5 mL of DCM. Then, 9.5 mL aliquot of ethanol was added. The mixture was vortexed for 3 min and then centrifuged at 8,300 RCF for 10 min at 25 °C. The drug was determined in the supernatant using spectrophotometric analysis (Lambda 35 UV/VIS, Perkin–Elmer, Woltham, USA) at 320 nm. A standard calibration curve in ethanol was used for the determination of drug concentration. The supernatant of the corresponding empty MPs (n = 3) was used as a blank, in order to eliminate any possible interference in the measurements.

Drug Loading (DL) and Encapsulation Efficiency (EE) were determined by applying the equations below:

$$\text{DL} = \left(\frac{\text{weight of drug in microparticles}}{\text{weight of microparticles}}\right) \times 100 \quad (2)$$

Table 2. Drug loading (DL) and encapsulation efficiency (EE) of MPs_T.

Sample	DL [%, w/w]	EE [%]
MPs_T	1.0 ± 0.3	10.1 ± 0.2

$$\text{EE} = \left(\frac{\text{actual drug loading}}{\text{theoretical drug loading}}\right) \times 100 \quad (3)$$

2.2.4. Thermal and Infrared Analyses

In order to investigate surfactant/polymer, drug/polymer interactions, and IND polymorphism, thermal analysis was performed using a Differential Scanning Calorimeter (DSC4000, Perkin–Elmer, Norwalk, Connecticut, USA). The DSC instrument was previously calibrated with indium. A heating rate of 10°C min^{-1} was employed over a temperature range of 30–180 °C with nitrogen purging (30 mL min^{-1}).

Fourier-Transform Infrared Spectroscopy (FT-IR) was carried out using a Spectrum Two spectrometer equipped with Universal Attenuated Total Reflection (ATR) sampling accessory (Perkin-Elmer, Milano, Italy) by depositing the samples onto the ATR crystal. The spectra were acquired in the spectral range 4000–450 cm^{-1} with a spectral resolution of 4 cm^{-1}. A 16 scan number value was selected to optimize the signal-to-noise ratio. The same conditions were applied for the background spectrum acquisition.

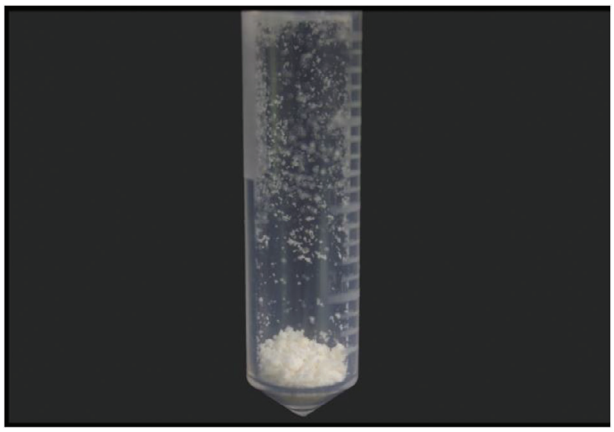

Figure 3. A picture representing a MPs_G sample recovered after freeze-drying.

