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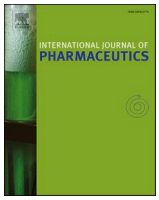

Microfluidic nanoparticle synthesis for oral solid dosage forms: A step toward clinical transition processes

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ABSTRACT

Nanomedicine provides various opportunities for addressing medical challenges associated with drug bioavailability, stability, and efficacy. In particular, oral nanoparticles (NPs) represent an alternative strategy to enhance the solubility and stability of active ingredients through the gastrointestinal tract. The nanocarriers could be used for both local and systemic targeting, enabling controlled release of encapsulated drugs. This approach allows more efficient therapies.

In this work, we aim to develop reliable oral solid dosage forms incorporating NPs produced by either one pot synthesis or continuous production, following protocols that yield highly consistent outcomes, promoting their technology transfer and clinical use.

Microfluidics technology was selected to allow an automated and highly productive synthetic approach suitable for the highly throughput production.

In particular, innovative systems, which combine advantage of NPs and solid dosage formulation, were designed, developed, and characterized demonstrating the possibility to obtaining oral administration. The resulting NPs were thus carried on oral dosage forms, *i.e.*, pellets and minitablets. NPs resulted stable after dosage forms manufacturing, leading to confidence also on protection of encapsulated drugs. Indomethacin was used as a tracer to test biopharmaceutical behaviour.

Anti-inflammatories or cytotoxic chemotherapeutics could be vehiculated leading to a breakthrough in the treatment of severe diseases allowing the oral administration of these drugs. We believe that the advancement achieved with the results of our work paves the way for the progression of nanoproducts into clinical transition processes.

1. Introduction

Oral delivery is a preferred route for drug administration because of its non-invasive nature and excellent patient's compliance and convenience, which contribute to the therapeutic efficacy of medicines (Patel, Joshi,and Sawant, 2020; Yang, Dai, Wang, et al. 2023). Despite these obvious advantages, oral route is characterized by some drawbacks, mostly relating to the stability of labile drugs along the gastrointestinal (GI) tract and to an intrinsic limitation associated with poor solubility of hydrophobic drugs, which are often indicated in cancer chemotherapy (Pourjavadi,Amin,and Hosseini, 2018).

Nanotechnology aims to solve these issues using oral nanoparticles (NPs) suitable to improve drug stability protecting it from GI environment, to increase the drug solubility characteristics and/or to deliver drugs to a specific site in the GI tract by active or passive targeting that could prevent first pass metabolism of encapsulated drugs (Date,Hanes, and Ensign, 2016; Bakhru et al., 2013). Recently, oral nanomedicine has been applied for targeted modulation of gut microbiota–brain crosstalk in intestinal diseases (He et al., 2023; Yang, Dai, Cheng, et al. 2023). In addition, it could be useful for masking the bad taste of drugs, such as antiretroviral agents or non-steroidal anti-inflammatory drugs (NSAIDs) (Nasr, ElMeshad, and Fares 2022).

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Nanocarriers could be formulated in a well-defined dosage form suitable for an easy administration and/or useful to obtaining modified drug release (Usman et al., 2016).

Generally, for their administration, NPs should be dispersed in aqueous liquid to obtain the final dosage form, which requires strict storage conditions to prevent aggregation, contamination, or degradation (Zielińska et al., 2020); for this reason, vehiculating NPs in solid oral dosage forms could represent a valid alternative. In recent years, there have been few studies on this approach (Ahmad et al., 2023; Horster et al., 2019) that could preserving NPs stability and taking advantage of improved patient compliance and the possibility to obtaining time- or site-specific release (Hua et al., 2018).

The first step necessary to obtain a successful product, intended to be incorporated in oral dosage forms, in terms of quality, yield, and reproducibility relies on the development of a protocol for NPs automated synthesis. Microfluidics technology allows achieving higher process yield, provides better reproducibility and increased drug encapsulation efficiency compared to traditional methods (Streck et al., 2019). The enhanced potential of microfluidics resides in the opportunity to control the process parameters, as flow rate ratio and total flow rate, allowing the optimizing of the experimental settings to obtain NPs with the desired features, such as size distribution.

The aim of this work is to assess the use of microfluidics to produce nanoparticles intended to be formulated into oral dosage forms. In particular, we have incorporated a poorly soluble drug selected as a tracer, *i.e.,* Indomethacin, into poly lactic-*co*-glycolic acid (PLGA) NPs and formulated the resulting drug-loaded NPs into immediate release oral dosage forms. PLGA-NPs were selected because they were approved by FDA in the formulation of medicines; thus allowing their relatively fast transfer to preclinical and clinical investigation (Sharma et al., 2016). The formulation stability and the release profiles of drug tracer loaded by the nanocarrier was compared to that of the drug carried on conventional oral dosage forms.

2. Materials and methods

2.1. Materials

Poly lactic-co-glycolic acid (PLGA, 38–54 KDa, 50:50, acid terminated); Polyvinyl alcohol (PVA, 9–10 KDa) were bought from Sigma-Aldrich (St. Louis, MO, USA). Indomethacin (IND) was bought from Thermo Scientific (Waltham, MA, USA). Acetonitrile (ACN, HPCL grade) and Methanol (MeOH, HPCL grade) from Carlo Erba Reagents (Milan, IT).

