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Star-shaped poly(ε-caprolactones) with well-defined architecture as potential drug carriers

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Abstract: The present study reports the potential application of star-shaped poly(ε-caprolactones) with different number of arms as new drug delivery matrix. Linear and star-shaped PCL ibuprofen loaded microspheres were prepared using oil-in-water (o/w) solvent evaporation technique and characterized with FTIR, DSC, XRD and SEM analysis. High yield, encapsulation efficiency and drug loadings were obtained for all microspheres. FTIR analysis revealed the existence of interactions between polymer matrix and drug, while the DSC analysis suggested that drug was encapsulated in an amorphous form. SEM analysis confirmed that regular, spherical in shape star-shaped microspheres, with diameter between 80 and 90 μm, were obtained, while quite larger microspheres, 110 μm, were prepared from linear PCL. The advantage of using star-shaped PCL microspheres instead of linear PCL was seen from drug release profiles which demonstrated higher amount of drug released from star-shaped polymer matrix as a consequence of their branched, flexible structure. Microspheres prepared from the polymers with the most branched structure showed the highest amount of the released drug after 24 h. Finally, cytotoxicity tests, performed using normal human fibroblasts (MRC5), indicated the absence of cytotoxicity at lower concentrations of microspheres proving the great potential of star-shaped PCL systems in comparison to linear ones.

Keywords: star-shaped PCLs; PCL microspheres; drug carriers; ibuprofen; cytotoxicity.

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INTRODUCTION

The development of controlled release and targeted drug delivery has evolved from the need to efficiently deliver a drug to a targeted site and thus increase the therapeutic effects, to prolong and better control the drug administration, but also to overcome the limitations found in conventional therapy such as toxic drug levels.[1–3] Different polymeric materials fulfill the challenges of drug storage and delivery, from which large number have already been used in pharmaceutical researches, whether as FDA approved formulations or in an early stage of investigations.[4] Star-shaped polymers have been intensively investigated as drug carriers due to their unique structural and chemical properties, which put them apart from linear analogues.[5] Well-defined structure, smaller hydrodynamic volume, internal and peripheral functionality, stimuli-responsiveness, lower melt and solution viscosities which further result in better loading efficiency and release properties, are among the main advantages that suggest star-shaped polymers as promising materials in drug delivery systems.[2,5,6]

Poly(ε-caprolactone), PCL, is a biodegradable, aliphatic and hydrophobic polyester that degrades very slowly in the human body. Despite the hydrophobic nature and relatively high degree of crystallinity which remarkably restrict its biomedical application, PCL has attracted a lot of attention as a synthetic biomaterial in controlled drug delivery systems due to its biocompatibility, good permeability for variety of drugs and non-toxicity.[7,8] One of the approaches to solve the problem of slow degradation is by synthesizing star-shaped PCLs containing one single point of branching from which linear chains originate. The branched structure with three or more arms departing from the center has more free chain ends which disrupt well-ordered crystal structure of linear PCL.[9] Different multifunctional alcohols, such as trimethylolpropane (TMP), pentaerythritol (PERT), ditrimethylolpropane (diTMP), dipentaerythritol (diPERT), sorbitol, xylitol, glycerol, *etc.*, could be applied as initiators in the ring-opening polymerization of ε-caprolactone (ε-CL) resulting in different molecular architectures.[9–11] Star-shaped polymer micelles have appeared as a perfect polymer matrix for hydrophobic drug encapsulation.[12] In addition, the change in molecular architecture (number of arms) and molecular weight of star-shaped polymer micelles could be a powerful tool for control of micelles size, but could also lead to increase of drug loading and affected release kinetics.[13] Most of the studies related to star-shaped PCL polymers for potential drug delivery applications were focused on micelle formulations made of star-shaped amphiphilic copolymers[14,15] and very limited literature is related to PCL based star-shaped polymers, designed for drug encapsulation in the form of micro/nanoparticles.[16] Ibuprofen, selected as a model hydrophobic drug for this study, is a nonsteroidal anti-inflammatory drug (NSAID) available in the treatment of rheumatoid arthritis and osteoarthritis.[17] Short half-lives and poor bioavailability of ibuprofen provided by consuming

conventional formulations, can be overcome by ibuprofen encapsulation into a polymer matrix, PCL for example, and minimize the adverse side effects.[18–21]

In our previous study, the synthesis and characterization of star-shaped poly(ε-caprolactone)s, with a different number of arms (three, four and six) with similar molecular weight was reported.[22] However, the studies focused on the ibuprofen loaded microspheres prepared from star-shaped PCL with different number of arms and arms' length as polymer matrix, and drug release profiles of such systems, have been very limited. Therefore, this study was related to the use of star-shaped PCL polyesters with well-defined structure as a novel drug carrier. The designed novel star-shaped PCL material was used for ibuprofen encapsulation (10 wt. %) in the form of microspheres prepared by the o/w emulsion solvent evaporation method. The influence of molecular architecture on the efficiency of encapsulation, microspheres size and morphology, and drug release kinetics, was investigated as well as their cytotoxicity by using healthy human fibroblasts (MRC5) for cell survival assessment.