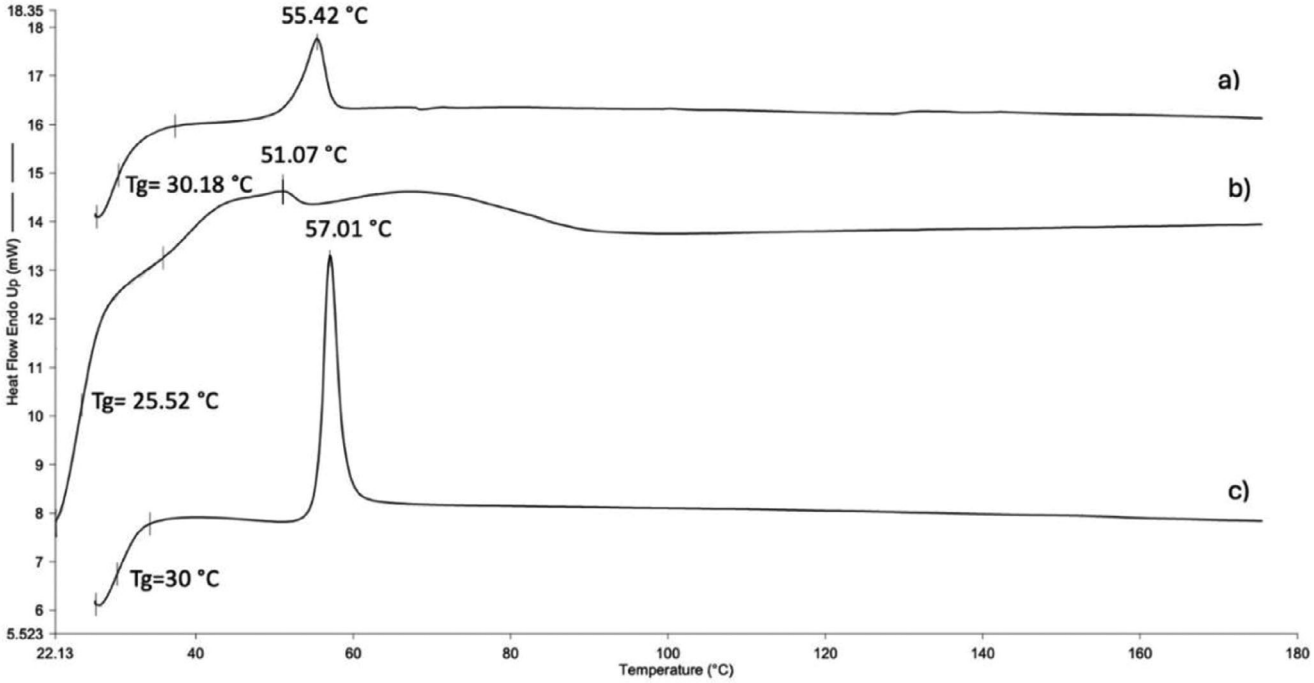

Figure 4. DSC thermograms of a) raw PLGA; b) empty MPs_T; and c) empty MPs_G.

Plain materials, MPs samples, a physical mixture of PLGA and INDγ, and solid dispersions (in the same component ratio used for MPs) were analyzed. In particular, the first solid dispersion was prepared by dissolving INDγ in DCM and then removing the solvent using a Concentrator plus instrument (Eppendorf AG, Germany) to obtain INDβ polymorph; the second solid dispersion was prepared using the same solvent method with the addition of PLGA for simulating MPs.

2.2.5. IND Solubility

The solubility of ɣ and β form of IND was measured by dissolving an excess amount of IND in MilliQ water at 37 ± 1 °C under magnetic stirring for 24 h. Then mixtures were centrifuged at 13000 rpm for 10 min (Mirko 120, Hettich Zentrifugen) and the amount of IND in solution was spectrophotometrically determined at 320 nm using a standard calibration curve in ethanol.

2.2.6. In Vitro Release Study

An exactly weighed amount of MPs containing ≈5 mg of IND was incubated in 50 mL of Phosphate buffer pH 7.4 at 37 ± 1 °C under magnetic stirring. At fixed time intervals (0.5, 1, 2, 3, 4, 5, 6, 24h, and each 24h for the subsequent 10 days) an aliquot of the suspension (1 mL) was withdrawn and centrifuged at 13500 RCF for 10 min to separate MPs from the medium. The drug content in the supernatant was determined spectrophotometrically at 320 nm from a standard calibration curve prepared in Phosphate

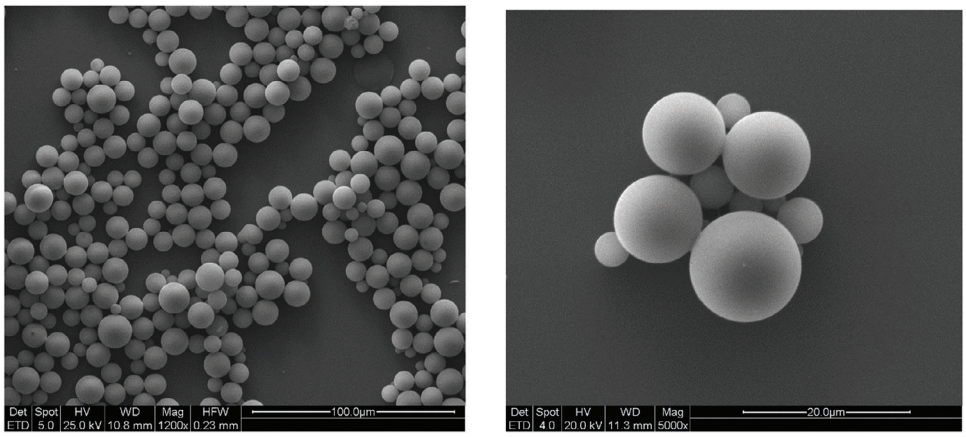

Figure 5. ESEM images of MPs_G sample at two different magnifications (1200x and 5000x).

Table 3. Drug loading (DL) and encapsulation efficiency (EE) of MPs_G.

Sample	DL [%, w/w]	EE [%]
MPs_G	2.7 ± 0.3	30.2 ± 3.4

buffer and using as blank the supernatant of the corresponding empty MPs (n = 3). After each analysis, supernatant and pellet were accurately mixed and added back into the release medium to maintain constant the volume and the amount of MPs.

2.2.7. Statistical Analysis

Data obtained were evaluated statistically using one-way analysis of variance (ANOVA). Significance was indicated by $p < 0.05$ ($^{*}p < 0.05$; $^{**}p < 0.02$; $^{***}p < 0.01$).

3. Results

The aim of the work was to produce, characterize, and optimize PLGA-MPs for the intra-articular prolonged release of IND. To prepare the MPs, the conventional single emulsion followed by solvent evaporation was chosen as the formulation technique. The settled formulation conditions are the result of a step-by-step understanding of IND-PLGA interaction.

