

Influence of different formulations and process parameters during the preparation of drug-loaded PLGA microspheres evaluated by multivariate data analysis

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The main objective of this study was to evaluate the influence of the formulation and process parameters on PLGA microparticles containing a practically insoluble model drug (ibuprofen) prepared by the o/w solvent evaporation method. Multivariate data analysis was used. The effects of altered stirring speed of a mechanical stirrer (600, 1000 rpm), emulsifier concentrations (PVA concentration 0.1 %, 1 %) and solvent selection (dichloromethane, ethyl acetate) on microparticle characteristics (encapsulation efficiency, drug loading, burst effect) were observed. It was found that with increased stirring speed, the PVA concentration or the use of ethyl acetate had a significantly negative effect on encapsulation efficiency. In addition, ethyl acetate had an adverse effect on the burst effect, while increased stirring speed had the opposite effect. Drug load was not affected by any particular variable, but rather by the interactions of evaluated variables.

Keywords: solvent evaporation, encapsulation, PLGA, controlled release, burst effect

In the last decade, poly(lactic-co-glycolic acid) (PLGA) received great interest in the medical and pharmaceutical field because of its biodegradability, toxicological safety and desirable biocompatibility. It is one of the small number of synthetic polymers that has been approved by the FDA for human clinical use. Nowadays, PLGA is widely applied in controlled drug delivery systems, including nano- and micro-particles and implants (1). PLGA-based microparticles are well established as advanced drug delivery systems, allowing drug protection and controlled release upon parenteral administration (2). PLGA microspheres are considered to be an important carrier for the encapsulation of water-insoluble drug candidates in the early stages of drug development (3). They offer various advantages over other controlled drug delivery systems, including: (i) the possibility to

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accurately control the drug release kinetics over a period of days to months; (ii) complete biodegradability (avoiding the removal of empty remnants upon drug exhaust); (iii) good biocompatibility, even within brain tissue (4, 5). However, the design of this type of advanced drug delivery system is not straightforward, because many factors influence the resulting drug release rate from PLGA microparticles, including the method of preparation. One of the most common methods of preparing PLGA microparticles is the single emulsion solvent evaporation method, which consists of four major steps: (i) dissolution or dispersion of the bioactive compound in an organic solvent containing the matrix-forming polymer; (ii) emulsification of this organic phase in a second, continuous (frequently aqueous) phase immiscible with the first phase; (iii) extraction of the solvent from the dispersed phase by the continuous phase, which is optionally accompanied by solvent evaporation transforming the droplets into solid microspheres; (iv) harvesting and drying of microspheres (6). The first two steps primarily affect the microparticle size distribution while the last two steps determine the microparticle morphology and have an important influence on the microparticle encapsulation and release behavior (7).

The main objective of the experiment was to prepare drug-loaded PLGA microspheres containing practically insoluble ibuprofen (IBU), which served as the model drug. Several formulation and process parameters of the oil in water (o/w) single emulsion method were modified, such as alteration of the stirring speed during solvent evaporation, emulsifier concentration and the type of organic solvent to enhance ibuprofen encapsulation. The main section is devoted to the expression of the statistical significance of the above-mentioned formulation and process parameters on PLGA particle characteristics, such as shape and size, encapsulation efficacy and *in vitro* drug release. The full factorial design method and partial least squares PLS-2 regression were regarded the best methods for evaluating the impact of parameters that were otherwise difficult to compare.

EXPERIMENTAL

Materials

Ibuprofen (Zentiva, Czech Republic) was the model drug, PLGA Resomer[®] RG 504 S (Boehringer Ingelheim, Pharma GmbH & Co, Germany) was used as the polymer carrier. Dichloromethane (DM, Penta, Czech Republic) and ethyl acetate (EA, Dr. Kulich Pharma, Czech Republic) were used as solvents and polyvinylalcohol (PVA) served as emulsifier. A phosphate buffer at pH 6.8 (dodecahydrate sodium hydrogenphosphate, potassium dihydrogenphosphate, both Merck KGaA, Germany) was applied as the dissolution medium. All materials were of Ph. Eur. quality.

