e-Polymers **2013**, no. 030

http://www.e-polymers.org
ISSN 1618-7229

Preparation of ciprofloxacin-encapsulated poly-ε-caprolactone microcapsules by the solvent evaporation technique

Marta Przybyslawska,[1*] *Aleksandra Amelian,*[1] *Katarzyna Winnicka*[1]

[1*]Department of Pharmaceutical Technology, Medical University of Bialystok, Mickiewicza 2C, 15-222 Bialystok, Poland; fax: 48 85 748 76 15; email: marta.przybyslawska@umb.edu.pl

(Received: 26 September, 2012; published: 11 July, 2013)

Abstract: The objective of this study was to prepare ciprofloxacin (CIP) encapsulated poly-ε-caprolactone (PCL) microcapsules by the single emulsion oil-in-water (o/w) solvent evaporation method. The obtained microcapsules were characterized for size, morphology, drug loading and entrapment efficiency. The physical state of microcapsules was determined by differential scanning calorimetry (DSC) and thermogravimetric analysis (TG). Storage stability, the *in vitro* drug release and mathematical modeling of drug release were also tested. It was found that obtained microcapsules had spherical shape and their size range was from 57.5 µm to 234.7 µm. The drug loading of microcapsules was from 1.72% to 11.02%. The optimal conditions of the encapsulation process include the drug/polymer ratio 2/1, using homogenizer for 5 min at 15000 rpm to disperse CIP in PCL solution and aqueous phase at pH 5.5. The results of CIP release study indicate that obtained microcapsules might be successfully used for designing sustained release dosage forms.

Introduction

Microencapsulation is a process of enclosing micron sized particles in a polymeric shell, which is widely used in pharmaceutical industry. Microcapsules are solid spherical particles of 1-1000 µm diameters and consist of two parts, core material and coat [1, 2]. Different types of natural products or synthetic compounds are used for the preparation of microcapsules. The selection of appropriate coating materials decides on the physical and chemical properties of the obtained microcapsules. Polymers used in the microencapsulation should be capable of forming a film which is cohesive with the core material, chemically compatible and non-reactive with the microencapsulated drug. Polymers should also provide the desired coating properties such as strength, flexibility and stability [3, 4].

Choice of the process of microencapsulation depends on many factors, including the properties of the drug, polymer and end use of the product. One of the main microencapsulation processes is emulsion solvent evaporation technique, which can be divided into two methods, single (oil-in-water – o/w) and double emulsion (water-in-oil-in-water – w/o/w) [3–5].

In the solvent evaporation process the biodegradable polymers: polylactic acid (PLA), poly(lactic-co-glycolic acid) (PLGA) and poly-ε-caprolactone (PCL) are mainly used. PCL is suitable for pharmaceuticals due to its high permeability to many small drug molecules, in-vitro stability and non-toxicity. The advantages of PCL include also slow degradation, which enables its application in prolonged drug delivery dosage forms [6–9]. So far many drugs, such as antihypertensive drugs, antibiotics, peptides and even live cells have been encapsulated in PCL for targeted drug delivery or for controlled drug release [7, 10]. As PCL is biodegradable and biocompatible, it might be widely used in tissue engineering, drug delivery in odontology or dentistry and in the production of implants and micro- or nanocapsules for ocular drug delivery [11–15].

Ciprofloxacin (CIP) is a synthetic, antibacterial agent of the fluoroquinolone class which inhibits bacterial DNA gyrase. It has broad spectrum against both G(-) and G(+) pathogens, including *Pseudomonas aeruginosa, Staphylococcus* and *Escherichia coli.* CIP is often used in the treatment of a wide variety of infections [16, 17], but it is currently available in only non-modified release tablets, parenteral infusions, suspensions, ophthalmic solutions and ointments [18]. Microencapsulation of CIP in biodegradable polymers might be a promising strategy to improve its oral bioavailability and to reduce drug toxicity and side effects [19]. Therefore, the objective of this study was to prepare CIP loaded PCL microcapsules by the single emulsion oil-in-water (o/w) solvent evaporation technique. The effect of the different parameters, like: drug/polymer ratio, method of dispersion of the drug in polymer solution (mechanical stirrer or homogenizer) and pH of aqueous phase (5.5 or 12.0) on microcapsules formation was evaluated. The obtained microcapsules were characterized for size, morphology, drug loading and entrapment efficiency. The physical state of microcapsules was determined by differential scanning calorimetry (DSC) and thermogravimetric analysis (TG). Storage stability for one month, the *in vitro* drug release and mathematical modeling of drug release were also tested.