3.1. Characterization of Initial Preparation (MPs_T sample)

Table 1 shows the adopted formulation conditions of each sample-. It is known that the preparation of MPs with homogenous size is not possible without surfactant. Therefore, Tween 80, one of the most commonly used non-ionic surfactants for its biocompatibility and poor toxicity,[17] was added first in the aqueous phase. However, after freeze-drying, the preparation appeared sticky and rubbery as illustrated in **Figure 1**.

Despite appearances, ESEM analysis on the samples showed that MPs formation has occurred, although they are clearly glued to each other with a translucent external shell (**Figure 2**).

Moreover, the obtained MPs revealed a low drug loading (DL) with only 10% drug encapsulation efficiency (EE) (**Table 2**).

3.2. Characterization of MPs_G Preparation

Assuming that Tween 80/PLGA interaction is involved in the rubbery state of the freeze-dried MPs and also in the low drug encapsulation, the surfactant was replaced with Gelatin, a protein known for its surfactant features and suitable for injection use.[10] Gelatin type A is obtained through acid hydrolysis and has an isoelectric point between pH 7.5 and 9.4, on the contrary, Gelatin type B is obtained by basic hydrolysis and has an isoelectric point at pH 4.8–5.2. When the pH of the aqueous phase is close to the isoelectric point of Gelatin, it loses its surfactant properties. Therefore, Gelatin A was selected owing to the possible acidification action of IND in the external aqueous phase during the preparation process.

As shown in **Figure 3**, after freeze-drying, the aspect of MPs prepared with Gelatin (MPs_G) was similar to a dry powder.

To elucidate the influence of the surfactant on the physical state of the formulation, DSC analyses of raw PLGA, empty MPs_T, and empty MPs_G were performed (**Figure 4**). The thermogram of raw PLGA (Figure 4a) shows a transition referred to the Tg of the polymer at ≈30 °C. The thermograms of the two MPs show different behavior: in the thermogram of MPs formulated with Tween 80 (MPs_T, Figure 4b), the Tg of the polymer occurs at a lower value (≈25 °C) compared to that of raw PLGA; conversely, MPs formulated with Gelatin (MPs_G, Figure 4c) do not lead to any modification of the Tg compared to raw PLGA.

The same consideration can be made in terms of PLGA melting temperature (Tm), which is ≈56 °C for raw PLGA and MPs_G, while a decrease of melting temperature (at ≈51 °C) was observed for MPs_T, due to Tween 80/PLGA interaction.

Even in this case, ESEM analysis of MPs_G was performed to evaluate the effect on the particle morphology resulting from the replacement of Tween 80 with Gelatin (**Figure 5**). The images showed MPs perfectly formed, regular in shape, and smooth on the surface, confirming the success in avoiding the rubbery aspect of the sample.

However, the new preparation conditions led only to a slight improvement in terms of DL and EE (**Table 3**). The permanence of the values at unsatisfactory levels can be attributed to the presence of a polymorphic form different from INDγ. To confirm this hypothesis a DSC analysis was conducted. INDγ was solubilized in DCM, the solvent used as an organic phase during the formulation, and after the solvent evaporation the obtained IND powder was analyzed by DSC and infrared spectroscopy, and compared to the initial stable γ form (**Figure 6**). The γ form of IND shows an endothermic peak at ≈163 °C due to its fusion, whereas the IND obtained after dissolution in DCM shows a different thermogram. In detail, an endo- and an exothermic peak, respectively at 91 and 101 °C were observed. Comparing this thermogram with data in the literature, it can be assumed that it represents the thermogram of the metastable β form of IND.[6,18] The peak at ≈154 °C indicates the conversion of the β form into the α form, which, in turn, converts into the γ form (peak at ≈160 °C).

Both IND polymorphs were analyzed also with FT-IR spectroscopy. The spectrum of β form has a strong vibration band at 1676 cm^{-1} that can be assigned as amide carbonyl and a second peak at 1698 cm^{-1} corresponding to the asymmetric stretch of hydrogen-bonded acid carbonyl. The main absorption peaks for the γ form are at 1689 cm^{-1} due to a single vibration mode for the amide carbonyl group, and at 1712 cm^{-1} that corresponds to another stretching band for the carboxylic acid carbonyl group (**Figure 7**).[19]

Eventually, solubility tests in water were carried out on both polymorphs (β and ɣ). These analyses confirmed the higher solubility of the metastable β form (9 μg mL^{-1}) compared to the stable γ one (5 μg mL^{-1}).

Therefore, the low DL observed for the MPs_G sample could be due to the increase in water solubility of IND during the formulation triggered by a polymorph transition.

To increase the DL, a strategy involving a reduction of drug solubility in the external water phase was adopted.