EXPERIMENTAL

Materials

Linear PCL and star-shaped PCLs with three, four and six arms were used as polymer matrix for drug encapsulation (Supplementary material to this paper Table S-I). They were synthesized by ring-opening polymerization (ROP) of ε-CL in bulk, initiated with hydroxyl groups of multifunctional alcohols and catalyzed using $Sn(Oct)_2$, as previously reported.[22] Variation in the number of arms was achieved using multifunctional alcohol, such as trimethylolpropane (TMP), ditrimethylolpropane (diTMP), penthaerytritol (PERT) and dipentaerytritol (diPERT), while the PCL arms' length was fixed (5000 g/mol). Dichloromethane (Fischer Chemicals) and chloroform (Sigma Aldrich) were used without further purification. Poly(vinyl alcohol) (PVA, M_w = 130000 g/mol, Mowiol® 18-88) was purchased from Sigma Aldrich. Ibuprofen, IB (99.9 %), was a gift from Hemofarm, Stada Group (Vršac, Serbia). Phosphate buffer solution (PBS, pH 7.4) was used as the drug release medium.

Preparation of polymer microspheres

Microspheres of linear PCL and star-shaped PCLs were prepared by the classical oil-in-water (o/w) emulsion solvent evaporation technique under the previously set conditions.[23] The PCL arms' lengths were fixed at 5000 g/mol, while the different multifunctional alcohols (TMP, diTMP, PERT and diPERT) provided branched architecture and different polymer molecular weight of the resulted star-shaped polyesters (Supplementary material, Table S-1). The polymers (2.000 g) and ibuprofen (200 mg, 10 wt. % calculated to polymer weight) were dissolved in dichloromethane. The organic phase (20 ml) was added dropwise into the water phase consisting of PVA solution (400 ml of 0.5 wt. % PVA solution) placed in 1 L reactor and stirred mechanically (1000 rpm) for 6 h, at room temperature, until complete evaporation of dichloromethane. The produced microspheres were filtrated, washed three times with 40 ml of distilled water and dried in vacuum oven. The same procedure was applied to prepare empty microspheres of linear PCL (PCL blank) and star-shaped PCLs (TMP/PCL blank, diTMP/PCL blank, PERT/PCL blank, diPERT/PCL blank).

*Determination of efficiency of encapsulation (*EE*) and drug loadings (*DL*)*

Ibuprofen encapsulation efficiency (*EE*) in the microspheres was assessed by dissolving 20 mg of drug-loaded microspheres in a small volume of dichloromethane (DCM), placed in 25 ml volumetric flask and filled with PBS. After all DCM was evaporated by purging with nitrogen, the ibuprofen concentration was determined by UV–Vis method. All measurements were done in duplicates. The encapsulation efficiency was calculated as:

$$EE = 100\frac{\text{Amount of drug in microspheres}}{\text{Amount of drug in feed}} \quad (1)$$

The drug loadings (*DL*) were obtained applying:

$$DL = 100\frac{\text{Mass of drug in microspheres}}{\text{Mass of drug in feed}} \quad (2)$$

ATR- Infrared spectroscopy (ATR-FTIR)

The Fourier transform infrared (FTIR) spectroscopy was applied to detect the possible interactions between the polymer matrix and the drug. The samples were recorded using an IR-affinity spectrophotometer (Thermo Scientific, Nicolet iS10). The number of scans was 32, collected in the range of 4000 to 400 cm^{-1} with a scan step of 4 cm^{-1}, at room temperature.

Differential scanning calorimetry (DSC)

DSC analysis of polymer microspheres was performed on a TA Instruments SDT Q600. Approximately 5 mg of each sample was heated in a temperature range of 20 to 200 °C, at a rate of 10 °C/min, in nitrogen atmosphere.

Wide angle X-ray diffractometry (XRD)

The X-ray diffraction was performed on an ItalStructure APD2000 diffractometer in a Bragg–Brentano geometry with CuKα (λ = 0.15418 nm) radiation. The microspheres were scanned from 2θ 5 to 40° with step-time of 2.0 s and step width: 0.02°. The degree of crystallinity, X_c, of the prepared microspheres was calculated by the peak deconvolution using PeakFit 4.12 program as a ratio of the area under the crystalline peaks to the overall diffraction pattern area. The relative areas under the crystalline reflections and the amorphous halo peak (range 2θ 15 to 30°) were determined.

Scanning electron microscopy analysis (SEM)

The shape, morphology and size of microspheres was visualized using a field emission scanning electron microscopy (FESEM, Mira3 Tescan) with an accelerating voltage of 10 kV. Prior to imaging, all microspheres were fixed on supports and sputter-coated with a thin layer of gold. Images were captured at different magnifications and ImageJ software was used to estimate the average diameter and size distribution of microspheres. For determination, at least 200 separate microspheres were measured.