Sample preparation

Drug-loaded PLGA microparticles were prepared by the o/w solvent evaporation method. In order to form an o/w-type emulsion, 150 mg of the drug and 700 mg of the polymer were dissolved in 5 mL of either dichloromethane or ethyl acetate. The solution was homogenized for 1 minute using an Ultra-Turrax (T25 basic, IKA-Werke, Germany) at 20,000 rpm for one minute to dissolve the polymer completely and then it was emulsified all at once into 800 mL of the aqueous continuous phase containing either 0.1 % (m/m) or

1 % (*m/m*) PVA. The emulsion was stirred with a mechanical stirrer with a pitched-blade impeller (Heidolph RZR 2021, Sigma Aldrich, USA) at either 600 or 1000 rpm for 2 hours to ensure complete solvent evaporation. The prepared microparticles were collected on a fine mesh sieve with 80 μm -sized openings, washed three times with purified water and dried at 25 °C in a cabinet drier (HORO-048B, Dr. Hofmann GmbH, Germany) for 24 h before further testing. All samples were prepared in triplicate and marked according to the altered parameters: DM/ET stands for organic solvent (dichloromethane or ethyl acetate), L/H expresses low or high PVA concentration and 600/1000 determines the stirring speed per minute. Sample characteristics are shown in Table I.

Table I. Variables in the preparation of microparticle samples

Sample	Organic solvent	PVA concentration (%)	Stirring speed (rpm)
DM-L-600	DM	0.1	600
DM-L-1000	DM	0.1	1000
EA-L-600	EA	0.1	600
EA-L-1000	EA	0.1	1000
DM-H-600	DM	1	600
DM-H-1000	DM	1	1000
EA-H-600	EA	1	600
EA-H-1000	EA	1	1000

Particle distribution

The size-based mean diameter and size distribution of the microparticles were measured with a laser diffraction particle size distribution analyzer (Horiba Partica LA-300, Horiba Ltd., Japan). 100 μL of a prediluted microparticle sample (0.5 g mL^{-1} of phosphate buffered saline) was injected into a cuvette-type fraction cell (filled with 10 mL of degassed and filtered phosphate buffered saline, pH 7.2) provided with a magnetic stirrer to prevent non-homogenous distribution due to the sedimentation of particles. All samples were measured immediately after the application into the cuvette and were analyzed for number-weighted size distribution.

Scanning electron microscopy

The morphology and surface topography of the microparticles were examined by scanning electron microscopy. The samples were mounted directly onto the SEM sample holder using double-sided tape and then coated with a 10-nm thick layer of gold powder and images were taken using a scanning electron microscope (MIRA3, Tescan, Czech Republic) at accelerating voltage of 5.0 kV.

Drug content

Ibuprofen content in the drug loaded microparticles was determined by dissolving an appropriate amount of dried PLGA-based microparticles (~10 mg of ibuprofen) in 25 mL of an appropriate organic solvent (ethyl acetate or dichloromethane). The absorbance of samples was measured at 264 nm using a UV/Vis spectrometer (Lambda 25, Perkin Elmer, USA). Encapsulation efficiency (EE) and drug load (DL) were determined from the values obtained using the following equations (8, 9):

$$\text{Encapsulation efficiency} = \frac{c_s}{c_t} 100 (\%) \quad (1)$$

where c_s corresponds to the actual ibuprofen content and c_t corresponds to the theoretical drug load. The assay was carried out in triplicate and the results were expressed as mean values and standard deviations (SD).

$$\text{Drug load} = \frac{m_1}{m_2} 100 (\%) \quad (2)$$

where m_1 is the drug mass in microparticles, m_2 is the gross mass of microparticles. The assay was carried out in triplicate. The results are expressed as mean values and SD.

The effectiveness of the process was also evaluated by the yield, calculated by the following equation (10):

$$\text{Yield} = \frac{m_2}{m_t} 100 (\%) \quad (3)$$

where m_2 is the gross mass of microparticles, m_t is the total mass of the drug and polymer used for microparticle preparation.

In vitro release studies

The *in vitro* release studies of drug-loaded beads were carried out using the paddle method in an automatic dissolution apparatus (SOTAX AT 7 On-Line System, Donau Lab, Switzerland) at 100 rpm. The dissolution medium volume used was 500 mL of phosphate buffer (pH 6.8) kept at 37.0 ± 0.5 °C. Samples were analyzed using a UV spectrophotometer (Lambda 25, Perkin Elmer, USA) at 264 nm. The samples for the dissolution test were weighed with respect to their encapsulation efficiency, so that 50 mg of ibuprofen was in each sample. The *in vitro* drug release was observed for 72 hours. The dissolution test was carried out with six samples of each batch and the results were expressed as average values and SD.