Colloidal silica (Aerosil 200) gifted by Evonik (Essen, DE); Explotab CLV and Avicel CL 611 were kindly donated by Rettenmeier Italia (Castenedolo, IT) and by IMCD Italia (Milan, IT), respectively. Whereas Magnesium stearate and Lactose were bought from ACEF (Fiorenzuola d'Arda, IT).

2.2. Settings of microfluidic system

Initializing the microfluidic system requires setting the software based on the type of solvent used for the synthesis. In particular, when an organic solution is used, this setting becomes indispensable. On the other hand, when an aqueous solution or a particular set of solvents is employed, this is not necessary. The settings have been configured to match the actual flow rate to the one pre-set by the software. In particular, the solution was flowed for 5 min setting different flow values, in the range 8–50 μL/min, and then the actual volume was measured, in order to draw a calibration curve. This setup was performed with a solution of PLGA in acetone, 1.0 or 0.5 % w/V. (Fig. S1).

In general, the microfluidic system (bought from Dolomite, Royston, UK) is composed by different pumps which, with external air source, converge two phases into the chip, in order to mix and synthetize NPs. The chip used is the 5 Input 3D 150 μm, a glass microfluidics device. Its design enables consistent diffusive mixing at the two liquid interfaces for the nanoprecipitation of polymer particles. (Jahn et al., 2007).

2.3. Polymeric nanoparticles: Synthesis and purification

Solution of PLGA in acetone (5 mg/mL), adding eventually Indomethacin, has been prepared and flowed in the microfluidic system and it has been mixed with PVA solution (1.0 or 0.5 %) for different time lengths following nanoprecipitation method with 5 input 3D chip. Different total flow rate (TFR) and flow rate ratio (FRR) have been tested to verify the better compromised to formulate polymeric nanoparticles. For each trial, the productivity of the process is calculated as the mass of nanoparticles obtained per unit of time (mg/min).The microfluidic products were centrifuged at 34,957 g for 50 min thrice by Avanti J-20 Centrifuge (Beckman Coulter, Indianapolis, IN, USA); the resulting supernatant has been removed, and pellets re-suspended in MilliQ water in order to eliminate excess of PVA and concentrate the final NPs suspension.

The samples with IND, in order to be purified from the unencapsulated drug, were appropriately diluted, before centrifugation, so that the drug concentration was below its solubility. The samples have been lyophilized (-60 °C, 1.4 mBar, overnight) and stored at −20 °C.

2.4. Nanoparticles characterization

All of nanoparticle's batches were characterized by Dynamic Light Scattering (DLS) and Nanoparticles Tracking Analysis (NTA). The first one measures hydrodynamic diameter with a Zetasizer Nano ZS ZEN3600 (Malvern Panalytical, Malvern, UK) operating at a light source wavelength of 633 nm and fixed scattering angle of 173°. The results were expressed as mean ± SD of 3 independent measurements.

NTA characterized NPs size distribution employing Nanosight NS300 (Malvern Panalytical, Malvern, UK) using 405 nm laser excitation and high sensitivity CMOS camera. The results were expressed as mean ± SD of 3 independent measurements.

2.5. FT-IR analysis

PLGA and PVA powder, PLGA NPs and supernatant lyophilized, obtained after each centrifugation, were analysed by FT-IR analysis with Jasco IR4100 (Jasco Europe, Cremella, IT).

2.6. PVA-iodine analysis

In order to verify qualitative efficiency of purification methods form PVA, it has been performed a colorimetric assay based on the formation of coloured complex between two adjacent hydroxyl groups of PVA and an iodine molecule, which allows to quantify molecules of PVA associated with nanoparticles. After each centrifugation, several samples of NPs were lyophilized in order to calculate the amount of PVA, based on a previously prepared calibration curve. For this analysis, it has been followed method report by Sahoo et al. (Sahoo et al., 2002). In particular, 0.5 mg of lyophilized nanoparticles sample were treated with 0.5 mL of NaOH (0.5 M) for 15 min at 60 °C. Each sample was neutralized with HCl 1 N, and the final volume was adjusted with water. To each sample, 0.75 mL of boric acid (0.65 M), 0.125 mL of I_2/KI (0.05 M/0.15 M), and 0.375 mL of water were added. For the quantification, a calibration curve was calculated analysing standard solutions with amount of PVA in the range between 9.9 and 150 μg/mL and setting the UV detector at 690 nm. A standard sample (100 μg/mL) of PVA was prepared for positive control.

2.7. Quantification of Indomethacin by HPLC analysis

HPLC protocol has been set by modify the one described in USP monograph (United States Pharmacopeial Convention, Rockfield, MD,

Table 1
Quali-quantitative composition of minitablets carrying on different amount of Indomethacin (MiniTBL) or nanoparticles containing Indomethacin (MiniTBL_NPs), 0.75 % or 1.50 %, w/w, respectively. All values are expressed in % (w/w).

	Components (%, w/w)					
Formulations (%, w/w IND)	IND	NPs	Explotab CLV	Lactose	Magnesium stearate	Colloidal silica
MiniTBL (0.75 %)	0.75	–	10	86.75	1.50	1
MiniTBL (1.50 %)	1.50	–	10	86.00	1.50	1
MiniTBL_NPs (0.75 %)	–	25 (=0.75 IND)	10	62.50	1.50	1
MiniTBL_NPs (1.50 %)	–	50 (=1.50 IND)	10	37.50	1.50	1

Table 2
Quali-quantitative composition of pellets carrying on Indomethacin (0.75 %, w/w) (Pellets) or nanoparticles containing Indomethacin (Pellets_NPs). All of values are expressed in % (w/w).