In vitro *drug release profiles*

The drug release studies were performed in phosphate buffer solution (PBS, pH 7.4), at 37 °C for 24 h. About 50 mg of polymer microspheres were placed into a vial into which 50 mL of PBS was added. At predetermined time intervals, 0.5 mL of the release media was removed from the incubated vials and replaced by fresh media in an equal volume. The concentration of ibuprofen in the release medium was determined by UV–Vis spectroscopy (UV--1800, Shimadzu, Japan) at a λ_{max} = 221 nm using a calibration curve previously established for ibuprofen in PBS. The results were an average value of two measurements. The cumul-

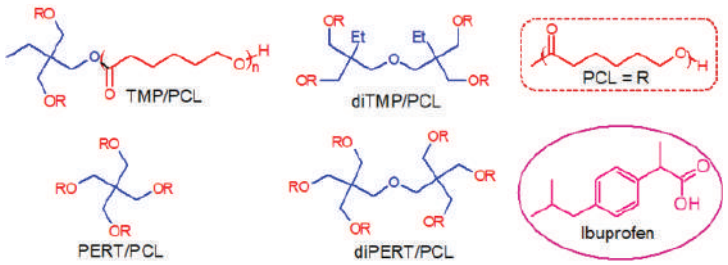
ative drug release values expressed in % were calculated based on the *EE* values, obtained for each sample of IB loaded microspheres.

In vitro *cytotoxic activity assays*

In order to assess cytotoxicity (antiproliferative activity), standard MTT assay[24] and methods suitable for materials testing[25] were used. Human lung fibroblasts (MRC5) cells (obtained from ATCC culture collection) were plated in a 96-well flat-bottom plate at a concentration of 10^4 cells per well, grown in humidified atmosphere of 95 % air and 5 % CO_2 at 37 °C, and maintained as monolayer cultures in RPMI-1640 medium, supplemented with 100 μg/mL streptomycin, 100 U/mL penicillin, and 10 vol. % fetal bovine serum (FBS). MRC5 cells were treated with 100, 50, 25 and 12.5 vol. % sample extract as well as with 600, 400, 200 and 100 μg/ml ibuprofen and incubated for 48 h. Control cultures were treated only with growth medium and blank wells also contained growth medium (200 μL). Cells proliferation was determined using MTT reduction assay by measuring the absorbance at 540 nm on Tecan Infinite 200 Pro multiplate reader (Tecan Group, Männedorf, Switzerland). The MTT assay was performed in triplicates and the results were presented as percentage of the control (untreated cells) that was arbitrarily set to 100 %. Morphological appearance of the treated cells was observed by DM IL LED inverted microscope (Leica Microsystems, Wetzlar, Germany).

RESULTS AND DISCUSSION

In the presented study, linear PCL and star-shaped PCLs with different number of arms (three, four and six) served as suitable polymer matrix for the ibuprofen encapsulation (10 wt. % calculated to polymer weight, Scheme 1).



Scheme 1. Structure of star-shaped PCLs with different number of arms and model drug – ibuprofen.

The aim of the investigation was to clarify the influence of variability of arms number and molecular weight of star-shaped polymer matrix on the efficiency of encapsulation, particle size and drug release profiles.

Preparation of microspheres

Microspheres were prepared using the classical oil-in-water (o/w) solvent evaporation technique, since this method is reproducible and recognized as the simplest way to encapsulate hydrophobic drug, such as ibuprofen, into a chosen polymer matrix. The applied technique resulted in high yields of obtained microspheres (ranged from 83 to 97 %), as well as the efficiency of encapsulation

ranged from 83 to 96 % (Table I). Also, drug loading values (7.5 to 8.7) were very similar to theoretical value of 9.1.

TABLE I. Microspheres characterization: yield, efficiency of encapsulation, mean diameter, drug loadings

Microspheres type	Yield, %	*EE* / %	Mean diameter[a], μm	*DL*[b] / %
PCL	97	-	110 ± 34	
TMP/PCL blank	88	-	89 ± 19	
diTMP/PCL blank	86	-	83 ± 20	
PERT/PCL blank	77	-	77 ± 16	
diPERT/PCL blank	89	-	76 ± 16	
PCL10	87	90	154 ± 51	8.2
TMP/PCL10	86	91	79 ± 14	8.3
diTMP/PCL10	83	83	77 ± 16	7.5
PERT/PCL10	88	96	80 ± 17	8.7
diPERT/PCL10	89	93	79 ± 15	8.5

[a]Determined by SEM analysis; [b]theoretical value was 9.1

Analyzing *EE* and *DL* values, it may be concluded that the interactions between polymer matrix and drug were responsible for high efficiency of encapsulation. Although the obtained *EE* and *DL* values were very similar for all obtained microspheres, it could be assumed that the branched architecture of polymer matrix resulted in the higher *EE* and *DL* values in comparison to the microspheres prepared from linear PCL.

FTIR analysis of prepared microspheres

Fourier transform infrared spectroscopy was employed to detect and understand any possible interactions between the polymer matrix and the encapsulated drug. FTIR spectra of PCL and all star-shaped PCL microspheres (Fig. 1a) showed characteristic peak of carbonyl group at 1720 cm^{-1}, and two CH_2 symmetric and asymmetric stretching bands at around 2943 and 2864 cm^{-1}.