Similarity factor analysis

Similarity factor f_2 was originally designed for dissolution profiles comparing the originals and the generics (11). It can be also used for the drug release profile comparison of samples prepared with different parameters. Similarity factor values ranged between 0 and 100. If $f_2 \geq 50$, drug release profiles were over 90 % similar. If $f_2 < 50$, release profiles were not similar and the observed influence of the formulation or process variables was considered to be significant. Standard deviations of the mean data did not exceed 9.1 %, so

the conditions for the variation (20 % maximum for the earlier points, 10 % maximum for the rest) were met. No more than one measurement was considered after 85 % of dissolution (12).

$$f_2 = 50 \times \log \left\{ \left[1 + \left(\frac{1}{n} \right) \sum_{i=1}^n |R_i - T_i|^2 \right]^{-0,5} \times 100 \right\} \quad (4)$$

R_i – drug amount (%) released at time interval i ; reference sample

T_i – drug amount (%) released at time interval i ; tested sample

n – total number of samplings

Factorial design

The experiment was planned as a 2^2 full factorial design with three replicated measurements, *i.e.*, 12 data points (Table I), to evaluate the influence of several variables (PVA concentration, stirring speed) and their interaction with response variables (encapsulation efficiency, drug loading, burst effect value) for each solvent separately. Individual experiments were performed in random order. The resulting data were analyzed by Unscrambler X (v. 1.3, Camo software) with the application of multiple linear regressions. For each response-variable, the results were expressed as the regression model in the form:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + e \quad (5)$$

where Y is the response variable, X_1 to X_3 are technological variables, β_0 is a constant (intercept), β_1 to β_{12} are regression coefficients representing an individual effect or interaction, e is the residual, *i.e.*, the difference between the observed and predicted values of Y . MLR models can be described on the basis of goodness-of-fit coefficients. R-square describes variability explained by the model, R-square of prediction expresses predictive ability of the model. C.V. (Coefficient of Variation) is the average modeling error expressed as percentage of the mean (13). Model significance was tested using ANOVA (Analysis of Variance); it was also used for evaluation of individual and interaction effects.

Analyzed data, including yield and particle size (Table I), were also evaluated by a principal component analysis (PCA) to study systematic variability and the relationships between variables and objects.

RESULTS AND DISCUSSION

Particle size and morphological properties

Effect of stirring speed. – The prepared microparticles with the mean size ranging between 90.7 to 382.8 μm showed particle size dependence on the stirring speed during solvent evaporation. The results in Table II confirmed that the microparticle mean size decreased with an increase in the stirring speed, which is a very well-documented effect (14, 15, 16). The force of higher stirring speed distributes the internal phase into smaller droplets, resulting in the formation of smaller microparticles (14).

Table II. Characteristics of drug-loaded microparticles

Sample	DL (%)	EE (%)	Yield (%)	Mean size (µm)	Initial drug release after 30 min (%)
DM-L-600	18.2 ± 0.1	79.8 ± 0.5	77.3 ± 5.1	382.8	20.0 ± 3.5
DM-L-1000	17.0 ± 0.3	57.4 ± 0.3	59.6 ± 5.2	254.9	23.6 ± 3.4
EA-L-600	17.5 ± 0.4	58.2 ± 1.6	40.7 ± 6.6	174.6	9.0 ± 3.9
EA-L-1000	16.2 ± 0.4	22.2 ± 1.4	24.2 ± 4.6	169.7	14.7 ± 3.9
DM-H-600	13.9 ± 0.5	44.6 ± 0.6	56.5 ± 5.0	284.3	18.0 ± 3.8
DM-H-1000	20.0 ± 2.4	23.2 ± 2.5	29.3 ± 6.2	173.5	29.1 ± 9.7
EA-H-600	19.0 ± 0.4	40.4 ± 0.4	54.0 ± 3.8	90.7	9.0 ± 3.5
EA-H-1000	17.5 ± 0.4	16.7 ± 0.5	16.8 ± 7.1	146.6	9.5 ± 0.8

Effect of PVA concentration. – It is also evident from Table II that particle size was dependent on the external phase viscosity, as the increasing PVA concentration decreased particle size. The particle size of samples prepared with 0.1 % PVA ranged between 169.7 and 382.8 µm, while the size of microparticles prepared with 1 % PVA had values from 90.7