	Components (%, w/w)				
Formulations	IND	NPs	Explotab CLV	Lactose	Avicel CL 611
Pellet	0.75	–	12	47.25	40
Pellets_NPs	–	25 (=0.75 IND)	12	23	40

USA). The analysis was performed using HPLC instrument (Infinity II 1260, Agilent Technologies, Milan, IT) where mobile phase was composed by ACN and buffer phosphate pH 6.2 (40:60) using C-18 as column (25 °C), flow 1 mL/min. At least lyophilized NPs was dispersed in ACN to have 1 mg/mL and it was sonicated for 5 min, at 30 °C; MeOH was added to have a mixture ACN/MeOH 1:2 (v/v) to precipitate the polymer and, after centrifugation (20 min, 7000 g, 4 °C), to collect the supernatant where the drug is dissolved. After that, 300 μL of phosphate buffer (pH 6.2) was added to obtain the final solution (ACN:MeOH: buffer 26:51:23 v/v/v). For the quantification of IND, a calibration curve was calculated with standard solutions of drug in ACN:MeOH (1:2) whose concentrations were in the 3–96 μg/mL range. The UV detector was set to analyse the samples at 230 nm wavelength. The obtained results could be used to calculate the efficiency of encapsulation (EE%), that is the percentage ratio between the amount of drug encapsulated and the amount of drug used for synthesis (w/w, %), and the loading that is the ratio between drug encapsulated and the amount of PLGA NPs (w/w, %).

To quantify the drug released, a standard concentration curve of IND (0.8 – 3 μg/mL) has been prepared in pH 7.2 buffer phosphate (the same used for dissolution test). After conducting dissolution tests, all samples were filtered (PVDF 0.22 μm) to remove any particles.

2.8. Formulation and characterization of solid oral dosage forms

2.8.1. Mini-tablets

Flowability of freeze-dried NPs and powders formulations was assessed by calculation of angle of repose (2.9.36: European Pharmacopeia Ed 11.2). Mini-tablets were prepared using the 4 formulations listed in Table 1. Two of them included nanoparticles containing Indomethacin and are named MiniTBL_NPs, and the other two contain the corresponding amount of free active ingredient and are named MiniTBL.

Powders (2 g total for MiniTBL and 500 mg for MiniTBL_NPs, comprehensively) are mixed in a mortar for 5 min. All of mini tablets were prepared using a rotary tablet press (AM8S, Officine Meccaniche Ronchi, Cinisello Balsamo, IT) equipped with concave 2 mm size punches composed by 16 pins applying 12 KN compression force (0.75 KN for pin). The die cavities were filled manually, and the position of lower punch was fixed to the minimum value allowed by the machine. Mini-tablets were characterized by weight (Europe 500, Gibertini, IT) and size by a digital caliper (Mitutoyo Absolute Digimatic, Mitutoyo Italia, Lainate, IT).

Disintegration test was performed in a disintegrator apparatus (Sotax DT3, Aesch, CH): 6 mini tablets were tested in a becher vessel containing 800 mL of water (37 ± 2 °C); the basket-rack was equipped with 0.5 mm mesh.

2.8.2. Pellets

Pellets with or without NPs was prepared using the 2 formulations listed in Table 2. One of them included nanoparticles containing Indomethacin (named Pellets_NPs) and the other the corresponding amount of free active ingredient (named Pellets).

Powders (5 g, comprehensively) were mixed in a mortar for 5 min and wetted with 3 mL of water using a pestle. The resulting wetted mass was extruded through a 1000 μm sieve. Spheronization was performed in a spheronizer (Nica S320, GEA, Dusseldorf, DE) with a cross-hatched plate (250 rpm for 4 min). Pellets were finally dried in a static oven at 40 °C for 24 h.

Equivalent spherical diameter to area; equivalent spherical diameter to perimeter; aspect ratio (AR) and circularity were determined using an image analysis system. Digital photomicrographs (n = 25, DinoCapture, Taipei City, Taiwan) were analyzed by ImageJ software (NIH, Bethesda, MD, USA) that allow calculation of dimension and shape parameters.

Aspect ratio (AR) is the ratio of the Maximum to the Minimum Feret Diameter of the particles imagine:

$$Aspect\ \ Ratio = Major\ \ Axis / Minor\ \ Axis$$

Circularity is the 2D equivalent of the true sphericity index. Circularity values range from 1 (perfect circle) to 0 (elongated shape, monodimensional shape) and it was calculated as:

$$Circularity = 4\pi^* \frac{Area}{perimeter^2}$$

Disintegration test was performed in a disintegrator apparatus (Sotax DT3, Aesch, CH): several of pellets previous sieved (1000 μm) were tested in a vessel containing 800 mL of water (37 ± 2 °C), the basket-rack was equipped with 0.1 mm mesh, and time of disintegration has been measured visually until absence of materials.