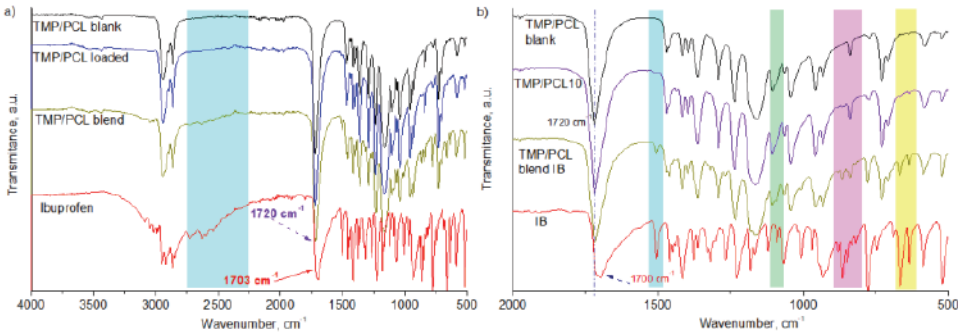


Fig. 1. Representative FTIR spectra of: a) TMP/PCL microspheres (blank, 10 wt. % IB loaded and TMP/PCL blend with 10 wt. % IB, b) 2000–500 cm^{-1} wavenumber region.

Yet, the carbonyl group of pure ibuprofen was displayed at 1700 cm^{-1} and after the encapsulation into a polymer matrix, this characteristic carbonyl peak overlapped with the carbonyl peak of polymer matrix in the region of 1660 to 1763 cm^{-1}. These changes in the characteristic peak frequencies of polymer and drug suggested the existence of the interactions between functional groups of drug and polymer. For comparison, the physical mixture of PCL and all star--shaped polymer matrixes and ibuprofen in the concentration of 10 wt. % were also recorded. As it could be noticed from the representative TMP/PCL blend IB spectra, some peaks inherent to IB (at 1090, 866 cm^{-1} and in the region of 664 to 634 cm^{-1}) still existed in these mixture, while they were not visible in the drug loaded microspheres, additionally confirming the interactions of IB with the polymer matrix and the successful encapsulation of drug into a polymer matrix.

DSC Analysis

The DSC thermograms (Fig. 2) of both linear PCL and star-shaped microspheres showed single, endothermic peak in the range of 64.3 to 68.5 °C with the highest T_m value measured for linear PCL (Table II), while the melting temperatures, T_m of star-shaped microspheres were very similar. For the pure ibuprofen, a sharp, endothermic peak was observed at 80.5 °C. After the drug encapsulation into a polymer matrix, only the melting endotherms of polymers were detected, indicating that ibuprofen was encapsulated into an amorphous form, or it was dissolved in the polymer melt during the heating. The drug encapsulation into linear PCL and star-shaped polymer matrixes caused slight decrease in melting temperatures, with the changes no greater than two degrees. Contrary to the melting temperatures, the melting enthalpies were significantly changed after ibuprofen encapsulation, with the greatest change, from 78.4 to 90.4 J/g, detected for linear PCL microspheres.

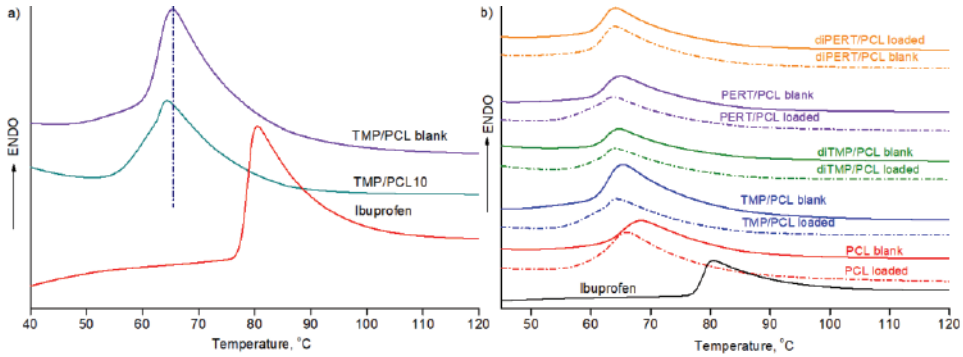


Fig. 2. DSC thermograms of: a) TMP/PCL microspheres and IB, and b) all microspheres.

The increase in melting enthalpy after drug incorporation was observed for all microspheres, but less prominent for star-shaped polyesters. Also, higher

values of melting enthalpies of some blank star-shaped microspheres in comparison with linear PCLs could be attributed to symmetric, branched structure of star-shaped PCLs, which provided better packaging of polymer chains.