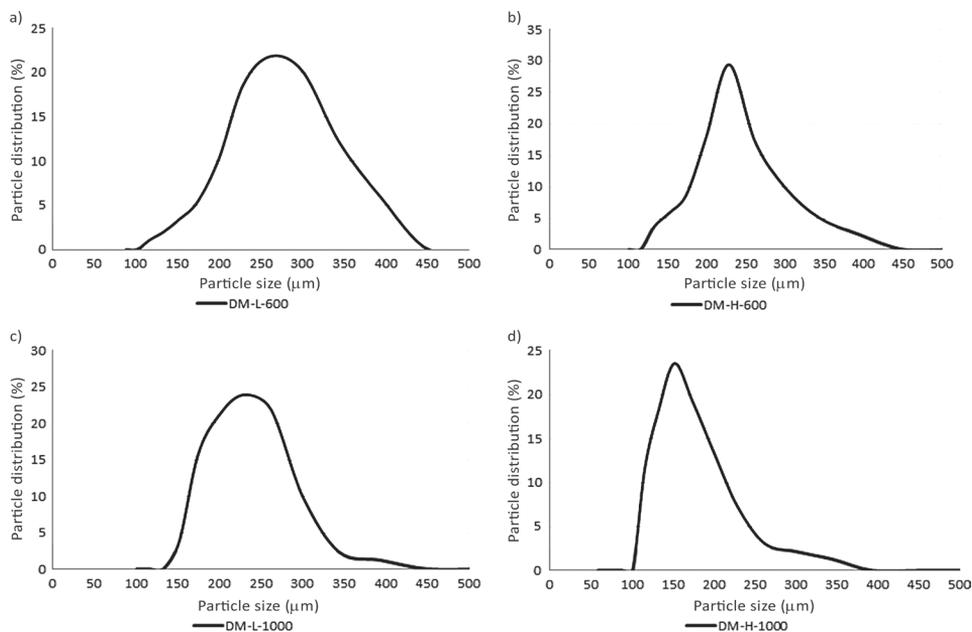


Fig. 1. Particle size distribution of PLGA (Resomer® RG 504S) microparticles prepared with dichloromethane as organic solvent: a) DM-L-600, b) DM-H-600, c) DM-L-1000, d) DM-H-1000.

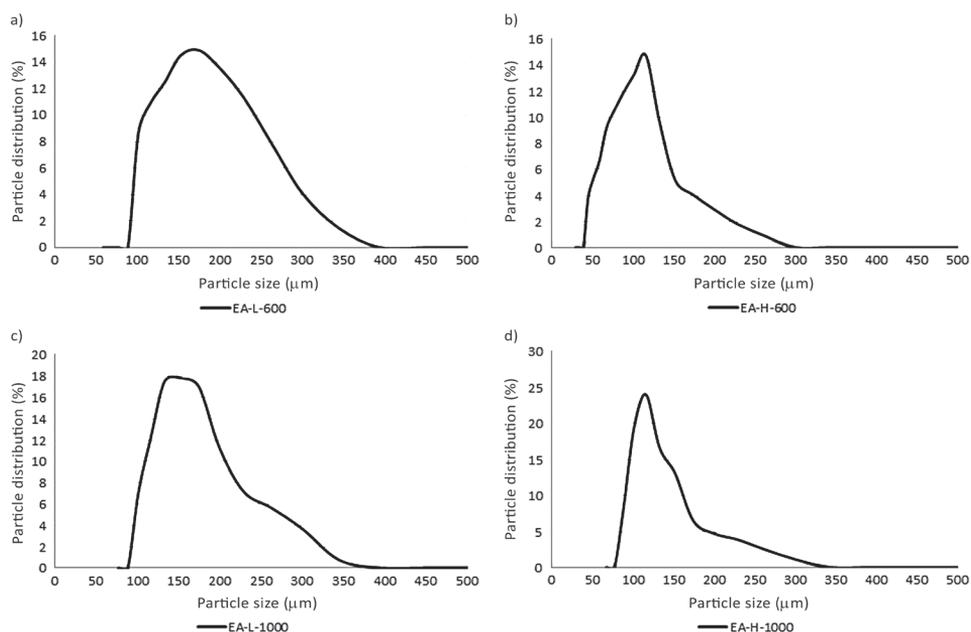


Fig. 2. Particle size distribution of PLGA (Resomer® RG 504S) microparticles prepared with ethyl acetate as organic solvent: a) EA-L-600, b) EA-H-600, c) EA-L-1000, d) EA-H-1000.

to 284.3 μm . Increased PVA concentration ensured better system stabilization against coalescence of the emulsion and therefore led to formation of smaller microparticles (17). Better emulsion stability also resulted in narrower particle size distribution in all samples, as seen in Figs. 1 and 2. Another well-observed effect (18) was that the value of the PVA concentration influenced also the shape of prepared microparticles. Fig. 3 shows that DM-L-600 provided spherical microparticles with holes in the surface, while DM-H-600 gave compact, spherical, regular and more uniform microparticles.