Results and discussion

The solvent evaporation technique is a common method used to obtain microcapsules and might be used to encapsulation both hydrophilic and hydrophobic drugs. However, there are various process parameters that could affect the drug entrapment in microcapsules and their properties [5]. The main disadvantage of this method includes penetration of water-soluble substances to the aqueous phase, the consequence is the cause of low drug loading in microcapsules. As CIP hydrochloride is soluble in aqueous solvents at low pH values, and only slightly soluble at alkaline pH [20, 21], therefore to reduce the diffusion of CIP into the aqueous phase, pH of the PVA solution was increased from the 5.5 (initial pH of the solution) to 12.0.

The obtained microcapsules were observed using an optical microscope and SEM. All formulations of microcapsules had spherical shape, smooth surface and did not aggregate (Fig. 1, 2).

The mean diameter of obtained microcapsules was below 200 µm (Table 1), and their size range was from 57.5 µm to 234.7 µm. When pH of the aqueous phase was changed from 5.5 to 12.0, the particle size of the final microcapsules did not change significantly. Interestingly, it was found that the increase of pH to 12.0, did not result in higher percent loading of CIP, which might be caused by relatively long time (up to 3 h) evaporation of the DCM and in the consequence the diffusion of CIP into aqueous phase.

Another important process parameter is drug/polymer ratio and the method of dispersion of CIP in PCL solution. The drug/polymer ratio is a key factor influencing the characteristics of microcapsules. The minimum drug loading was in formulation D2, when drug/polymer ratio was 1/8 and maximum drug loading – in formulation G5, when drug/polymer ratio was 2/1. Further increase in drug/polymer ratio did not result in higher drug loading. The encapsulation efficiency ranges from 1.87% to even 43.24% (Table 1). The increase in the drug/polymer ratio resulted in an improvement of CIP percent loading, but entrapment efficiency was significantly decreased. These observations could be explained by the insufficient amount of polymer to cover CIP particles completely. The lower drug/polymer ratio might result in a more rapid solidification of the polymer layer and in the consequence inhibit the diffusion of the drug into the aqueous phase [22, 23].

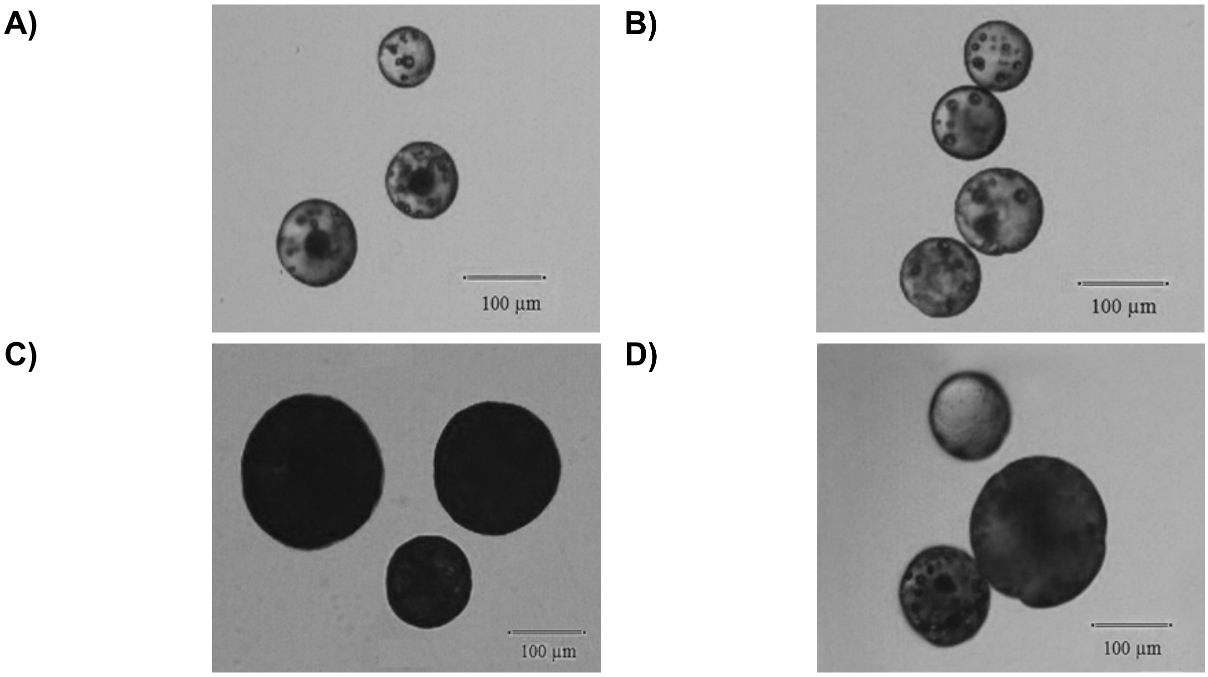

Fig. 1. Images of microcapsules formulation D5 (A), E5 (B), F5 (C) and G5 (D) under magnification 10 ×.