2.9. Dissolution test

Each sample (three samples for each batch, namely NPs, MiniTBLs, MiniTBL_NPs, Pellets, Pellets_NPs) was located in different vessels in 750 mL of phosphate buffer pH 7.2 (0.2 M) and water (1:4, v/v) (37 °C, 100 rpm) in order to perform dissolution test (Dissolution system 2500, North Brunswick, NJ, USA) as stated by USP Monograph of Indomethacin Capsules. Two mL of dissolution media were withdrawn at

Table 3
Characterization of NPs before and after the process of lyophilization (mean ± SD, n = 9).

	Hydrodynamic diameter (DLS analysis, nm)	Hydrodynamic diameter (NTA analysis, nm)	PDI	Yield of process (mg PLGA/mg NPs, %)	Productivity (mg NPs/min)
Before lyo	179.8 ± 13.7	183.2 ± 5.1	0.148 ± 0.050	–	–
After lyo	194.1 ± 15.2	195.7 ± 16.8	0.158 ± 0.071	64.6 ± 5.7	0.86 ± 0.07

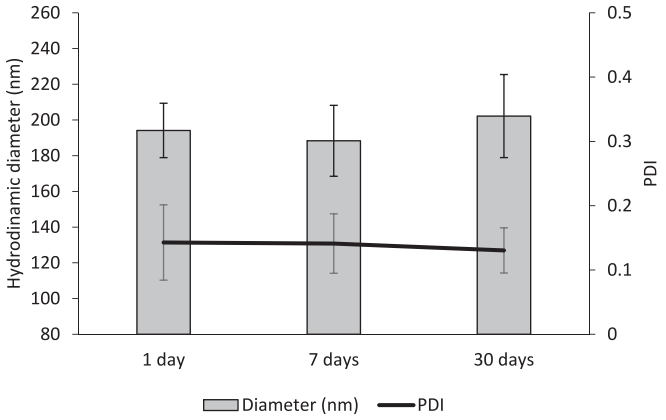

Fig. 1. Hydrodynamic mean diameter and PDI of PLGA NPs (TFF 1066 μL/min and FRR 3) 1, 7 and 30 days after their manufacturing (mean ± SD, n = 9).

predetermined times (until 24 h) to be analyzed in terms of drug concentration. Samples without NPs were directly analyzed by HPLC analysis, after filtration; samples with NPs were centrifuged (25000 g, 15 min, 4 °C) to separate drug released from NPs. Pellets precipitated was re-suspended by 2 mL of dissolution buffer and added to vessel, supernatants with drug released have been analyzed by HPLC, as described before (Par 2.7).

3. Results and discussion

3.1. Synthesis of poly lactic-co-glycolic nanoparticles by microfluidics

Protocol for polymeric NPs synthesis was optimized to set up the solution parameters, including flow rate ratio (FRR) and total flow rate (TFR) (Table S1, Fig. S2). Based on the results obtained in this preliminary test, in order to obtain a good outcome, including narrow size distribution and high productivity in term of amount of NPs obtained in unit of time, FRR and TFR were set at 3 and at 1066 μL/min, respectively, for the NP synthesis. Polymer, with a 50:50 ratio between glycolic acid and lactic acid monomers, has been selected because it is characterized by higher degradation process and consequently faster drug release compared to other ratio monomers (Makadia and Siegel, 2011). The aqueous phase was constituted by PVA solution (1 %, m/v), while organic phase was a PLGA solution in acetone (0.5 %, v/v); it represents optimal concentration to avoid chip clogging. Centrifugation was chosen as a method for the purification of PLGA NPs: qualitative FT-IR (Fig. S3-S5) and quantitative (PVA-iodine assay) analyses (Fig. S6) were performed to evaluate the good efficacy and efficiency of the selected method.

Process and material parameters were adjusted as mentioned above and several batches of NPs were characterized in terms of hydrodynamic average diameter and polydispersity index (PDI) before lyophilisation (Table 3., *before lyo*) to confirm reproducibility of the protocol. In order to enable granulation and compression processes, while preserving stability upon incorporation into oral dosage forms, the NPs underwent lyophilization. This procedure was carried out without the addition of cryoprotectants and it demonstrates to have no relevant impact on the hydrodynamic dimensions of the NPs (Table 3., *after lyo*).

The products underwent the same characterization tests at different time points of shelf-life to evaluate their stability (Fig. 1). After 30 days, hydrodynamic mean diameter was slightly increased without a significant difference ($p > 0.05$), while the polydispersity index never exceeds 0.2.

Table 4
Materials parameters applied in the three production methods of three formulations of Indomethacin-loaded NPs and their characterization in terms of size and polydispersity index (PDI) and encapsulation efficiency (EE%) (mean ± SD, n = 3).

Formulations	IND (mg/mL)	PLGA/IND	Hydrodynamic diameter (DLS analysis, nm)	PDI	EE%
NPs10_IND30 (1)	0.17	30	137.6 ± 1.9	0.107 ± 0.024	10.4 ± 0.19
NPs10_IND20 (1)	0.25	20	165.9 ± 2.5	0.138 ± 0.061	12.7 ± 0.29
NPs10_IND10 (1)	0.50	10	175.8 ± 8.2	0.220 ± 0.013	11.8 ± 0.11

(1) NPs10_IND30/20/10: where NPs10 indicates the concentration of PVA (10 mg/mL) and IND30/20/10 the PLGA/IND weight ratio, respectively.

