

ORIGINAL ARTICLE

Influence of emulsification mode, stirring speed and volume on ibuprofen-loaded PLGA microparticles

Vliv způsobu emulgace, rychlosti míchání a objemu na PLGA mikročástice s ibuprofenem

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Summary

Microparticles based on biodegradable synthetic lactic acid and glycolic acid copolymer (PLGA) were successfully prepared by the solvent evaporation method. Ibuprofen was chosen as the model drug. Various formulation and process parameters have been used to prepare each sample with emphasis on size reduction. The effect of the emulsification method (direct emulsification or emulsification using an ULTRA-TURRAX or a NE-1000 dispenser), the volume of the aqueous phase (200, 800 ml) and the stirring speed of the emulsion system (600, 1000 rpm) on the characteristic properties of microparticles, such as encapsulation efficiency, drug loading and particle morphology, was observed. The resulting microparticles were evaluated by optical microscopy or laser diffraction and the dissolution test was performed. It was found that the sample prepared by direct emulsification with 800 ml of an aqueous phase at 600 rpm provided the most favorable results, meanwhile the emulsification pre-step using a homogenizer caused promising particle size reduction. Gradual emulsification was evaluated as inapplicable due to great losses.

Key words: microparticles • solvent evaporation • PLGA • ibuprofen • size reduction

Souhrn

Mikročástice na bázi biodegradovatelného syntetického kopolymeru kyseliny mléčné a kyseliny glykolové (PLGA) byly úspěšně připraveny metodou odpařování rozpouštědla. Modelovým léčivem pro enkapsulaci byl zvolen ibuprofen. Pro přípravu každého vzorku byly použity odlišné formulační a procesní parametry různě ovlivňující výsledné mikročástice. Během odpařování rozpouštědla byl konkrétně sledován vliv metody emulgování (přímé emulgování či přímé emulgování za využití přístroje ULTRA-TURRAX nebo NE-1000 dávkovače), objemu vodné fáze (200, 800 ml) a rychlosti míchání tohoto emulzního systému (600, 1000 ot/min) na charakteristické vlastnosti mikročástic, jako je enkapsulační účinnost, drug loading a morfologie částic. Vzniklé mikročástice byly hodnoceny pomocí optické mikroskopie, případně laserové difrakce, a byla také provedena disoluční zkouška. Nejpriznivější výsledky byly pozorovány u vzorku připraveného přímým emulgováním s 800 ml vodné fáze o rychlosti míchání 600 ot/min. Vzorek připravený s pre-emulzifikačním krokem na homogenizátoru se zase vyznačoval slibným zmenšením velikosti částic. Postupná emulzifikace byla naopak shledána jako nepoužitelná kvůli velkým ztrátám.

Klíčová slova: mikročástice • odpaření rozpouštědla • PLGA • ibuprofen • zmenšení velikosti

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Introduction

Microdispersion and nanodispersion dosage forms have a prominent place in contemporary research of controlled release^{1–4}. Microparticle drug delivery systems are generally used to prolong drug release, to enhance their stability, to target a specific site and to improve bioavailability⁵. With a growing need of drug incorporation into polymeric materials, several chemical and physical-mechanical methods have been gradually developed. The chemical methods include

coacervation, interphase polymerization and polycondensation and cross-linking methods. Physical-mechanical methods include, for example, extrusion and spheronization, spray drying and cooling, or molding and coating of microparticles in the fluidized bed. One of the most frequently used physical-mechanical methods is the solvent evaporation method, which is very important for the preparation of poly(lactic-co-glycolic acid) (PLGA) microparticles⁶. PLGA is a biodegradable polymer with very well-known degradation properties⁷ which has been extensively utilized for controlled drug delivery systems⁸ and belongs to the best-defined biodegradable materials available for similar systems⁹.

The basic method principle is the evaporation of that emulsion portion which contains a dissolved or dispersed drug and polymer. During evaporation, the dissolved polymer solidifies and forms a matrix which entraps the drug in the particle structure. Microparticle preparation by a simple way of this method can be divided into several steps¹⁰. In the first stage, the polymer is dissolved in a volatile organic solvent which is immiscible with water¹¹. Subsequently, the drug is dissolved or dispersed in this polymer solution. The resulting lipophilic phase is then emulsified into an aqueous continuous phase, usually combined with emulsifiers, to form a fine O/W emulsion (O is the reference for the oil phase and W is the water phase). To achieve the required emulsification level, for example, a propeller stirrer with adjustable speed, a homogenization device or an ultrasonic bath are used¹⁰. The organic solvent subsequently diffuses into the aqueous phase and vaporizes in the last step on the water/air interface. Lack of solvent and stirring causes a formation of microparticles from the polymer. The resulting microparticles, suspended in the continuous phase, are then filtered, washed and dried¹².