TABLE II. DSC and XRD results: melting temperature, T_m, melting enthalpy change, ΔH_m, and degree of crystallinity, X_c

Microspheres type	T_m / °C	ΔH / J g^{-1}	w_{PCL} / %	X_c[a] / %	X_c(PCL)[a] / %	X_c[b] / %
Ibuprofen	80.5	121.5	100			–
PCL blank	68.5	78.4		57.6		66.5
TMP/PCL blank	65.5	87.3		64.1		54.9
diTMP/PCL blank	64.9	70.6		51.8		49.2
PERT/PCL blank	65.2	79.9		58.7		56.2
diPERT/PCL blank	64.3	80.5		59.1		57.5
PCL10	66.1	90.4	91.8	66.4	72.4	70.0
TMP/PCL10	64.5	89.5	91.7	65.8	71.7	72.8
diTMP/PCL10	64.2	79.6	92.5	58.5	63.2	67.3
PERT/PCL10	63.9	87.0	91.3	63.9	70.0	71.0
diPERT/PCL10	63.6	84.5	91.5	62.1	67.9	67.3

[a]Determined from DSC analysis; [b]determined from XRD analysis

The total degree of crystallinity (X_c) of microspheres was calculated by comparing ΔH_m obtained by DSC with literature value for the perfectly crystalline PCL homopolymer (ΔH_m^o = 136.1 J/g).[26] The relative degree of crystallinity (X_c^{PCL}) was calculated according to the Eq. (3), where w_{PCL} is the amount of PCL in loaded microspheres:

$$X_c^{PCL} = \frac{\Delta H_m}{\Delta H_m^o w_{PCL}} \quad (3)$$

Besides the unloaded diTMP/PCL microspheres with the lowest degree of crystallinity in comparison to other unloaded microspheres, the unloaded linear PCL microspheres exhibited the lowest total X_c of 57.6 %. The X_c value of linear PCL microspheres was the result of the linear structure which provided less organized lamellar structure and packaging of polymer chains in comparison to star-shaped PCLs (Table II).

In the series of unloaded star-shaped PCL microspheres, the X_c value was in the range of 52.5 to 64.7 %, which means that PCL could not crystallize completely and that some fractions of PCL were incorporated into the amorphous phase. In the series of IB-loaded microspheres, the relative degrees of crystallinity (X_c^{PCL}) were in the range of 63.2 to 72.4 % and were quite higher in comparison to their unloaded analogues. The increase in X_c values after IB encapsulation could be explained by the IB nucleation-promoter role (drug acts as nucleating agents in polymer matrix).[20,27]

XRD Analysis

XRD measurements were conducted in order to get an insight into a physical state of drug encapsulated into different polymer matrixes (Fig. 3). Bikiaris *et al.*[28] proposed that the coupling XRD analysis with DSC would allow complete physicochemical characterization of drug loaded systems, since XRD reveals their physical state at room temperature, while DSC reveals it during heating.

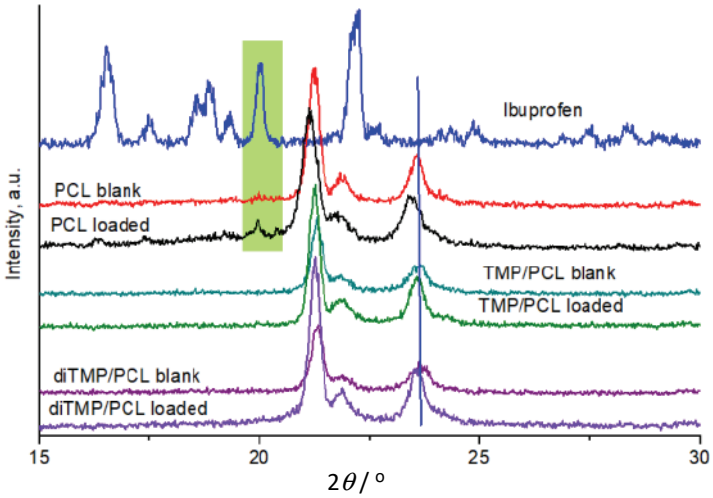


Fig. 3. Representative XRD diffractograms of ibuprofen and ibuprofen loaded microspheres.

Therefore, the degrees of crystallinity of loaded and unloaded PCL based microspheres determined from the XRD analysis (Table II) were lower than those values obtained from DSC measurements. The obtained results showed that ibuprofen is a high crystalline with several characteristic peaks at 16.51, 17.48, 18.82, 20.04 and 22.23°,[29] while the drug loaded star-shaped microspheres showed sharp peaks at 21.15, 21.88 and 23.46° which corresponded to (110), (111) and (200) planes, respectively, coming from the PCL orthorhombic crystalline lattice.[30] Fig. 3 depicted the representative diffractograms of star-shaped PCLs, while the others from the series were similar to the presented. All ibuprofen loaded star-shaped microspheres did not show any peaks corresponding to IB, referring that IB was probably incorporated into a matrix in an amorphous form. Contrary to IB loaded star-shaped microspheres the linear PCL loaded microspheres, in which ibuprofen characteristic reflection at 18.82° of small intensity was detected, indicating that ibuprofen was partially dispersed in crystalline state within the linear PCL microspheres, although SEM photos displayed the absence of any drug crystals deposition (Fig. 4). The presented XRD diffractograms, in which sharp and more intense reflections were observed for IB loaded microspheres, also pointed that the star-shaped PCLs exhibited increased crystallinity

after the ibuprofen encapsulation. The drug-loaded PCL10 microspheres had degree of crystallinity of 70.0 %, while the degrees of crystallinity of the IB loaded star-shaped microspheres were in the range of 67.3 to 72.8 %. The calculated X_c values of IB loaded microspheres increased in comparison to unloaded analogues, which was in a line with the results obtained by DSC.