Table 5
Characterization of three different batches of NPs-loaded Indomethacin in terms of size and polydispersity index; analysis of Encapsulation Efficiency (EE%) and loading (%) (mean ± SD, *NPs5_IND30 and NPs5_IND20*, n = 3; *NPs5_IND10*, n = 20).

Formulations	Hydrodynamic diameter (DLS analysis, nm)	PDI	EE%	Loading (%)
NPs5_IND30 (2)	215.7 ± 17.8	0.17 ± 0.02	14.07 ± 1.38	0.64 ± 0.08
NPs5_IND20 (2)	215.7 ± 30.7	0.21 ± 0.05	22.90 ± 6.10	1.50 ± 0.50
NPs5_IND10 (2)	188.4 ± 16.4	0.12 ± 0.04	31.76 ± 8.73	3.49 ± 0.86

(2) NPs5_IND30/20/10: where NPs5 is for concentration of PVA (5 mg/mL) and IND30, 20, 10 is for ratio between PLGA/IND respectively.

3.2. Synthesis and characterization of PLGA NPs loaded with Indomethacin

After setting the synthesis method, it was deemed useful to load a drug tracer into the NPs to better study their behaviour. Indomethacin (IND) is a poorly soluble in water and easy to detect by UV–Vis analysis. Its hydrophobic character makes it a good candidate to investigate the potential of nanoparticles to improve oral administration of insoluble drugs. Applying the One Factor At Time (OFAT) methods, 3 different PLGA/IND weight ratios (*i.e.*, 10, 20 and 30) were tested in three different experiments, maintaining the same concentration of PVA and PLGA (10 and 5 mg/mL, respectively), to select the best formulation in terms of encapsulation efficiency and particle size distribution. In particular, the three produced batches were characterized by DLS and by HPLC to measure the particle size distribution and to quantify the encapsulation efficiency (EE%) obtaining the results listed in Table 4. Although the results attest to the good quality of the products, they were worse than those found in literature (Corrigan and Li, 2009; Badri et al.,

Table 6
Characterization of minitablets with or without NPs in terms of size and weight (mean ± SD, n = 3).

	MiniTBL (0.75 %)	MiniTBL (1.50 %)	MiniTBL_NPs (0.75 %)	MiniTBL_NPs (1.50 %)
Height (mm)	2.25 ± 0.01	1.98 ± 0.11	1.74 ± 0.13	1.41 ± 0.41
Weight (mg)	8.81 ± 1.98	8.86 ± 2.25	7.22 ± 2.41	4.84 ± 1.92

2018).

We supposed that the concentration of PVA used (10 mg/mL) could increase the stability of IND in aqueous solution (Brough et al., 2016) during the synthesis and it could prevent its precipitation with PLGA polymer for NP formation, causing consequent low drug loading; for this reason, the next synthesis trials were conducted using 5 mg/mL of PVA.

The reduction of concentration of PVA leads to enhanced encapsulation efficiency (Table 5) obtaining the best result when the minimum ratio between PLGA and Indomethacin was used (*NPs5_IND10*). This type of NPs has been selected for the subsequent experiments, since the high number of synthesis replicates (n = 20), confirm its high reproducibility, loading efficiency, and overall good quality. Moreover, yield of process (80.35 ± 7.33 mg NPs/mg PLGA) and productivity (1.18 ± 0.25 mg NPs/min) have been calculated.

The results obtained, demonstrate that microfluidic technique provides great opportunities for synthesizing NPs, also when loading a poorly soluble drug, allowing tightly controlled conditions and with moderate consumption of materials and time. In particular, in view of vehiculating the NPs in an oral dosage form, this technique is suitable to have relatively high amount of product endowed with extreme homogeneity in terms of size and drug loading.

3.3. Formulations of oral dosage forms with polymeric NPs

Flow property of freeze-dried NPs is passable (angle of repose 42°) but when mixed with excipients for tablets it becomes excellent (angle of repose 26°). Subsequently, freeze-dried NPs were formulated into oral dosage forms, in particular pellets and mini tablets, and their behaviour after technological manipulation was studied.

Mini tablets and granules were specifically chosen because they are useful for the preparation of multiple-unit dosage forms, offering various advantages in addition to the possibility of obtaining a modified release, such as therapeutic dose adjustments by varying the number of sub-units administered (e.g., for paediatric patients), the possibility of combining different active ingredients, the reduction of the risk of dose dumping and, finally, the uniformity of the transit time along the gastrointestinal tract (Zuccari et al., 2022; Palugan et al., 2015).

To evaluate the NPs behaviour, mini-tablets and pellets with or without NPs were prepared. The same amounts of drug tracer (IND) were carried on both dosage forms, assuming that the drug loading was reproducible for different batches manufactured.

3.3.1. Characterization of minitablets

Minitablets with or without NPs were manufactured and characterized in terms of height and weight. Some differences were found among analyzed samples: MiniTBL_NPs (1.50 % IND) were the lightest, probably due to low density of powders composed by 50 % of NPs (Table 6). However, the disintegration time was not affected, being less than 2 min for all formulations.

3.3.2. Characterization of pellets

Shape of pellets was measured by analyzing digital photomicrographs image (Fig. 2) by ImageJ software (Table 7).