Preparation process is influenced by a great number of both the process and formulation parameters, including stirring speed, emulsification approach, or ratios of used excipients. Altering these variables can seriously affect encapsulation efficiency, yield and particle size, resulting in different dissolution profiles^{12, 13}.

The aim of this study was to prepare ibuprofen-loaded PLGA microparticles by the solvent evaporation method and to evaluate the influence of the outer aqueous phase volume, stirring speed and the mode of the emulsification on the measured particle parameters, mainly encapsulation efficiency, drug loading and particle size, and on dissolution profiles. Drug release profiles were then analyzed using kinetic model equations to approximate the drug release mechanism.

Experimental part

Materials

Ibuprofen (Zentiva, Czech Republic) served as the model drug, poly(lactide-co-glycolide) acid PLGA; L-lactide/glycolide = 50/50 (Resomer® RG 504 H, Boehringer Ingelheim Pharma GmbH & Co., Germany) was used for the formation of the polymer matrix in the oil phase. Dichloromethane – DM (Penta, Czech Republic) was as the organic solvent used for the oil phase and polyvinyl alcohol – PVA (Mw 31.000–50.000) (Sigma Aldrich, USA) served as the emulsifier. Phosphate buffer of pH 6.8 for dissolution test was prepared from sodium phosphate dodecahydrate and potassium dihydrogen phosphate (both Merck KGaA, Germany). All materials were of Ph. Eur. quality.

Microparticle preparation

Microparticles were prepared by the single emulsion (O/W) solvent evaporation technique. The samples preparation differed in the formulation parameter of the outer phase volume (200 or 800 ml), and the process parameters, namely the stirring speed (600 or 1000 revolutions per minute) and the mode of emulsification – direct emulsification, direct emulsification with a pre-emulsification step and emulsification using a NE-1000 dispenser (New Era Pump Systems, USA) with a dispensing rate of 1.12 ml/min. The samples characteristics and their designation are listed in Table 1.

For the formation of the oil phase, 200 mg of ibuprofen and 700 mg of PLGA were dissolved in 5 ml of dichloromethane. In the next step, the thus

Table 1. Preparation characteristics of microparticle samples

| Sample | Aqueous phase (ml) | Stirring speed (rpm) | Emulsification method | Pre-step |
|--------|--------------------|----------------------|------------------------------------|----------|
| A | 800 | 600 | Direct emulsification | No |
| B | 800 | 600 | NE-1000 dispenser | No |
| C | 200 | 600 | Direct emulsification | No |
| D | 200 | 600 | NE-1000 dispenser | No |
| E | 800 | 1000 | Direct emulsification | No |
| F | 800 | 1000 | NE-1000 dispenser | No |
| G | 200 | 1000 | Direct emulsification | No |
| H | 200 | 1000 | NE-1000 dispenser | No |
| I | 200 | 600 | Direct emulsification ULTRA-TURRAX | Yes |

formed O phase was emulsified into the aqueous phase of a 0.1% PVA solution by one of the three above-mentioned emulsification modes. The organic solvent was evaporated under a mechanical stirrer (Heidolph RZR 2021, Sigma Aldrich, USA) for 90 minutes at 450 rpm. After evaporation, the prepared microparticles were collected using an 80 µm mesh sieve, washed three times with purified water and then dried at 25 °C in a cabinet drier for 24 hours (HORO – 048B, Dr. Hofmann GmbH, Germany). To compare the results with our previous studies¹⁴, sample A was prepared as a reference under previously tested conditions. The preparation of the last sample (I) was similar to other samples; however, the internal phase was directly emulsified by a rotor-stator homogenizer ULTRA-TURRAX (T25 basic, IKA-Werke, Germany) for 60 seconds at 10 000 rpm to ensure a formation of a fine micro emulsion (pre-emulsion step). As a result of this modification, at the end of the preparation, the microparticles were not detectable by the naked eye. Isolation of the resulting micro suspension by sieve was not possible, but it was accomplished by centrifugation (EBA 20 Hettich, Germany) at 6000 rpm for 2 minutes. Particles were re-suspended in a small amount of water and collected by filtration on a Buchner funnel with membrane filter paper. Each sample was prepared in quadruplicate, combined after the collection to represent one sample. A total of 9 PLGA samples were prepared.

Microparticle characteristics

Drug content analysis

The ibuprofen content in PLGA microparticles was determined using an UV/Vis spectrometer (Lambda 25, Perkin Elmer Instruments, USA). Each sample was prepared by weighing an exact quantity of dried microparticles (50 mg) into a 50 ml volumetric flask with dichloromethane added to the mark. Each sample was analyzed in triplicate, absorbance was measured at 264 nm (the absorption maximum for ibuprofen) and the obtained results were expressed as mean values and their standard deviations. According to the data obtained from calibration curve, the drug content in the microparticles was determined.