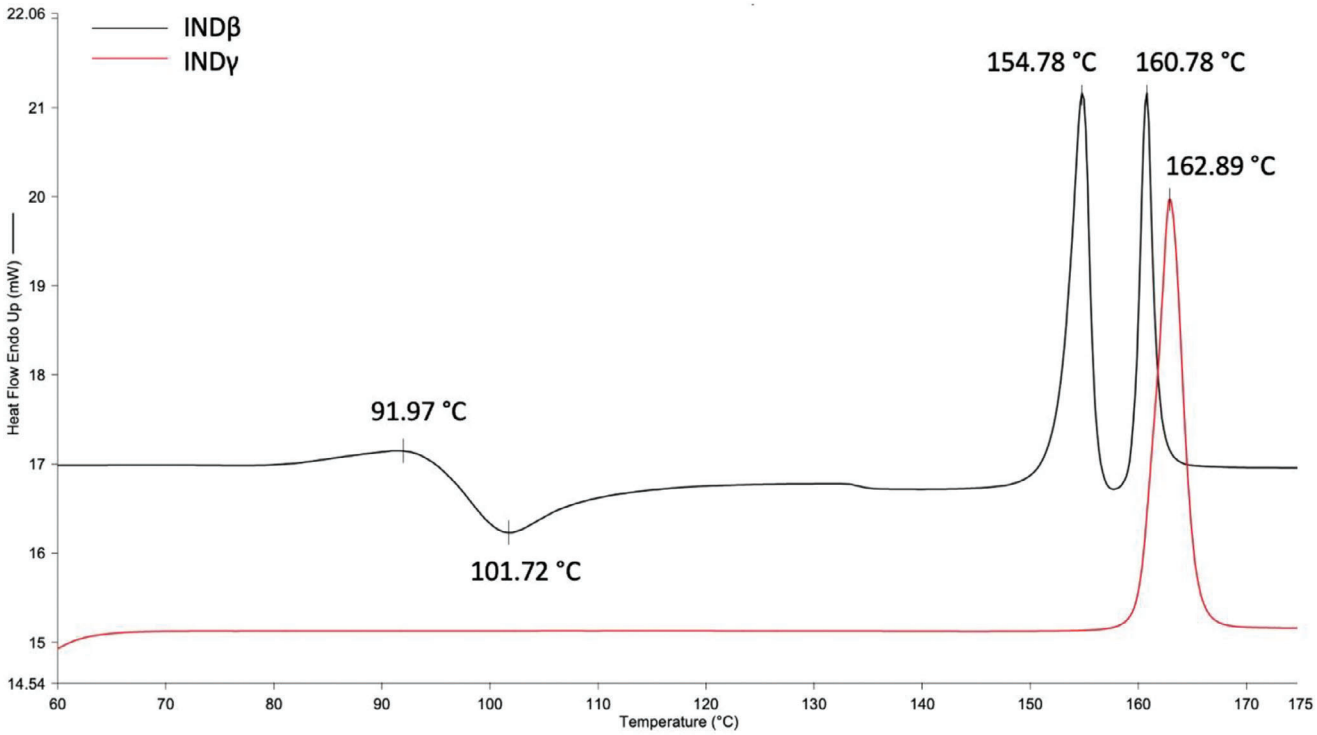

Figure 6. DSC thermograms of IND polymorphs: INDβ in black and INDγ in red.

3.3. Optimization of Drug Loading (MPs_G_* Samples)

Since the solubility of a weak acid drug such as IND is strongly affected by the pH, different pH values of the external aqueous phase, below and above the pKa of IND (pKa = 4.5), were investigated.[20] All MPs_G samples were prepared using the same solvent evaporation method and the DL and EE are reported in **Table 4**.

As can be observed from Table 4, the acidification of the aqueous phase is a relevant factor to improve DL and EE. Indeed, at values below the pKa of the drug (i.e. pH 1 and pH 3), high EE values were obtained (≈90%), while above the pKa, EE values