As attested by AR and circularity values and also as evidenced by pictures, pellets without NPs (Fig. 2A) were closer to a spherical shape and decisively different than those with NPs (Fig. 2B), probably due to the worse properties in spheronization of NPs compared to the excipient; nonetheless, the pellet shape could be considered acceptable.

3.3.3. Characterization of NPs after minitablets and pellets manufacturing

The dosage forms manufactured were then tested to assess NPs stability in terms of maintenance of size distribution. Before DLS analysis, the solution containing pellets formulations with NPs was filtered to

Table 7
Characterization of Pellets and Pellets_NPs. d_A, diameter spherical equivalent area; d_P diameter spherical equivalent perimeter; AR, aspect ratio and circularity (mean ± SD, n = 25).

	Dimensional and shape characterization			
Batch	d_A (mm)	d_P (mm)	AR	Circularity
Pellets	1.05 ± 0.05	1.14 ± 0.06	1.19 ± 0.15	0.74 ± 0.03
Pellets_NPs	1.11 ± 0.06	1.37 ± 0.08	1.35 ± 0.20	0.60 ± 0.06

Table 8
Characterization of NPs after minitablets manufacturing in terms of size distribution (mean ± SD, n = 3).

Batch	Hydrodynamic diameter (DLS analysis, nm)	PDI
MiniTBL_NPs (0.75 %)	220.1 ± 4.7	0.193 ± 0.06
MiniTBL_NPs (1.50 %)	201.8 ± 7.8	0.137 ± 0.03

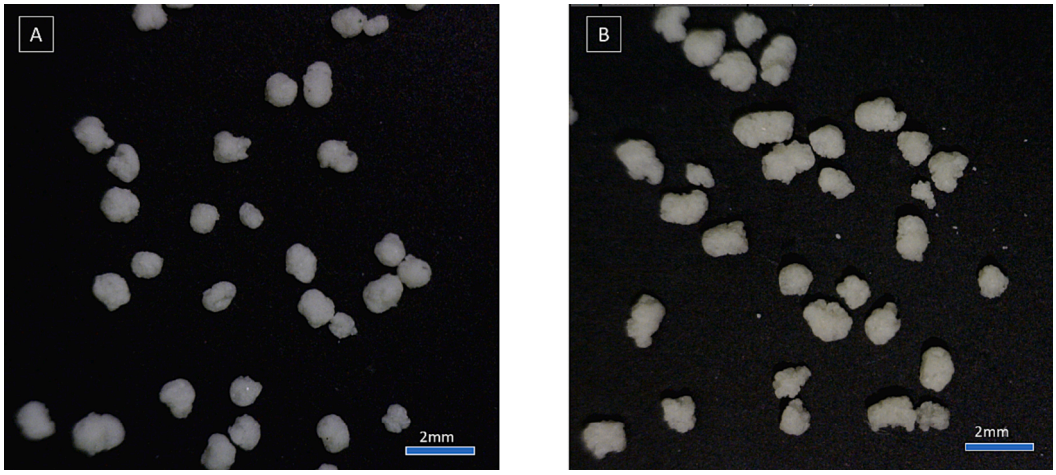

Fig. 2. Picture of pellets taken by digital photomicrograph. A) Pellets B) Pellets_NPs.

Table 9
Characterization of NPs after pellets manufacturing in terms of size distribution (mean ± SD, n = 3).

Batch	Hydrodynamic diameter (DLS analysis, nm)	PDI
Pellets_NPs	212.4 ± 20.5	0.208 ± 0.010

eliminate the particles of excipients, which could affect the results of the analysis.

As evidenced in Tables 8 and 9, after conveying the NPs in an oral dosage form, the occurrence of notable alterations due to mechanical stress during the manufacturing process seems to be ruled out as shown in Figures S7 and S8. Since nanoparticles appear to be stable, it could be predicted that their protective role toward encapsulated drug could persist after manufacturing of oral dosage form. These results open the doors to go forward in the development of NPs formulations for oral administration.

3.4. Dissolution test of NPs from minitablets and pellets

After the assessment of the particle size stability, the drug release behaviour of dosage forms with NPs was compared with that of formulations without NPs. In particular, the goal was to verify if the presence of NPs affects the drug release profile. Then, it could be designed a coating of the dosage form to reach the needed drug release behaviour.

The dissolution profile of three different formulations were compared with those of the relevant amount of encapsulated IND.

After 30 min the drug was completely dissolved from all formulations; indicating that NPs in these conditions slightly affects, with exclusion of a little slowing down, the dissolution profile from this kind of dosage forms.

In particular, the tracer dissolution profile from MiniTBL_NPs (0.75 %) (Fig. 3) was quite similar to the relevant formulation without NPs; while, when MiniTBL_NPs (1.50 %) was tested, a slight but significantly slowdown of the release was detected (Fig. 4). This could be attributed to the higher amount of NPs into these formulations (50 %, w/w), which may interact with the excipient ingredients, and/or to a lower amount of lactose in the final formulations, leading to a less prompt and complete drug release compared to the one obtained from MiniTBL_NPs (0.75 %) formulation.

The results of drug release test from pellets (Fig. 5) could be influenced by the presence of Avicel CL611, which is a co-processed mixture of microcrystalline cellulose and sodium carboxymethyl cellulose, that can form a weak matrix which could causes a slight slowdown in the dissolution of the drug.