Effect of the organic solvent choice. – As observed in Fig. 3 and Table II, microparticles prepared with dichloromethane provided regular, highly spherical microparticles with a smooth surface, characterized by larger particle size in contrast to the smaller, irregularly-shaped microparticles created when ethyl acetate was used. A typical feature of ethyl acetate microspheres is their rough surface. An explanation could be that the polarity of ethyl acetate is higher compared to dichloromethane, resulting in microparticles of lower quality (19).

Encapsulation process. – Drug loading of the prepared samples ranged between 13.9 and 20.0 %, encapsulation efficiency had values from 16.7 to 79.8 % (Table II). For evaluation of the encapsulation process, *i.e.*, influence of the parameters on encapsulation efficiency and drug loading, MLR models for both organic solvents were used separately, four models in all. Regression diagnostics of all models had very good values (R -square > 0.8, R -square predicted > 0.8, C.V. < 3 %, models p < 0.001).

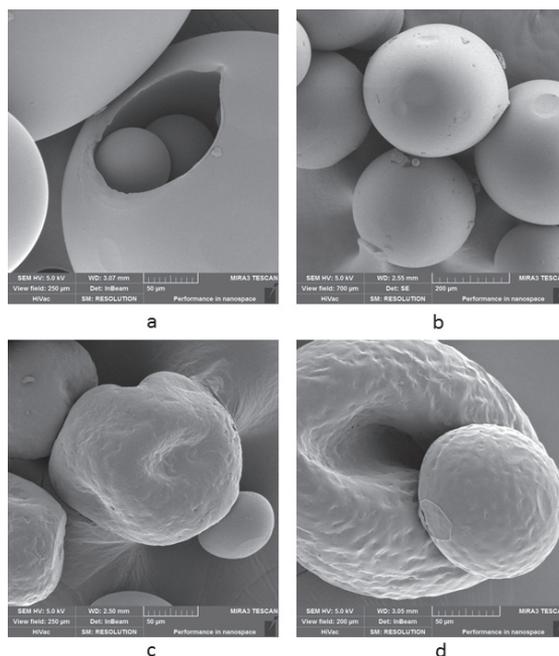


Fig. 3. SEM photographs of the surface of PLGA microspheres corresponding to: a) DM-L-600, b) DM-H-600, c) EA-L-600, d) EA-H-600.

Plots of interactions were used to compare the influence of variables on the effect. They represent an average value of factor-effect in dependence on the level of the second factor. In case of encapsulation efficiency for ethyl acetate samples (Fig. 4a), positive significance ($p < 0.001$) of the PVA concentration-stirring speed interaction was found. The plot shows that the influence of PVA concentration depends on the stirring speed. On the other hand, results for dichloromethane samples reveal a significant effect of individual variables ($p < 0.001$ for both), as shown in Fig. 4b. Both connecting lines between the effect averages are parallel, *i.e.*, for both PVA concentrations, the increase in stirring speed equals an approximately identical decrease in encapsulation efficiency. In case of both solvents, the maximum of encapsulation efficiency was reached with lower PVA concentration and slower stirring speed, which is in agreement with the literature. Increase in the stirring speed delivers greater energy to the system, resulting in an increased breakdown of the forming microspheres and lower encapsulation efficiency (20). It was also found that an excessive increase of PVA concentration can lead to lower encapsulation efficiency, probably due to higher viscosity (21, 22). Of both solvents used, ethyl acetate was found to provide lower values for encapsulation efficiency. Higher polarity of ethyl acetate, and therefore higher miscibility with the external phase, was probably responsible for the formation of a less stable o/w emulsion. In the EA/PVA system, the drug is transferred to the external phase more frequently and a smaller number of microspheres is produced, resulting in lower encapsulation efficiency (23).

In case of drug loading in samples prepared with ethyl acetate (Fig. 5a), the significant effect of individual variables is obvious ($p < 0.001$). This means that for both PVA concentra-

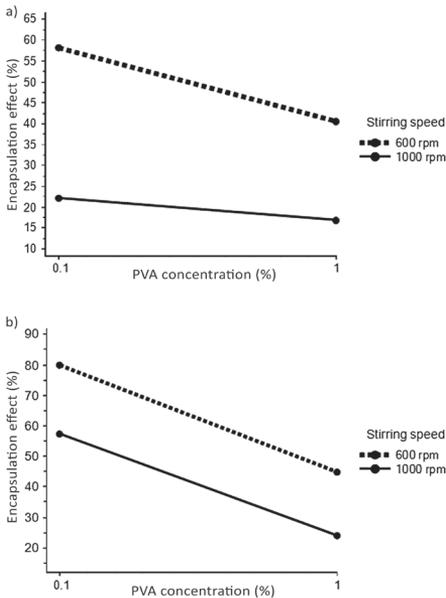


Fig. 4. Interaction plots; effect of PVA concentration on encapsulation efficiency, at various stirring speeds, for: a) ethyl acetate, b) dichloromethane.