The obtained values also served to determine encapsulation efficiency (EE) [1], drug load (DL) [2] and practical yield [3] by using the equations below^{15–17}.

$$EE = \frac{w_1}{c_t} \times 100 \text{ [%]} \quad [1]$$

$$DL = \frac{w_1}{w_2} \times 100 \text{ [%]} \quad [2]$$

$$Yield = \frac{w_2}{w_t} \times 100 \text{ [%]} \quad [3]$$

where w_1 represents the actual weight of the drug in microparticles, c_t is the theoretical amount of the drug, w_2 is the total weight of prepared microparticles and w_t is the theoretical yield (total amount of the drug and polymer used for the microparticle preparation).

Optical microscope analysis

Morphological properties of the prepared microparticles such as sphericity factor and equivalent size distribution were evaluated by a NIKON SMZ 1500 optical stereomicroscope (Nikon, Japan) and a 72AUC02 USB camera (The Imaging Source, Germany). Randomly selected 200 microparticles were evaluated by the computer software NIS-Elements AR 4.0 (Nikon, Japan). Equivalent diameter and sphericity were calculated from the measured values according to equations [4, 5] and expressed as arithmetic mean ± standard deviation^{18, 19}. Due to a smaller size, optical analysis of sample I was performed using a NIKON ECLIPSE E200 (Nikon, Japan) optical microscope. A picture of each sample was taken.

$$\text{Equivalent diameter} = \sqrt{\frac{4A}{\pi}} \text{ [mm]} \quad [4]$$

$$S = \frac{4\pi A}{p^2} \quad [5]$$

where S means sphericity, A is the area of particles and p their perimeter.

Scanning electron microscopy

The morphology and dimension of sample I was also evaluated by scanning electron microscopy (SEM; MIRA3, Tescan Orsay Holding, Czech Republic) equipped with a secondary electron detector (SED). The sample was mounted on a SEM specimen stub using carbon conductive double-faced adhesive tape (Agar Scientific, United Kingdom). To eliminate charging artefacts, microparticles were coated with a 20 nm layer of gold using the metal sputtering coating method with argon atmosphere (Q150R ES Rotary-Pumped Sputter Coater/Carbon Coater, Quorum Technologies, United Kingdom). SEM images were obtained at accelerating voltage of 3 kV.

Laser diffraction

To determine sample I particle size distribution, laser diffraction was performed using a Mastersizer 2000 (Malvern, United Kingdom). To obtain a clear signal, approximately 0.5 g of sample was poured into a beaker filled with 300 ml of degassed purified water. The sample was measured immediately and it was analyzed for volume-weighted size distribution. The measurement was performed in triplicate.

Multivariate data analysis

For evaluation of process/formulation variables (stirring speed, aqueous phase volume and emulsification method) and their interaction with response variables (EE, DL, yield, diameter), factor analysis was used, including rotation of factors (Varimax normalized). The data matrix for multivariate analysis was composed of 8 measurements/objects. Prior to modelling, variables were automatically adjusted by autoscaling. Statistical evaluation of data was performed using the program Statistica 12 (StatSoft, USA).

In vitro release studies

The samples were weighed to obtain 15 mg of the drug, placed in 500 ml of 6.8 pH buffer and tested in an automated dissolution device (SOTAX AT 7 SMART On-Line System, Donau Lab, Switzerland) equipped with baskets. The temperature was kept at 37.0 ± 0.5 °C and the stirring speed at 75 rpm. Sampling was performed at pre-set time intervals: every 15 minutes for 1 hour, every 60 minutes for the rest of the first 24 hours and every 120 minutes for next two days to obtain 72-hour profiles. Analysis of the samples was performed by a UV spectrophotometer (Lambda 25, PerkinElmer, USA) at 222 nm. The measurement result was a dissolution curve expressing the cumulative drug release in time. Sustained drug release rate was expected since ibuprofen is practically insoluble in water. The obtained data were correlated with the equations of the drug release mathematical models²⁰⁾:

Zero order equation:

$$\frac{M_t}{M_\infty} = K_0 \times t \quad [6]$$

First order equation:

$$\frac{M_t}{M_\infty} = 1 - e^{-K_1 \times t} \quad [7]$$

Higuchi model:

$$\frac{M_t}{M_\infty} = K_H \times \sqrt{t} \quad [8]$$

Korsmeyer-Peppas equation:

$$\frac{M_t}{M_\infty} = K_{KP} \times t^n \quad [9]$$

Baker-Lonsdale model:

$$\frac{3}{2} \left[1 - \left(1 - \frac{M_t}{M_\infty} \right)^{2/3} \right] \frac{M_t}{M_\infty} = K_{BL} t \quad [10]$$

where M_0 is the initial amount of the drug; M_t is the amount of the drug released in time t ; M_∞ is the absolute cumulative amount of the drug released at an infinite time; K_0 , K_1 , K_H , K_{KP} , K_S and K_{BL} are the zero order, first order, Higuchi, Korsmeyer-Peppas, Hixson-Crowell and Baker-Lonsdale release constants. Release exponent n of the Korsmeyer-Peppas model describes the mechanism of the drug release: $n = 0.5$ corresponds with the Fickian diffusion, $0.5 < n < 1.0$ suggests an anomalous transport,

$n = 1.0$ means the zero-order release kinetics and $n > 1.0$ suggests the super Case II transport.