In order to investigate the influence of process and excipients used to manufacture oral dosage forms on the behaviour of NPs, the drug release profiles were compared to that resulted from NPs that was used as

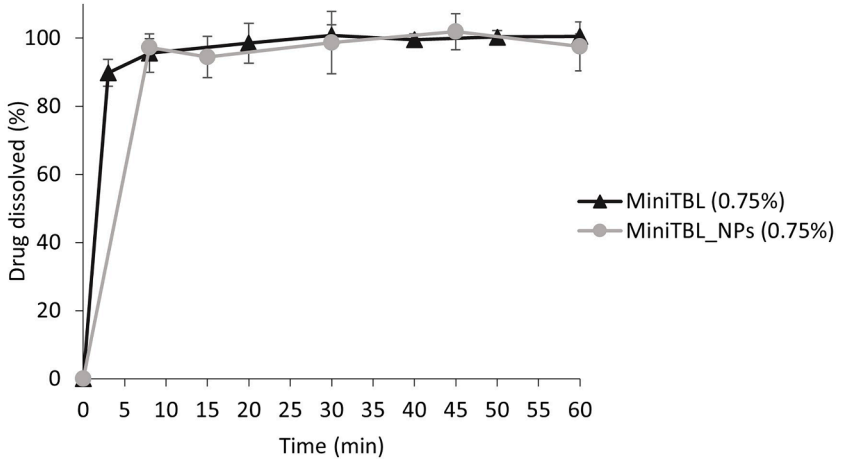

Fig. 3. Indomethacin released from minitablets with or without NPs (0.75 % IND) evaluated by HPLC analysis. Amount of the released drug (%) is calculated on 100 % released after 24 h (mean ± SD, n = 5).

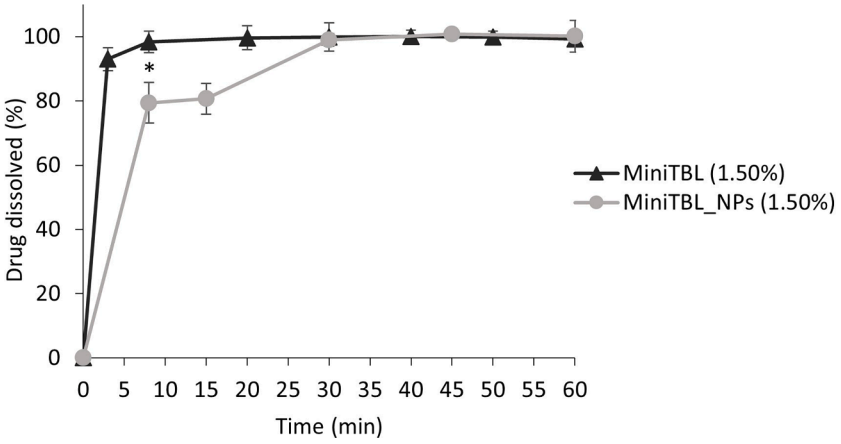

Fig. 4. Indomethacin released from minitablets with or without NPs (1.50 % IND) evaluated by HPLC analysis. Amount of the released drug (%) is calculated on 100 % released after 24 h (mean ± SD, n = 5). * $p < 0.05$ vs MiniTBL (1.50 %), calculated with Student's *t*-test.

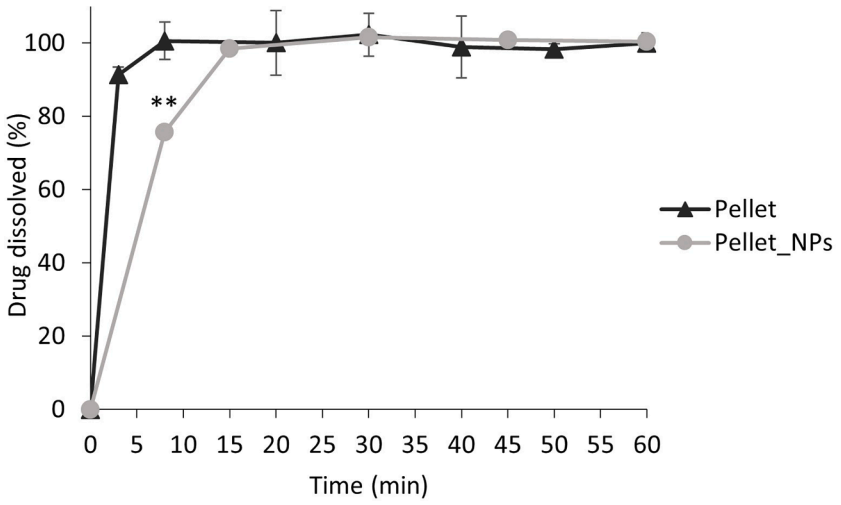

Fig. 5. Indomethacin released from pellets with or without NPs (1.50 % IND) evaluated by HPLC analysis. Amount of the released drug (%) is calculated on 100 % released after 24 h (mean ± SD, n = 5). **p < 0.005 vs Pellet, calculated with Student's *t*-test.