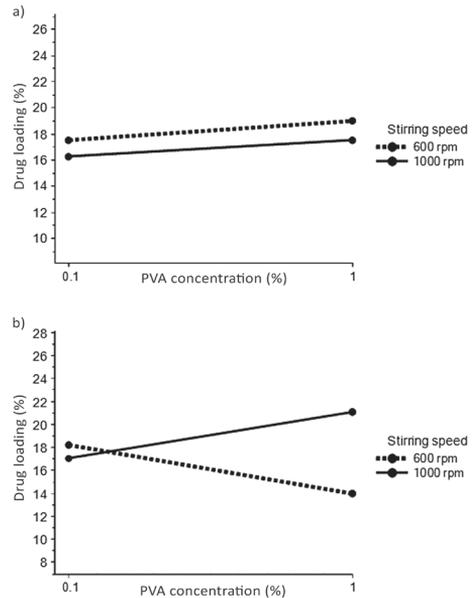


Fig. 5. Interaction plots; effect of PVA concentration on drug loading, at various stirring speeds, for: a) ethyl acetate, b) dichloromethane.

tions an increase in stirring speed led to an equivalent decrease in drug loading. Maximum drug loading was achieved with a high PVA concentration and low stirring speed. In dichloromethane samples (Fig. 5b), the effect of PVA concentration-stirring speed interaction is apparent ($p < 0.001$). At lower PVA concentration, the influence of the stirring speed on drug loading is relatively low compared to higher PVA concentration, where an increase in the stirring speed greatly increases drug loading. However, drug loading does not reflect the loss rate of the encapsulation process and therefore it serves as a complementary characteristic to encapsulation efficiency.

Drug-release behavior of microparticles

Figs. 6 and 7 show the drug dissolution profiles performed at pH 6.8. The obtained release profiles were characterized as predominantly bi-phasic profiles with apparent burst release, followed by sustained drug release for at least 72 hours. The basic release pattern of PLGA microparticles is a tri-phasic profile. However, factors such as pore formation and/or closure, drug solubility, drug amount, and polymer molecular mass and/or its density can transform a tri-phasic profile into a bi-phasic one (24–26).

Burst effect. – MLR models for burst effect have good coefficients of determination, but predictive characteristic limits are reached due to greater variability between replicates. ($R^2 > 0.75$, R^2 predicted < 0.5 , C. V. $< 18\%$, model p -values: $0.05 < p < 0.1$, *i.e.*, possibly significant). Despite this fact, model plots provide sufficient representation of average factor effects and

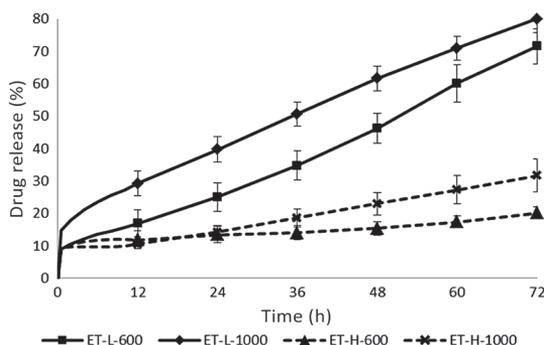


Fig. 6. *In vitro* dissolution profiles of ibuprofen from PLGA samples prepared with dichloromethane as organic solvent.

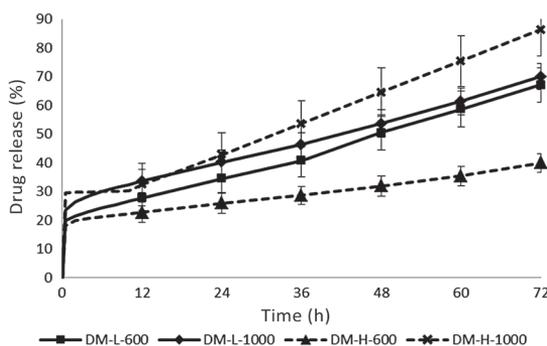


Fig. 7. *In vitro* dissolution profiles of ibuprofen from PLGA samples prepared with ethyl acetate as organic solvent.