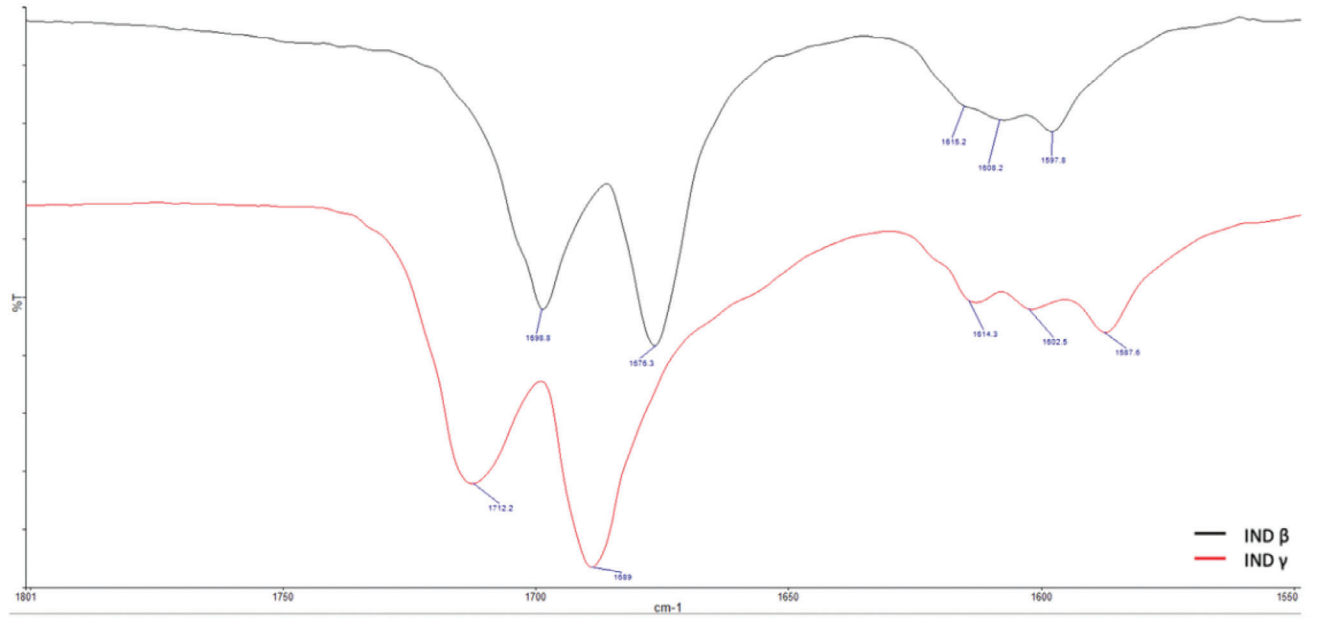

Figure 7. FT-IR spectra of IND polymorphs in the spectral region between 1800 and 1550 cm^{-1}: INDβ in black and INDγ in red.

Table 4. Drug loading (DL) and encapsulation efficiency (EE) of MPs_G_* samples.

Samples	pH of aqueous phase	DL [%, w/w]	EE [%]
MPs_G_1	1	8.3 ± 0.3	91.7 ± 3.5
MPs_G_3	3	8.2 ± 0.7	90.6 ± 7.7
MPs_G_5.5	5.5	6.2 ± 1.6	68.4 ± 3.0
MPs_G	7	2.7 ± 0.3	30.2 ± 3.4
MPs_G_9	9	0.4 ± 0.3	4.7 ± 1.7

decrease as the pH increases. Therefore, considering the similar high drug loading of MPs_G_1 and MPs_G_3, a less drastic pH 3 value was selected as the optimized final formulation to be analyzed from a physical-chemical point of view.

3.4. Physical-Chemical Characterization of Optimized Formulation

Size distribution of MPs_G_3 was achieved by measuring at least 100 particles from ESEM images and processed using ImageJ.

As observed in **Table 5**, the sample containing IND exhibited a diameter of ≈7 µm with a low homogeneity in size distribution. This observation was confirmed qualitatively in **Figure 8**a and quantified by the *SPAN* value (0.7). The same size, but different size distribution, was obtained for empty MPs_G_3 that present a lower *SPAN* value (0.5) and therefore greater dimensional homogeneity (Figure 8b). Morphology analysis by ESEM confirmed the previous data. Indeed, empty MPs appear perfectly spherical (**Figure 9**a), while IND-loaded MPs show a poor spherical shape (most of the particles are similar to a coffee bean) along with a wrinkled surface (Figure 9b).

IND-loaded MPs_G_3 was analyzed by DSC and compared to the physical mixture and empty MPs to verify IND incorporation. As observed in **Figure 10**, in the thermogram of a physical mixture, two distinct endothermic peaks can be clearly observed, attributed to the fusion of the single components, PLGA between 53 and 57 °C (Figure 4a) and INDγ slightly below 162 °C (Figure 6). As expected, empty MPs show only the peak related to the melting of the crystalline fraction of the polymer. Also for MPs_G_3 only a single endothermic peak at ≈54 °C is visible (Tm). A slight reduction in this value is observable compared to plain PLGA (55.4 °C) and a marked reduction compared to the polymer in empty microparticles (57 °C). No peak attributable to Tm of IND is detected, indicating the amorphization of the drug in the polymer. Also when PLGA was added to IND in DCM and then evaporated (solid dispersion), no IND peak can be observed, suggesting the drug amorphization in the polymer matrix.

Regarding the Tg, as previously reported in Figure 4, empty MPs_G_3 formulated with Gelatin does not lead to any modification of the value (30 °C) compared to raw PLGA.

Conversely, when formulating with IND, as observed in the solid dispersion and in the IND-loaded MPs_G_3 sample, a slight reduction in the polymer's Tg occurs (approximately from 30 to 28.5 °C) underscoring a drug/polymer interaction. However, IND simply added in a physical mixture does not affect the Tg.