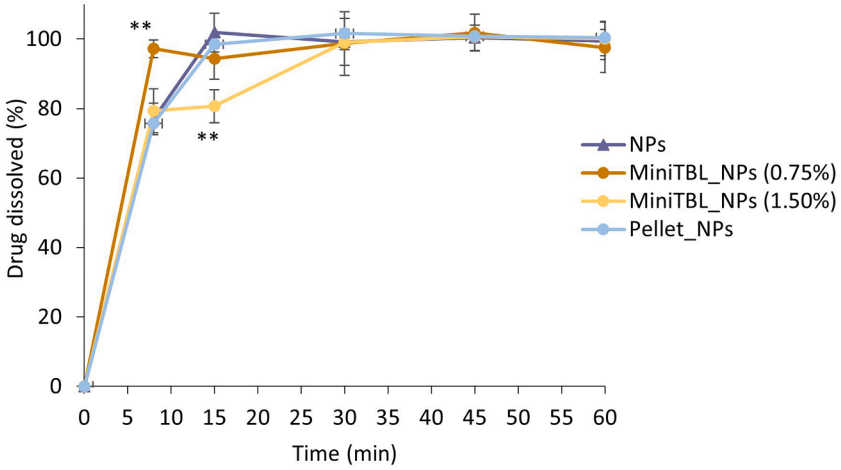

Fig. 6. Indomethacin released form NPs and pellets and minitablets with NPs evaluated by HPLC analysis. Percentage of released drug is calculated on 100 % released after 24 h (NPs and MiniTBL_NPs mean ± SD, n = 5; for Pellet_NPs mean ± SD, n = 5, from one batch). **p < 0.005 vs NPs, calculated with Student's *t*-test.

reference. In Fig. 6, at first time point, MiniTBL_NPs (0.75 %) showed a higher amount of tracer released compared to that of NPs, probably due to a larger amount of hydrophilic excipient, which attracted water improving drug release. On the contrary, at the second time point, MiniTBL_NPs (1.50 %) exhibited significantly lower released amount of Indomethacin compared to NPs, for which we advanced the same interpretation provided for the result of the above experiment, *i.e.*, the lower amount of lactose which could help the interaction with water. The release profile of Pellets_NPs was found to be identical to that of NPs not formulated. Overall, the release test results confirm that the selected ingredients in these formulations slightly impact the disintegration and drug release, which can be attributed mainly to the presence of NPs. Nevertheless, all the formulations, except a slowing down for MiniTBL_NPs (1.50 %), released more than 85 % of drug in 15 min, thus they could be defined as immediate release dosage forms (Services, Administration, and (CDER) 1997).

In conclusion, the obtained results demonstrate the feasibility of incorporating NPs into oral formulations, potentially opening the possibility of coating the dosage forms with a specific external film to modify and control release in the GI tract.

4. Conclusions

To evaluate the potential and the synergistic effect of NPs for oral delivery, we combined microfluidics technique for NPs synthesis and the formulation in multiple-units dosage form.

This study demonstrated that microfluidics is suitable to manufacture FDA-approved PLGA NPs in large quantities of homogenous and uniform NPs endowed with optimal size distribution and drug encapsulation efficiency. In particular, PLGA NPs encapsulating a poorly soluble drug tracer, *i.e.* Indomethacin, were prepared with hydrodynamic flow-focusing microchannel designed using a 5-input 3D chip. On the horizon of this research, one of the possible outcomes involves the incorporation of microfluidics into an elaborated system for continuous production. Optimization of chip geometry and experimental setup, exploiting 3D printing engineering technology, could be implemented. (Kara et al., 2021) Work is ongoing in this direction.

For the formulation in multi-units dosage forms, the effect of applied processes (compaction and extrusion/spheronization) and of selected excipients on the nano-particles size distribution to evaluate their stability and the drug release from the dosage forms. Overall, the results highlight that the drug incorporation into PLGA NPs does not lead to noticeable changes to its release profile from conventional oral dosage forms.

The synergistic combination of automated synthesis of NPs encapsulating a drug with formulation into solid dosage forms paves the way for the development of orally administered NPs, increasing the patient compliance, their stability in GI tract (*e.g.*, biological molecules), their solubility characteristics, and masking the drug bad taste. In conclusion, this study, with successful manufacturing of oral nano-solid dosage forms, corroborates the assumption that the use of appropriate technologies for NPs synthesis and their possible combination in a suitable final formulation could represent a starting point for basic research to promoting a reappraisal of nanoproducts into clinical transition processes. This paradigm could represent an important point to improve the technological transition toward reliable NPs to be administered orally.

CRediT authorship contribution statement

Lucia Morelli: Writing – original draft, Methodology, Formal analysis, Data curation. **Evelyn Ochoa:** Writing – original draft, Methodology, Data curation. **Lucia Salvioni:** Writing – original draft, Methodology, Formal analysis, Data curation. **Marco Davide Giustra:** Methodology, Formal analysis. **Beatrice De Santes:** Methodology. **Francesca Spena:** Methodology. **Linda Barbieri:** Formal analysis, Data curation. **Stefania Garbujo:** Methodology, Investigation. **Giulia Tomaino:** Software, Formal analysis. **Brian Novati:** Methodology. **Leonardo Bolis:** Formal analysis. **Saliha Moutaharrik:** Formal analysis, Data curation. **Davide Prosperi:** Writing – review & editing, Conceptualization. **Luca Palugan:** Writing – original draft, Methodology, Formal analysis, Data curation. **Miriam Colombo:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpharm.2024.123850.

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