models are used for approximate evaluation of the factors' influence on the effects. In samples prepared with ethyl acetate, there was possibly a significant effect ($0.05 < p < 0.1$) of PVA concentration-stirring speed. At higher PVA concentration, the burst effect values were roughly the same for both stirring speeds used, whereas there was a remarkable difference at lower PVA concentration (Fig. 8a). Results of dichloromethane samples showed a significant influence of individual variable stirring speed ($p < 0.05$) on the burst effect (Fig. 8b). At 1000 rpm, the burst effect was at least six units greater than with samples prepared at 600 rpm. At 1000 rpm, smaller microparticles were prepared and therefore these samples had a larger surface area per mass unit than samples prepared at 600 rpm. This effect resulted in a higher burst effect followed by faster drug release (22). The burst effect is generally considered to be undesirable, especially because of local or systemic toxicity and shortening of release profiles (27). However, in some cases, the burst effect can be advantageous, primarily in wound treatment, targeted delivery or controlled release.

Sustained release. – Similar factor f_2 analysis also revealed the dependence of drug release on the stirring speed in dichloromethane samples prepared with 1 % PVA concentra-

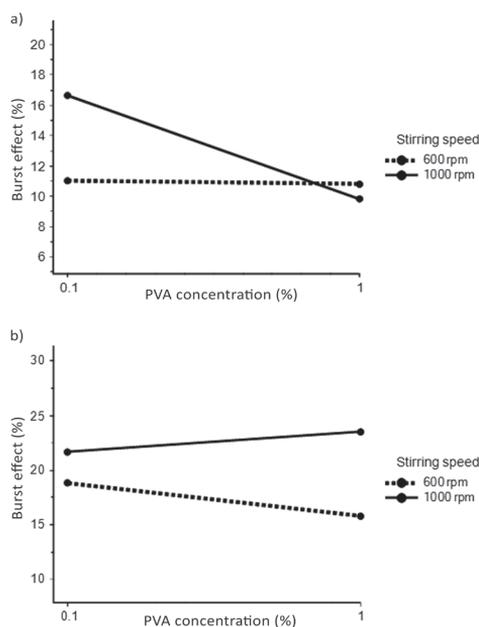


Fig. 8. Interaction plots; effect of PVA concentration on burst effect, at various stirring speeds, for: a) ethyl acetate, b) dichloromethane.

tion (DM-H-600 × DM-H-1000 $f_2 = 29.9$) and in ethyl acetate samples prepared with 0.1 % PVA (ET-L-600 × ET-L-1000 $f_2 = 46.1$). Ethyl acetate samples prepared with 1 % PVA and dichloromethane samples prepared with 0.1 % PVA were considered to be similar (ET-H-600 × ET-H-1000 $f_2 = 64.3$; DM-L-600 × DM-L-1000 $f_2 = 66.3$) and therefore the stirring speed alone probably had no significant influence on drug release.

PVA concentration had a noticeable impact on drug release, as shown in Figs. 6 and 7. All the samples prepared with 0.1 % PVA concentration exhibited faster drug release than their equivalent counterparts prepared with 1 % PVA concentration (with the exception of DM-H-1000, which showed the fastest release among all samples). For example, DM-L-600 released 67.1 % of IBU within 72 hours in comparison with DM-H-600, which released 40.0 % of the drug. This is surprising, as higher PVA concentration helps produce smaller microparticles with a larger surface, just like the surfactant effect of the remaining PVA on the surface (22), and so faster drug release is to be expected. The explanation of this phenomenon could be the more even distribution of the drug inside the matrix. Higher viscosity of the 1 % PVA solution can significantly lower the concentration gradient of the drug inside the particles (17). The significance of this effect was evaluated by the similarity factor f_2 analysis. Similarity factors f_2 between samples prepared with 0.1 and 1 % PVA were determined. For ethyl acetate samples, the similarity factor f_2 values were 30.2 (ET-L-600 × ET-H-600) and 25.7 (ET-L-1000 × ET-H-1000), which indicated that there was a significant dependence of the drug amount released on the PVA concentration. However, the dichloromethane samples' similarity factor f_2 had values of 42.6 (DM-L-600 × DM-H-600) and 53.7 (DM-L-1000 × DM-H-1000). This means that in dichloromethane samples, the dependence of drug release on emulsifier concentration disappears with the increase of rotation speed, confirming the multifactorial complexity of the process (24).