Figure 11 presents the drug release profile of MPs_G_3 in Phosphate buffer at 37 °C over two weeks.. The drug release follows a typical biphasic pattern, initially diffusion-controlled and later driven by chemical erosion.[14] Specifically, an initial phase of rapid release, known as the "burst effect," was observed, during which a significant percentage of the drug was released. Notably, 80% of IND was released within three days. This was followed by a slower, second phase, referred to as the "plateau phase," during which the remaining drug was gradually released, reaching a maximum of ≈85% after eleven days.

Table 5. Mean particle size and SPAN value of loaded MPs_G_3 and empty MPs_G_3 samples.

Sample	Mean particle size [µm] ± SD	SPAN value
IND loaded MPs_G_3	7.3 ± 2.1	0.7
Empty MPs_G_3	7.0 ± 1.3	0.5

4. Discussion

The compatibility between IND and PLGA is nowadays known to be poor, which is in alignment with the experimental results showing low drug loading in terms of amorphous drug content. It has been suggested in the literature that drug-polymer compatibility can significantly affect drug loading, MPs solid-state properties, and drug release.[21] Moreover, identifying and understanding the critical variables in MPs development is not an easy task. Therefore, the effect of each variable is often analyzed individually,[22] as in this study, leading to suitable formulation conditions that are the result of a step-by-step understanding of IND-PLGA interaction.

One of the fundamental properties to consider during the formulation process of MPs is the Tg of PLGA (between 30 and 60 °C). Depending on the handling techniques, type of drug loaded, and surfactants used, Tg temperature of the polymer may change.[5] According to literature, the rubbery state of the polymer represents a liquid-like structure with high molecular mobility and is more prone to physical and chemical changes than the glassy state.[23] Acting as a surfactant, Tween 80 helps to stabilize the emulsion but also acts as a plasticizer by embedding itself within the polymer matrix. A high concentration of Tween 80 can lower the Tg of the PLGA (from ≈30 to 25 °C), enhancing polymer chain mobility.[23] The ability of Tween 80 to act as a plasticizer appears to be responsible for a sticky and rubbery sample and not a classic powder. The complete replacement of Tween with Gelatin was the strategy adopted to avoid the rubbery state of the sample, since DSC analysis showed that the latter did not influence the Tg value of the polymer[24] leading, at the same time, to a slight increase in DL and EE.

Park et al. demonstrate that also IND interacts with PLGA, causing a slight reduction in the polymer's Tg.[25] The authors underlined that IND behaves as a plasticizer by intercalating between the polymer chains, increasing their mobility, and interacting at an intermolecular level. Regarding the plasticizing effect, authors hypothesized that the interactions responsible for this slight reduction of Tg are (polar) hydrogen bonds established

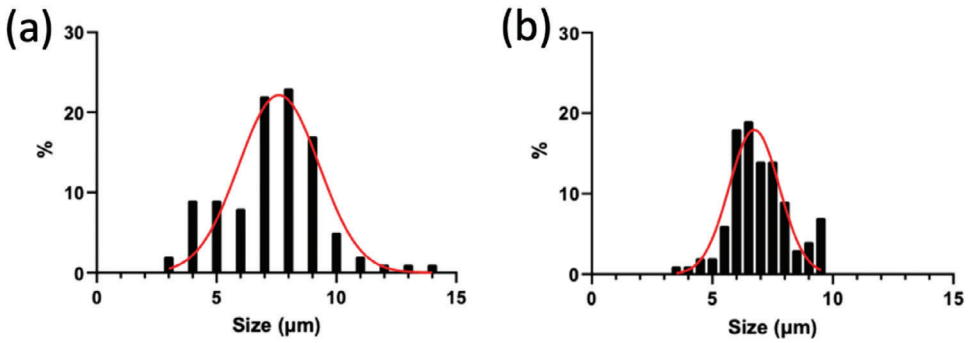

Figure 8. Size distribution of (a) IND loaded MPs_G_3 and (b) empty MPs_G_3 samples.

between the carboxyl group of IND and the ester groups of PLGA. Also, molecular dynamics simulations reported in the literature show that, at low temperatures, molecules of NSAID drugs around the PLGA are inclined to establish hydrogen bonds.[6] On the other side, at higher temperatures, the number of molecules around the PLGA increases even if the probability of establishing hydrogen bonds decreases. Since the results of the simulations suggest that the interaction of the drug with the polymer increases with temperature, it is hypothesized that hydrogen bonds are not the only interactions involved, and non-polar interactions may be responsible too as the temperature rises.[6] Since DCM can also act as a plasticizer during MPs formation, it induces high molecular mobility that allows the reorganization of uncharged IND molecules into dimers, essential for unit cell formation, and crystal growth. Furthermore, the stress applied during stirring is known to leave the system with a higher energy, i.e., a less stable state.[25] The β polymorph is structurally similar to the amorphous form and appears to be the least stable form. Since this form exhibits the highest degree of disorder, it has the lowest activation energy in the amorphous-crystalline barrier and is, therefore the one favored by the kinetic driving force.[19] The β polymorph could be responsible for a low IND encapsulation, being the metastable as well as the most soluble form.