The similarity factor f_2 [11] indicates the similarity percentage between two dissolution curves. The range of this factor can vary from 0 to 100. If a similarity factor is 100, it means that the dissolution profiles are identical²⁰⁾. If f_2 values range between 50 and 100, the dissolution profiles are evaluated as similar²¹⁾. If the value is 50 or higher ($f_2 \geq 50$), then it can be said that the drug release profiles are more than 90% similar. While if this value is lower than 50 ($f_2 < 50$) then the profiles are not similar and the observed influence of the process variable can be considered as significant.

$$f_2 = 50 \times \log \left\{ \left[1 + \left(\frac{1}{n} \right) \sum_{i=1}^n w_i |R_i - T_i|^2 \right]^{-0.5} \times 100 \right\} \quad [11]$$

where T_i is the drug amount (%) released at time interval i in the tested sample, R_i is the drug amount (%) released at time interval i in the reference sample, n is the total number of samplings and w_i is optional weight factor at time interval i .

Results and discussion

Encapsulation process, drug loading and yield

The encapsulation efficiency, drug loading and yield results are shown in Table 2. The encapsulation efficiency ranged between 15.69 ± 0.12 % and 54.07 ± 1.22 %, the drug loading took values from 15.19 ± 0.12 to 20.15 ± 0.32 and the yield was from 18.60% to 61.67%. From the results it is evident that the emulsification using a dispenser provided values vastly inferior to the direct emulsification method, primarily the yield values from which the poor encapsulation efficiency also resulted. It is possible that the gradual addition of the O phase disrupts already formed half-made particles, which leads to higher material losses through polymer merging. From Table 2 it can be also seen that at 600 rpm the samples prepared with a higher volume of the W phase provided much better values of encapsulation efficiency and yield compared to their corresponding samples prepared with 200 ml outer phase.

Table 2. Results of encapsulation efficiency, drug loading, yield, equivalent diameter and sphericity factor

| Sample | EE (%) | DL (%) | Yield (%) | Equivalent diameter (µm) | Sphericity factor |
|--------|--------------|--------------|-----------|--------------------------|-------------------|
| A | 54.07 ± 1.22 | 19.53 ± 0.44 | 61.67 | 262.7 ± 94.8 | 0.947 ± 0.043 |
| B | 26.91 ± 0.58 | 18.96 ± 0.41 | 31.55 | 296.1 ± 92.1 | 0.946 ± 0.037 |
| C | 38.57 ± 0.24 | 18.09 ± 0.11 | 47.46 | 352.1 ± 80.0 | 0.919 ± 0.047 |
| D | 15.87 ± 0.32 | 18.99 ± 0.38 | 18.60 | 301.9 ± 88.1 | 0.913 ± 0.048 |
| E | 31.99 ± 0.27 | 19.52 ± 0.16 | 36.45 | 213.2 ± 56.9 | 0.892 ± 0.045 |
| F | 22.10 ± 0.18 | 19.53 ± 0.16 | 25.23 | 192.0 ± 48.2 | 0.922 ± 0.039 |
| G | 43.72 ± 0.65 | 19.29 ± 0.28 | 50.47 | 187.5 ± 61.7 | 0.913 ± 0.048 |
| H | 23.32 ± 0.37 | 20.15 ± 0.32 | 25.75 | 142.6 ± 25.8 | 0.951 ± 0.023 |
| I | 15.69 ± 0.12 | 15.19 ± 0.12 | 23.01 | – | – |