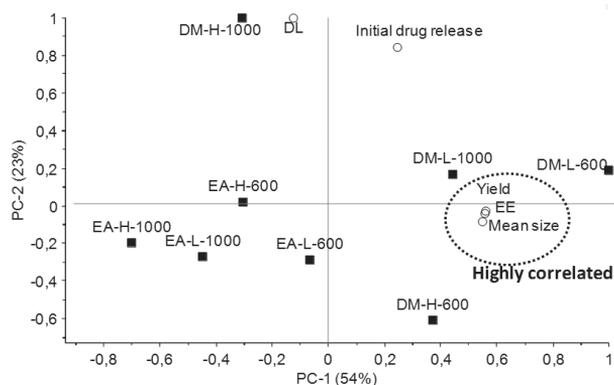


Fig. 9. PCA bi-plot displays a projection of objects (boxes) and response variables (circles) in the area of the first two components. Objects describe the performed experiment in the form: solvent (DM-dichloromethane; EA-ethyl acetate)-PVA concentration (L-low; H-high)-stirring speed (600; 1000). The first two components describe 77 % of total variability.

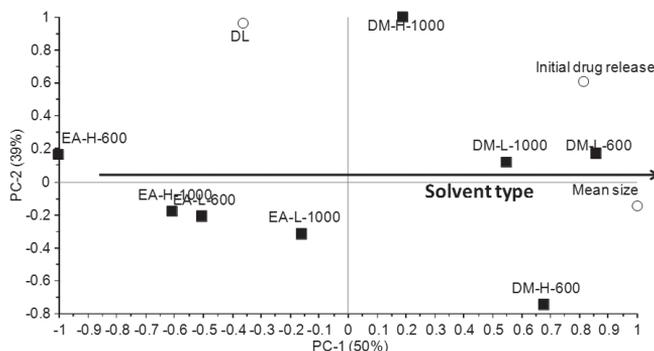


Fig. 10. PCA bi-plot displays a projection of objects (boxes) and response variables (circles) in the area of the first two components. Highly correlated variables from previous model (Fig. 9) were removed in order to take into account the remaining variability. Objects describe the performed experiment in the form: solvent (DM-dichloromethane; EA-ethyl acetate)-PVA concentration (L-low; H-high)-stirring speed (600; 1000). The first two components describe 89 % of total variability.

Figs. 6 and 7 show drug release differences between ethyl acetate and dichloromethane samples. The ethyl acetate samples prepared with 0.1 % PVA concentration were characterized by faster drug release than samples prepared with dichloromethane. This could be explained by the different distribution of IBU in the matrix of microparticles. In the DM samples with a perfectly spherical shape, the drug could be situated more in the peripheral matrix parts, resulting in higher burst effect. On the other hand, EA samples were smaller, deformed and collapsed, could have the drug spread out more evenly around the matrix. Although the behavior of samples was different, the similarity factor f_2 between

the ethyl acetate and the dichloromethane samples did not confirm the significance of this variable (ET-L-600 × DM-L-600 $f_2 = 54.1$; ET-L-1000 × DM-L-1000 $f_2 = 57.9$). Faster release from the ethyl acetate samples negated the difference caused by lower burst effect.

However, at a 1 % PVA concentration, the subsequent drug release rate was approximately the same for both ethyl acetate and dichloromethane samples. This means that the curves remained parallel with a relatively wide gap. Similarity factor f_2 analysis calculated between the EA and DM samples prepared with 1 % PVA revealed the significance of the solvent choice effect on dissolution profiles (ET-H-600 × DM-H-600 $f_2 = 43.0$; ET-H-1000 × DM-H-1000 $f_2 = 22.7$). Most likely, the higher PVA viscosity caused more evident differences in the dissolution profiles.

This interesting information was also obtained from the results of PCA analysis (Fig. 9). From the position of the symbols representing response variables, it is apparent that there was a correlation among variables-encapsulation efficiency, yield and mean microparticle size. The practical benefit of this finding could be seen in approximate prediction of these variables based on the knowledge of only one of them.

Another PCA model was designed for the study of variability among variables. Highly correlating variable encapsulation efficiency and yield were removed to prevent the overvaluation effect on the PC-1 component. This enabled a description of the mean size and initial drug release correlation (Fig. 10) and their influence on PC-1, at which the solvent type variability lies. A strong influence of organic solvent on the mentioned variables was manifested. Dichloromethane gave double values compared to ethyl acetate.

CONCLUSIONS

PLGA microparticle samples were successfully prepared by the solvent evaporation method. The full factorial design method and partial least squares PLS-2 regression were used to evaluate the impact of several formulations and process parameters used during the process. It was found that the formulation and process parameters influenced the microparticle characteristics as individual variables but also in interactions with other parameters. Multivariate data analysis was shown to be an applicable tool for identification of such relations. The results confirm the complexity of the process using the solvent evaporation method.

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