To increase the drug loading, a strategy involving the reduction of drug solubility in the external aqueous phase was adopted. The pH of the external aqueous phase during the preparation of MPs can significantly influence the drug encapsulation efficiency, especially for drugs with pH-dependent solubility characteristics like IND, a weak acid with a pKa of ≈4.5. At pH values below its pKa (e.g., pH 3), it predominantly exists in its unionized form, which has lower solubility in aqueous solutions. Consequently, in this form, IND is less likely to pass into the external aqueous phase of the emulsion during formulation, remaining mostly incorporated into the polymer matrix. MPs_G_3, being characterized by suitable formulation conditions to obtain a high EE (≈90%), was selected and analyzed from a chemical-physical point of view. DSC analysis provides further evidence of IND incorporation and FT-IR spectroscopy corroborates the DSC findings. MPs_G_3 exhibits a slightly irregular, coffee bean-like shape with a wrinkled surface, indicating the encapsulation process. The incorporation of a plasticizer drug affects the MPs internal and surface structure by interfering with the polymer's Tg and causing deformation. MPs_G_3 particles have an average diameter ≈7 μm, suitable for syringability and retention in the synovial capsule. Complete retention of MPs has been observed at diameters of at least 3 μm, regardless of the joint's inflammatory state.[26] MPs ranging from 3 to 100 μm can act as depots, being small enough for syringe injection but large enough to avoid drainage and clearance by phagocytic cells.[27]

The released form of IND is most likely the amorphous one, being the form with the highest degree of disorder, as discussed above. The drug release profile from MPs_G_3 shows a

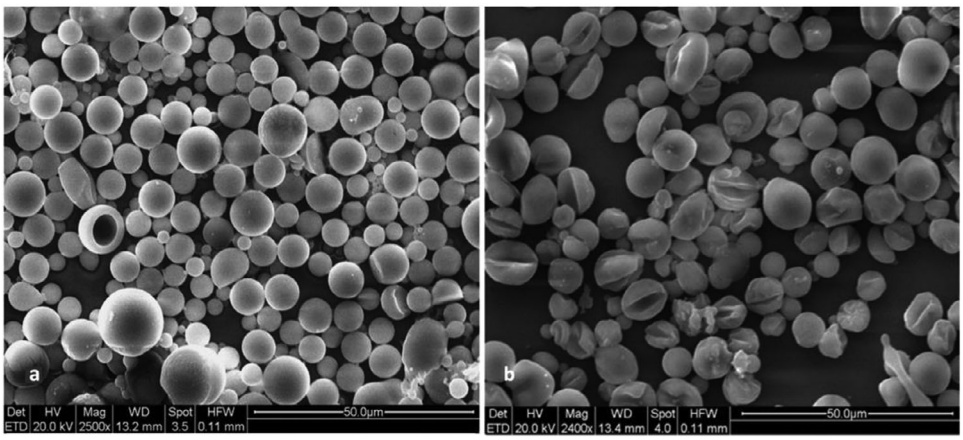

Figure 9. Representative pictures of a) empty MPs_G_3 and b) IND-loaded MPs_G_3 from ESEM analysis.