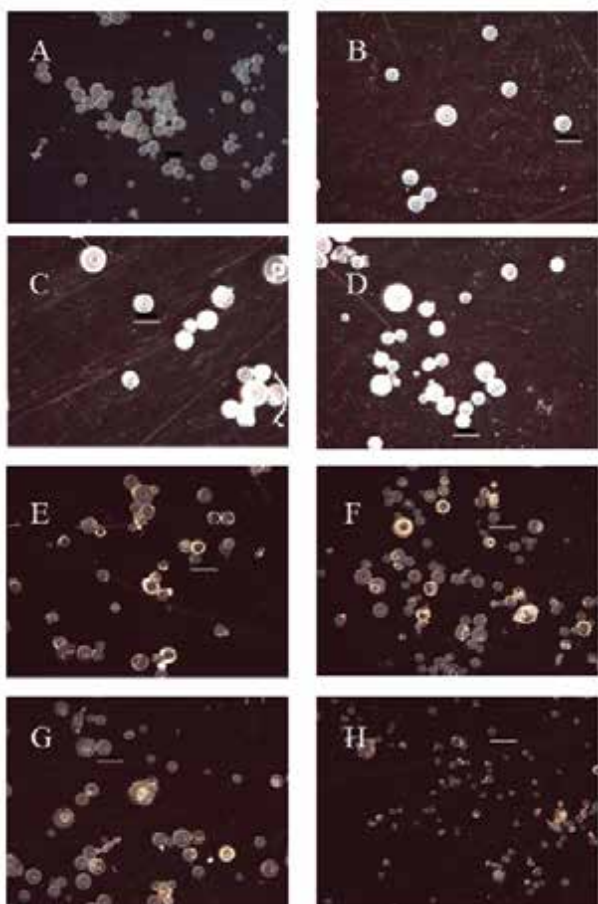


Fig. 1. Picture of optical stereomicroscope analysis of samples A–H; bar corresponds to 500 μm

Surprisingly, at 1000 rpm this trend is not evident and sample G prepared with 200 ml outer phase gives even better results than its corresponding sample. It could mean that stirring rate co-influences the observed characteristics together with the volume of the outer phase. This is further confirmed by interpreting the results from the perspective of the stirring rate. In the corresponding samples prepared with 800 ml outer phase the samples prepared at 600 rpm provide better results, however this trend is missing in the samples prepared with 200 ml outer phase.

Results for sample I stood apart the other samples. Emulsification pre-step ensured the smallest size, as is further discussed, and this fact influenced the observed characteristics considerably. Sample I suffered from the lowest encapsulation efficiency and drug loading values, which were probably caused by higher drug losses during preparation. As the particles were of a smaller size, they had a greater surface area on the same weight ratio compared to the other samples, thus providing a larger area for drug leakage²².

Sphericity, equivalent diameter and morphology

Sphericity and particle size of prepared microparticles were evaluated by optical microscope analysis using specialized software. Images from the microscope are shown in Figure 1. Results of sphericity and equivalent

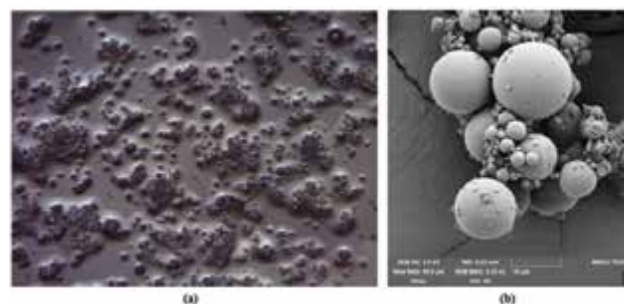


Fig. 2. (a) sample I picture by optical microscope Nikon Eclipse using objective with 40 \times zoom; (b) the scanning electron microscope image of sample I