SEM Analysis

The photomicrographs (Figs. 4 and 5) obtained by SEM analysis proved spherical, regular shape of all prepared microspheres with no agglomeration of particle.

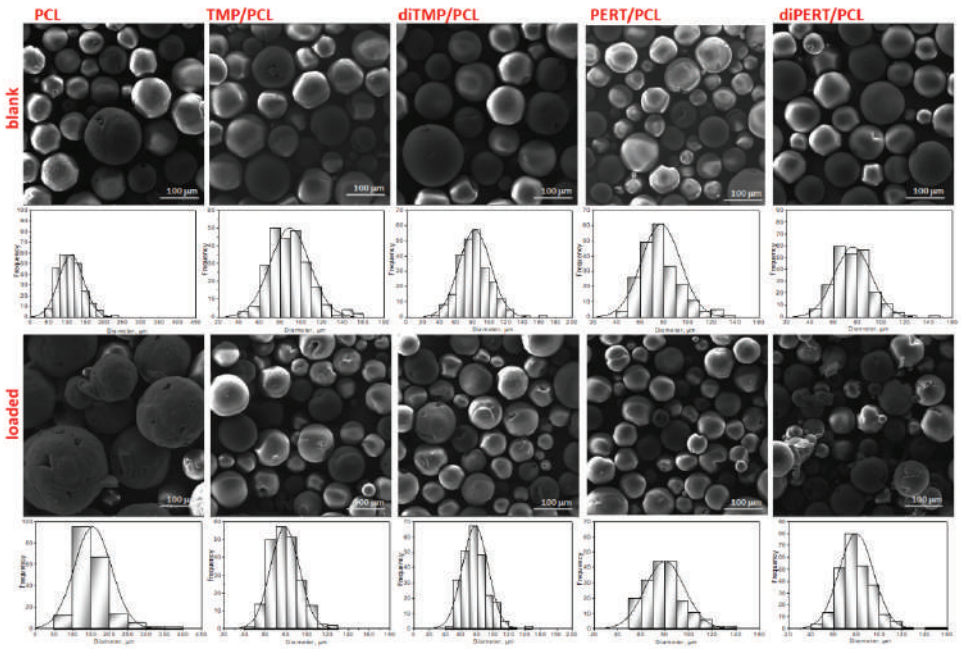


Fig. 4. SEM micrographs and size distribution of prepared microspheres.

From the SEM micrographs of blank microspheres, it could be seen that microspheres obtained from linear PCL possessed significantly higher diameter (around 110 μm) in contrary to microspheres prepared from star-shaped PCLs which size were in the range of 76 to 89 μm. Higher diameter of linear PCL microspheres was a consequence of higher molecular weight of PCL polymer which further resulted in higher viscosity of PCL solution (internal organic phase) and therefore larger microspheres were obtained. Also, the diameters of star-shaped microspheres with different number of arms were very similar and the influence of molecular weight was negligible. This result is a consequence of similar hydrodynamic volume, since the length of a single branch (here 5000

g/mol for all the synthesized polymers) of star-shaped PCL reflected on hydrodynamic volume. The ibuprofen loaded microspheres also possessed regular, spherical shape, with the absence of any agglomerates or drug deposits on microspheres surface. After the ibuprofen encapsulation into star-shaped PCLs, diameter remained almost unchanged, while the linear PCL loaded microspheres were remarkably larger in comparison to unloaded ones (154 to 110 μm). The microspheres size distribution presented on histograms below the corresponding SEM micrographs showed broad, unimodal size distribution.

In vitro *drug release profiles*

From the release profiles presented in Fig. 5, it could be observed that ibuprofen was released from both linear PCL and star-shaped PCLs polymer matrices as followed: the initial fast release, so-called burst effect, followed by more sustained release during few hours, after which a plateau with a constant drug concentration was reached.

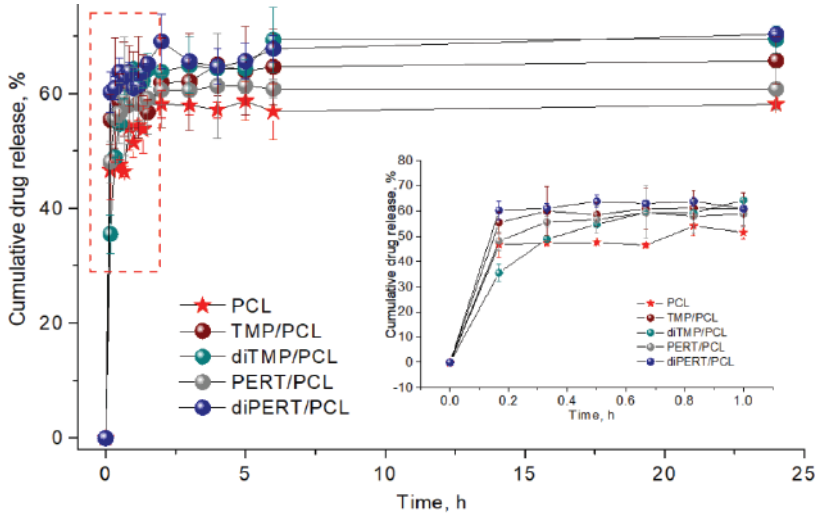


Fig. 5. Drug release profiles of IB from PCL and star-shaped PCL microspheres over 24 h.