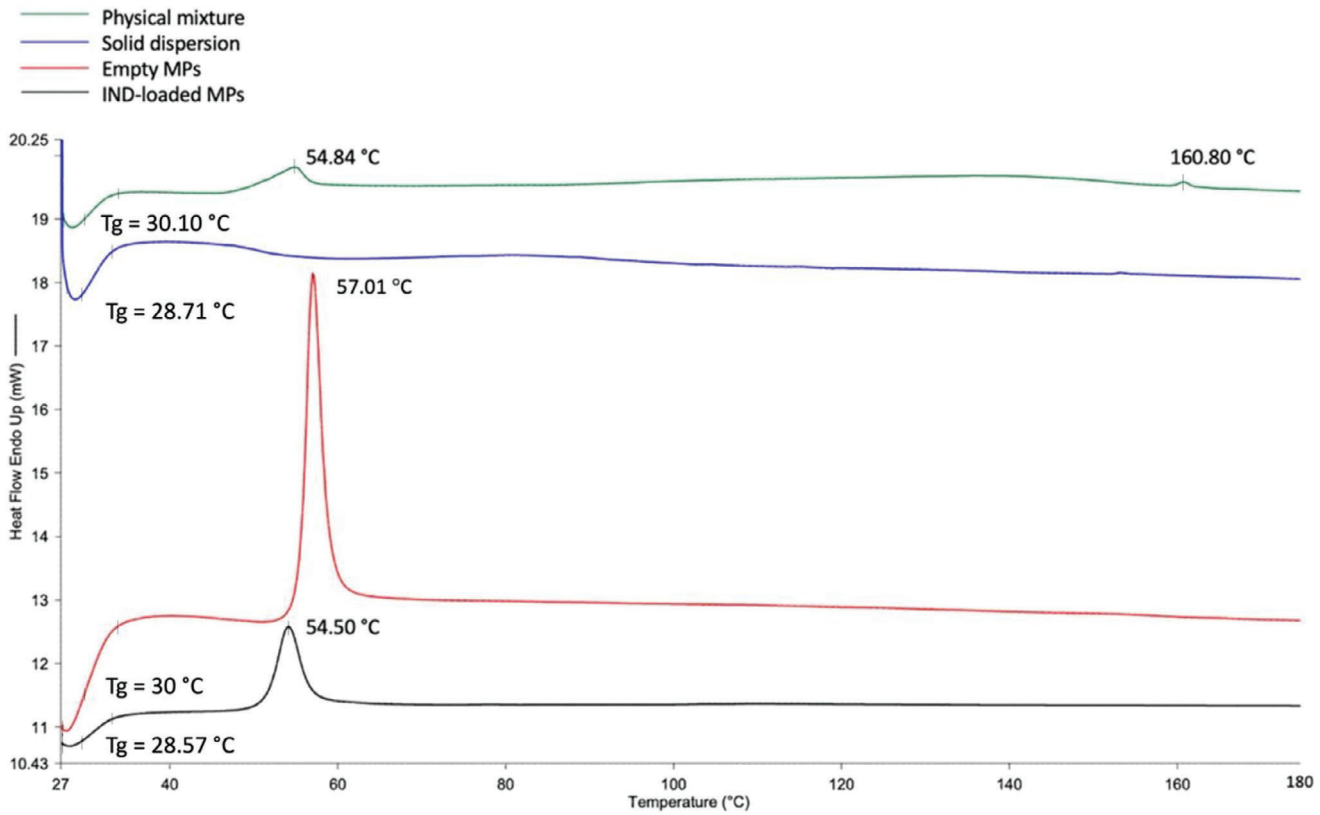

Figure 10. DSC thermograms of the physical mixture in green, solid dispersion in blue, empty MPs_G_3 in red, and IND-loaded MPs_G_3 in black.

two-stage mechanism. Initially, a rapid release phase, or "burst effect," occurs, with 80% of IND released within the first three days, likely due to the dissolution of surface-bound IND. This is followed by a slower release phase, where the remaining drug is gradually released, reaching ≈85% after eleven days. This biphasic profile, typical for PLGA-based systems, includes an initial diffusion-controlled release followed by a degradation-controlled release. The initial burst release ensures a quick therapeutic effect, while the sustained phase provides prolonged drug availability, potentially reducing administration frequency and enhancing patient compliance. The gradual drug release during the steady-state phase occurs as the polymer degrades enzymatically, and the release does not reach 100% in vitro.[25]

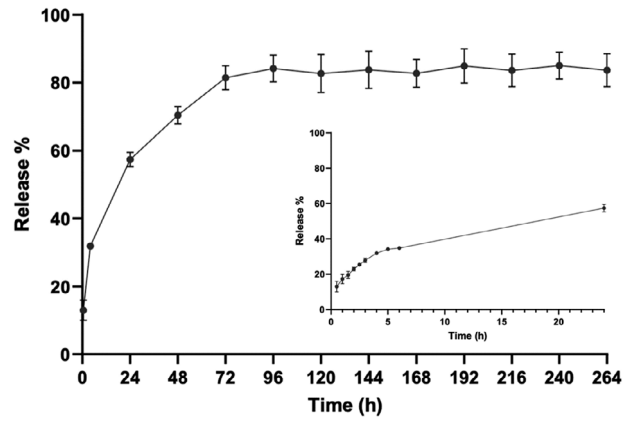

Figure 11. The drug release profile of sample MPs_G_3 with a focus on the first 24 h.

5. Conclusion

Despite the extensive literature on the development of polymeric MPs, there are no universal protocols for producing MPs with predictable DL, EE, particle size, size distribution, and drug release. Identifying and understanding the critical variables in MPs development is challenging and the design of experiments (DOE) or artificial intelligence (AI) methodologies could be good tools to solve this inconvenience. However, these approaches are not feasible for modeling the results and drawing correct conclusions when the variables involve the interaction among constituents.[22] Thereby, in this study, optimization of each specific formulation parameter, such as the type of surfactant or the pH of the aqueous phase, was complemented by an in-depth study of the physical-chemical implications. As a result, the obtained IND-loaded PLGA MPs exhibit a high encapsulation efficiency (over 90%) and sustained drug release profile and are potentially capable of providing a reduction of the administration dose and minimization of systemic side effects. In the future, computational approaches, such as Design of Experiment (DOE) o Artificial Intelligence (AI), may be applied to further improve particle features. Nevertheless, this study provides a foundational framework toward the optimization of the IND encapsulation in PLGA

MPs, potentially advancing future clinical applications of such drug delivery system.

Acknowledgements

M.A. and E.M. contributed equally to this work.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

differential scanning calorimetry, intra-articular administration, microencapsulation, NSAID, poly(lactic-co-glycolic) acid

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