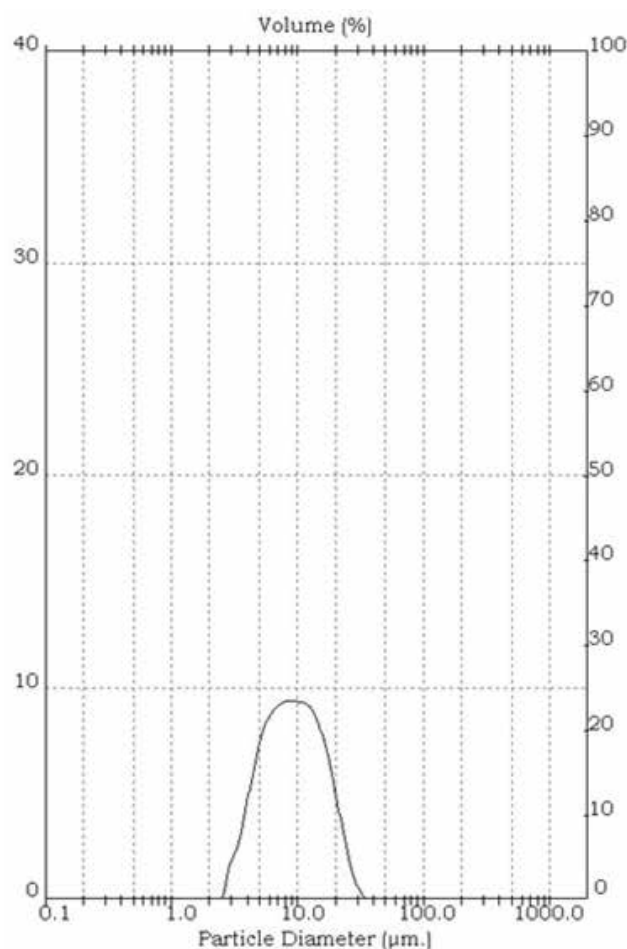


Fig. 3. Laser diffraction chart of sample I

diameter are presented in Table 2. Generally, all samples showed great sphericity as the sphericity factor ranged from 0.892 ± 0.045 to 0.951 ± 0.023 and it is difficult to find any dependency pattern. The particle size ranged from $142.6 \pm 25.8 \mu\text{m}$ to $352.1 \pm 80.0 \mu\text{m}$. Results clearly show that the larger particles were formed at the stirring rate of 600 rpm. It has been reported before that, among other parameters, the size of microparticles can be affected by stirring speed^{23, 24}. Increasing stirring speed reduces the mean diameter and yields lower microparticles polydispersity¹². Also, it could be concluded that at 600 rpm the outer phase volume can

influence the size as the samples A and B had a lower size compared to samples C and D; however, because of standard deviation values overlay a multidimensional analysis was needed for the confirmation (see below). Nevertheless, similar relationship was described in Yang's et al. article based on a greater probability of connecting microparticles with big pores in a smaller volume of water phase^{25,26}. In the samples prepared at 1000 rpm a possible dependency of the particle size on the volume of the aqueous phase was not shown.

Because of the significantly smaller size of the microparticles prepared by pre-emulsion step, it was not possible to establish sphericity and equivalent diameter measurement using stereomicroscopic analysis. Therefore, sample I was captured by a Nikon Eclipse optical microscope (Fig. 2). Particle size was determined by laser diffraction, results are shown in Figure 3. The frequency curve is characterized by a number-based particle size distribution. Significant part of the measured microparticles was in the size range of 3 to 30 μm , proving that emulsification via homogenizer yields a significantly lower particle size. This was further confirmed by surface morphology examination using scanning electron microscopy (SEM). SEM revealed that the microparticles of sample I had a spherical shape without visible cracks, the average size of the shown particles varied from 0.5 to 15 μm and the size distribution seemed unequable within this range. SEM also indicated smooth and creaseless (or unfolded) surface with no visible pores on it (Fig. 2).

Multivariate data analysis

To support the initial finding about possible complex co-influences, multivariate data analysis was performed. A data set of samples A to H with process variables representing the preparation method used, stirrer speed and aqueous phase volume were evaluated by factor analysis to find the relationships between the formulation/process variables and qualitative parameters of the prepared microparticles. Sphericity was not included in the factor analysis due to low inter-sample variability and the analysis did not evaluate sample I. The first two factors were used for the calculation, which together explain 79.3% of the total variability in the data. In the factor load graph (Fig. 4), the first factor is explained by the emulsification method used and the second factor is explained by the stirrer speed. In the graph of factor loads, a correlation of EE and the yield with the direct emulsification method and a negative correlation with the NE-1000 dispenser method can be observed. DL is correlated with the speed, while the particle diameter decreases with the speed. (However, changes in DL with changing speed are in the range of about 0.5% and are not of great practical importance for the microparticle preparation). According to Figure 4, the influence of the aqueous phase volume is insignificant. (This result probably comes from the fact that the qualitative parameters (EE, yield, ...) do not change in direct proportion to the

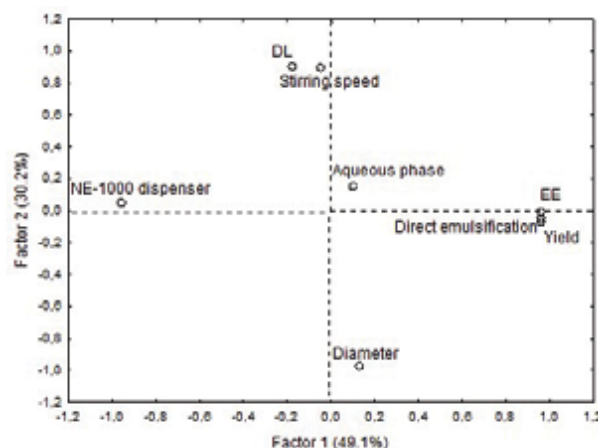


Fig. 4. Factor loading plot

volume, but the change in qualitative parameters with the change of volume is conditioned by certain stirring speed). Multivariate data analysis output further confirms the hypothesis about complex phenomenon with existing co-influences.