From the inserted graph which showed the release profiles in the first hour, it could be noticed that the sample diTMP/PCL indicated less prominent burst effect, but still, after only 10 min, more than 30 wt. % of drug was released. In the case of linear PCL microspheres, IB (ibuprofen) release kinetics was affected both by linear architecture and molecular weight of polymer matrix (high molecular weight resulted in large size of microspheres, which further had a great impact on the IB release kinetics and the lowest amount of drug was released over time). After 6 h, less than 60 wt. % of drug was released from PCL microspheres. In general, the drug release kinetics can be controlled and dictated by

several factors, such as compatibility and permeability of drug and polymer matrix, polymer molecular weight, degree of crystallinity of polymer, drug distribution into a polymer matrix, particles size and size distribution, *etc.*[32,33] In comparison to linear PCL, all star-shaped IB loaded microspheres released higher amount of drug. However, small differences could be explained by different architecture and crystallinity of loaded microsphere. Therefore, in the starting few hours of the IB release, the higher concentration of drug was released from diPERT/PCL10 polymer microspheres, pointing to the dominant role of polymer matrix architecture on release kinetics but also to the smallest degree of crystallinity (defined by XRD analysis). It is also important to mention that ibuprofen solubility in PBS,[34] as well as the existence of drug-polymer interactions proved by FTIR analysis, could play a great role in drug release kinetics, although polymer architecture and crystallinity appeared as dominant factors.

Kinetic analysis of the drug release profiles was performed by calculating kinetic parameters, such as sampling time, $t_{x\%}$, dissolution efficiency, *DE*, and the released amount of drug after 24 h. The sampling time corresponds to the time necessary to release a determined amount of drug ($t_{50\%}$, $t_{60\%}$), 50 and 60 % in this case, while the dissolution efficiency of a pharmaceutical dosage form was calculated by:

$$DE = \frac{\int_0^t y \, \mathrm{d}t}{y_{100}t} 100 \tag{4}$$

where $\int_0^t y \, \mathrm{d}t$ is the area under the dissolution curve up to a certain time (24 h in this case), t, and the value of $y_{100}t$ corresponds to the area of the rectangle described by 100 % dissolution in the same time; y is the drug percentage dissolved at time t.[35] The calculated kinetic parameters for linear PCL and star-shaped PCLs microspheres are listed in Table III. Time needed to release 50 % of IB from linear PCL microspheres was 90 min which was quite slower in comparison to all star-shaped PCLs, while the fastest release was achieved from diPERT/PCL microspheres as a consequence of the most branched architecture. Dissolution efficiency values, *DE*, indicated that the lowest *DE* value (57 %) was calculated for linear PCL microspheres due to the slow release of drug. Among the star-shaped PCLs, the highest percentage of dissolved drug was achieved for

TABLE III. Drug release kinetics: sampling time ($t_{50\%}$, $t_{60\%}$), *DE*, and released amount of drug after 24 h, c_{24}

Sample	$t_{50\%}$ / min	$t_{60\%}$ / min	*DE* / %	c_{24h} / ppm
PCL10	90	1440	57	58.1
TMP/PCL10	<10	20	64	65.7
diTMP/PCL10	20	40	67	69.4
PERT/PCL10	15	40	60	60.7
diPERT/PCL10	<10	10	68	70.3

diPERT/PCL10 with the value of 68 %, followed by diTMP/PCL10 and TMP/ /PCL10 with *DE* values of 67 and 64 %, respectively.

Cytotoxicity tests

Nonclinical safety tests, such as cytotoxicity, are required when new polymer materials are proposed for potential biomedical application (Fig. 6). Both empty and IB loaded microspheres were tested and compared to control group and also to cells treated with IB solution in different concentrations (100, 200, 400 and 600 μg/ml, where the drug loads of 10 wt. % corresponded to 300 μg/ml). The cell viability of the cells incubated with empty microspheres was very high for all microspheres, even at higher concentrations, when cell survival was above 70 %. Only diPERT/PCL unloaded microspheres indicated mild cytotoxicity at higher amounts of microspheres that could be attributed to the physical stress and space constrains caused by the microspheres presence, since the cells appeared with no changes in morphology at these higher concentrations (Supplementary material, Fig. S-1). From the histograms of ibuprofen loaded microspheres, lower cell survival was detected in comparison to blank microspheres due to the toxic effect of drug released from polymer matrix. While the cell survival of all IB loaded microspheres, apart from diTMP/PCL10, upon treatment with 100 and 200 μg/ml, was above 55 % indicating mild cytotoxicity, for the higher amounts of loaded microspheres, higher cytotoxicity was observed.

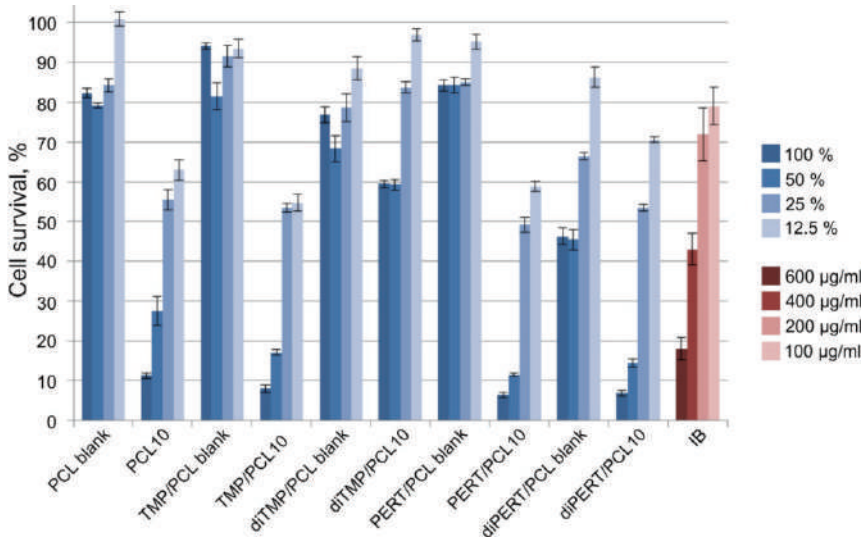


Fig. 6. Cytotoxic effects of blank and IB loaded PCL and star-shaped PCL microspheres (cell survival, %, after exposure at 100, 50, 25 and 12.5 vol. % (from dark blue to light) of microspheres sample extracts and 100 (light pink), 200 (dark pink), 400 μg/mL (light red) and 600 μg/mL (dark red) of IB).

Correlating these results with the drug release profiles, it could be concluded that the presence of burst effect and high concentration of drug released in the starting few hours was toxic for the applied cell system. Contrary, for the diTMP/ /PCL10 microspheres, the absence of cytotoxicity even for the highest concentrations was detected, which might be attributed to more sustained ibuprofen release in the starting few hours. The results of this study can be briefly supported by several studies which explain the high toxicity of ibuprofen rapidly delivered upon 48 h on cell viability.[19]

CONCLUSION

This study referred to the potential application of ibuprofen loaded star-shaped PCLs with variable number of arms (three, four and six) and all benefits which their use brought in comparison to linear PCL. The XRD results demonstrated that ibuprofen was encapsulated into an amorphous form in star-shaped PCLs, while one part of the drug remained partially crystalline after the encapsulation into linear PCL polymer. DSC analysis of the drug loaded star-shaped microspheres indicated negligible decrease in melting temperatures and increase of the melting enthalpies and the total degree of crystallinity, pointing that the encapsulated drug played nucleation-promotion role over the crystallization process. Drug encapsulation into a star-shaped PCL resulted in smaller microspheres diameter, when compared to linear PCL, which also affected drug release kinetics. The drug release profiles revealed that both polymer architecture and polymer molecular weight affected the overall degree of crystallinity of the polymer matrix, and all these factors had an impact on the IB release. The star-shaped PCLs exhibited no apparent cytotoxicity at lower concentrations. Summarizing all findings stated in this study, the star-shaped PCLs with well-defined and controlled architecture could be considered as promising biomaterial in ibuprofen delivery.

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ИЗВОД

РАЗГРАНАТИ ПОЛИ(ε-КАПРОЛАКТОНИ) СА ДОБРО ОДРЕЂЕНОМ АРХИТЕКТУРОМ КАО ПОТЕНЦИЈАЛНИ НОСАЧИ ЛЕКОВА

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У оквиру ове студије је приказана потенцијална примена разгранатих поли(ε-капролактона) са различитим бројем грана као новог полимерног матрикса за уношење

лекова у организам. Микросфере линеарног и разгранатих PCL са инкапсулираним ибупрофеном су припремљене применом технике отпаравања лако испарљивог растварача из емулзије "уље у води" (oil-in-water, o/w) и карактерисане помоћу FTIR, DSC, XRD и SEM анализе. За све микросфере су добијени висок принос, висока ефикасност инкапсулације и висок садржај лека. FTIR анализом је потврђено постојање интеракција између полимерног матрикса и лека, док је DSC анализа указала да је лек инкапсулиран у аморфном облику. SEM анализа је потврдила да су добијене микросфере разгранатих PCL правилног, сферичног облика, са пречником између 80 до 90 μm, док су знатно веће микросфере, 110 μm, припремљене од линеарног PCL. Предност употребе микросфера разгранатих PCL уместо линеарног PCL се види из профила отпуштања лека, који су показали већу количину отпуштеног лека из микросфера разгранатог полимерне матрице као последица њихове разгранате, флексибилне структуре. Микросфере припремљене од полимера са најразгранитијом структуром су показале највећу количину отпуштеног лека након 24 h. Тестови цитотоксичности, изведени коришћењем ћелија нормалних, хуманих фибробласта (MRC5), показали су одсуство цитотоксичности при нижим концентрацијама микросфера доказујући велики потенцијал разгранатих PCL система у поређењу са линеарним.

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