Drug release behavior

The dissolution profiles of all samples have shown prolonged drug release and in addition, samples A–H had only a mild *burst effect* (5–10%, Fig. 5, Table 3). The faster initial release was observed in samples E–H when compared to their corresponding samples from A–D group²⁶. The slowest release of ibuprofen was observed in sample A with lower stirring speed and higher aqueous volume, where only 27.40% of the substance was released after 72 hours. The fastest drug release was observed in sample I with the smallest particle size. During the first 30 minutes, 58.41% of drug was released. From the preparation perspective it can be concluded that the samples prepared with a higher stirring speed or with a pre-emulsification step had faster drug release, resulting from the smaller particle size²⁷. From the results it is also possible to find an eventual dependency on emulsification method and outer aqueous phase volume in samples prepared at 600 rpm. In samples prepared at 1000 rpm these differences are wiped, suggesting a stirring speed as an important co-factor.

Based on the obtained dissolution profiles, similarity factors were calculated (Table 4). For sample I, the values of f_2 ranged from 11.66 to 20.57, meaning that the dissolution profile shows no similarity, clearly apart of other samples. Generally, the low f_2 values suggest that the observed parameters could have a significant effect on the drug release, meaning the preparation with pre-emulsification step yields a unique profile. Majority of similarities found was divided mainly among two stirring speed groups, A–D and E–H, respectively. At 600 rpm it is observable that just one change of preparation parameters still yields a similar profile. At 1000 rpm this trend is not so evident, further confirming stirring speed as a major variable during microparticle preparation.

The most important information obtained from the dissolution profile using the kinetics equation is the expression of the drug release kinetics and mechanisms. The drug release from microparticles can occur by several mechanisms including physical erosion, hydrolysis of the polymer, diffusion through the surface of the pores or through the polymer, ion

exchange mechanism or by and a combination of those mechanisms^{28, 29}. A determination coefficient R^2 of a given equation served as a parameter for the correlation evaluation with the applied existing model. The resulting values are presented in Table 5. Neither sample showed any correlation with the first order kinetic model (perhaps except for sample B, which

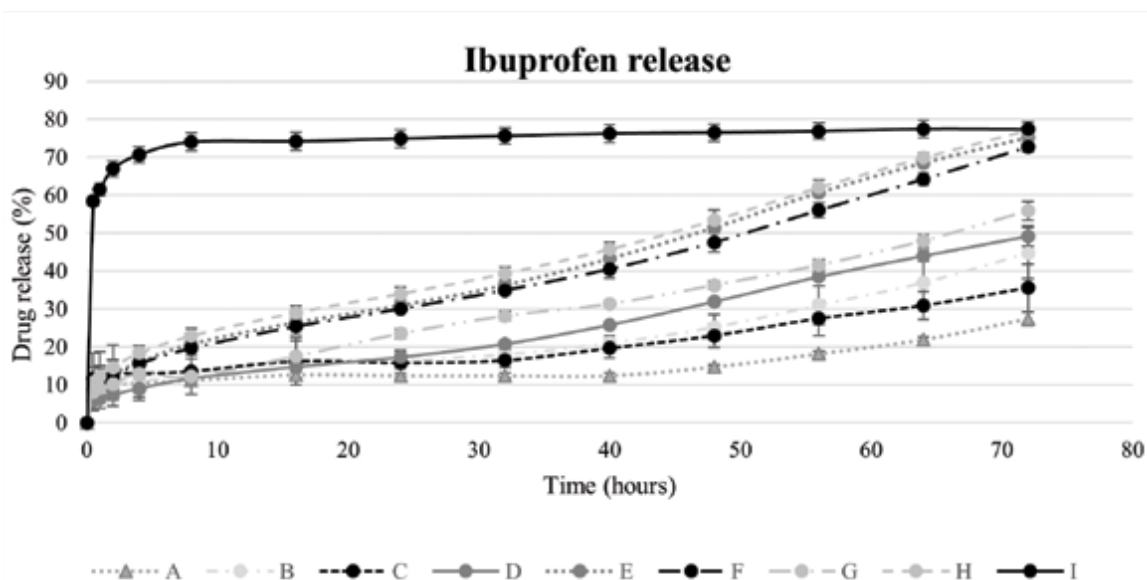


Fig. 5. Ibuprofen release dissolution profiles of the prepared samples

Table 3. Ibuprofen percentage release from the prepared samples within first day (each value in %)

| Time (hours) | A | B | C | D | E | F | G | H | I |
|--------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.5 | 9.94 | 9.02 | 11.63 | 5.90 | 10.17 | 8.50 | 8.03 | 10.65 | 58.41 |
| 1 | 9.85 | 9.41 | 11.96 | 6.39 | 11.12 | 9.99 | 9.17 | 12.23 | 61.52 |
| 2 | 10.14 | 10.42 | 13.12 | 7.39 | 12.97 | 12.19 | 10.15 | 14.62 | 66.89 |
| 4 | 10.49 | 10.97 | 12.96 | 9.03 | 15.92 | 15.55 | 12.96 | 18.36 | 70.65 |
| 8 | 11.18 | 12.61 | 13.55 | 11.68 | 20.77 | 19.65 | 12.19 | 22.91 | 73.98 |
| 16 | 12.60 | 16.40 | 16.17 | 14.74 | 26.38 | 25.40 | 17.54 | 29.06 | 74.18 |
| 24 | 12.39 | 16.55 | 15.77 | 17.30 | 30.91 | 30.02 | 23.53 | 34.02 | 74.89 |

Table 4. Similarity factor analysis (similar values are bold highlighted)

| Similarity factor f_2 | A | B | C | D | E | F | G | H | I |
|-------------------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|-------|
| A | | 53.34 | 61.86 | 45.30 | 28.08 | 29.96 | 39.95 | 26.79 | 11.66 |
| B | 53.34 | | 71.35 | 67.85 | 35.99 | 38.76 | 55.87 | 34.08 | 13.87 |
| C | 61.86 | 71.35 | | 55.97 | 32.97 | 35.29 | 48.75 | 31.39 | 13.55 |
| D | 45.30 | 67.85 | 55.97 | | 40.18 | 43.52 | 67.14 | 37.72 | 14.31 |
| E | 28.08 | 35.99 | 32.97 | 40.18 | | 79.00 | 46.27 | 81.23 | 19.56 |
| F | 29.96 | 38.76 | 35.29 | 43.52 | 79.00 | | 50.91 | 68.02 | 18.84 |
| G | 39.95 | 55.87 | 48.75 | 67.14 | 46.27 | 50.91 | | 43.26 | 15.93 |
| H | 26.79 | 34.08 | 31.39 | 37.72 | 81.23 | 68.02 | 43.26 | | 20.57 |
| I | 11.66 | 13.87 | 13.55 | 14.31 | 19.56 | 18.84 | 15.93 | 20.57 | |

Table 5. Kinetic models determination coefficients

| Sample | Zero order R ² | First order R ² | Higuchi R ² | Korsmeyer-Peppas | | Baker-Lonsdale R ² |
|--------|---------------------------|----------------------------|------------------------|------------------|-------|-------------------------------|
| | | | | R ² | n | |
| A | 0.808 | 0.885 | 0.674 | 0.599 | 0.147 | 0.701 |
| B | 0.939 | 0.980 | 0.837 | 0.826 | 0.285 | 0.796 |
| C | 0.930 | 0.972 | 0.814 | 0.727 | 0.189 | 0.843 |
| D | 0.987 | 0.957 | 0.913 | 0.919 | 0.423 | 0.879 |
| E | 0.994 | 0.937 | 0.943 | 0.963 | 0.349 | 0.888 |
| F | 0.991 | 0.912 | 0.945 | 0.974 | 0.385 | 0.873 |
| G | 0.992 | 0.954 | 0.933 | 0.910 | 0.379 | 0.889 |
| H | 0.994 | 0.921 | 0.955 | 0.978 | 0.345 | 0.895 |
| I | 0.579 | 0.552 | 0.750 | – | – | 0.644 |

nearly correlates at 0.987), nor the Higuchi, Baker-Lonsdale and Korsmeyer-Peppas release mechanism mathematical models. Samples E to H showed clear correlation with the zero-order kinetic model (0.991–0.994), suggesting at least partial gradual release not depending on the remaining drug concentration. Low correlation of the samples with the Higuchi model suggests that the diffusion is not a main release mechanism and the drug is probably released predominantly by erosion mechanism. General absence of the correlation with Korsmeyer-Peppas model could be explained by segmented, multi-phasic profiles of some samples. Moreover, the results were obtained using a classic linear regression; however, a more correct approach is the use of non-linear regression, as was reported recently³⁰.

Conclusion

A total of 9 ibuprofen-loaded samples was successfully prepared by the O/W solvent evaporation method. As the model drug substance in these microparticles ibuprofen was picked and the PLGA polymer was used as the polymer carrier. Different preparation parameters were applied, which were evaluated in terms of particles morphological properties such as sphericity, equivalent size distribution; and further the yield, encapsulation efficiency and drug loading were monitored. All prepared samples had very high values of sphericity factor. A very negative effect on the yield and encapsulation efficiency had the utilization of a NE-1000 dispenser and an ULTRA-TURRAX which, on the other hand, provided promising reduction of the microparticles size. A change in the microparticle mean size was also evident when increasing the stirring speed from 600 rpm to 1000 rpm. Smaller particles further influenced drug release profiles, causing faster ibuprofen release, compared to corresponding 600 rpm samples, and also raising a significant burst effect of sample I. The 1000 rpm samples correlated with the drug release kinetics of the zero order, the ideal type of kinetics for this type of dosage form. From the point of view

of morphology and drug content, the best result was shown in sample A prepared with 600 rpm and 800 ml of the aqueous phase, possessing the highest yield and encapsulation efficiency.

Conflict of interest: none.

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