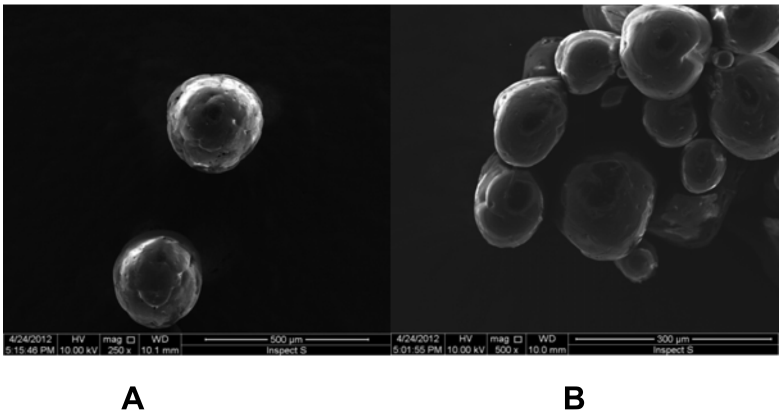

Fig. 2. SEM images of microcapsules formulation G5 under magnification 250 × (A) and 500 × (B).

The production of microcapsules can also be affected by intensity of agitation during dispersion of the drug in the polymer solution. It was noted, that the mean diameter of microcapsules decreased as the agitation intensity increased. Microcapsules of formulation G1, where magnetic stirrer was used to disperse CIP in PCL solution have mean diameter 195.2 µm, but formulation G5, when homogenizer at 15 000 rpm was used – 157.7 µm. Accurate dispersion of CIP in polymer solution results in the formation of smaller microcapsules and in the increase of percentage loading (from 1.72% in formulation F2 to 11.02% in formulation G5).

Tab. 1. Characteristics of CIP loaded PCL microcapsules.

Formulation	The way of dispersion of drug in polymer solution	pH of aqueous phase	Production yield (%)	Encapsulation efficiency (%)	Percent loading (%)	Mean diameter (µm)
			Drug/polymer ratio 1/8			
D1	MS	5.5	76.42	19.10	2.12	142.3
D2	MS	12.0	53.42	15.50	1.72	145.3
D3	H8	5.5	77.37	34.05	3.78	138.9
D4	H8	12.0	52.45	30.10	3.34	140.1
D5	H15	5.5	66.33	43.24	4.80	121.8
D6	H15	12.0	52.97	30.10	3.34	119.3
			Drug/polymer ratio 1/4			
E1	MS	5.5	60.00	26.45	5.29	153.1
E2	MS	12.0	52.68	19.50	3.90	149.3
E3	H8	5.5	60.10	30.00	5.77	158.9
E4	H8	12.0	37.70	9.25	1.85	158.4
E5	H15	5.5	61.80	28.85	6.00	139.3
E6	H15	12.0	51.01	13.85	2.77	150.3
			Drug/polymer ratio 1/1			
F1	MS	5.5	45.55	10.00	5.45	161.2
F2	MS	12.0	31.46	4.76	2.38	154.4
F3	H8	5.5	33.37	12.90	6.45	162.3
F4	H8	12.0	26.70	5.66	2.83	157.5
F5	H15	5.5	33.72	13.80	6.90	151.8
F6	H15	12.0	28.34	6.24	3.12	133.9
			Drug/polymer ratio 2/1			
G1	MS	5.5	30.42	7.5	5.50	195.2
G2	MS	12.0	23.67	1.87	2.35	181.7
G3	H8	5.5	20.74	11.02	7.35	183.6
G4	H8	12.0	15.42	4.96	3.31	173.8
G5	H15	5.5	26.05	26.53	11.02	157.7
G6	H15	12.0	19.67	3.78	2.52	151.5

MS—Magnetic stirrer 200 rpm 15 min.
H8—Homogenizer 8000 rpm 5 min.
H15—Homogenizer 15000 rpm 5 min.

Consequently, the optimal conditions of the process include the drug/polymer ratio 2/1, using homogenizer for 5 min at 15000 rpm to disperse CIP in PCL solution and aqueous phase at pH 5.5 (formulation G5). Formulation G5 with the highest drug loading was chosen for further investigations. The results of the sieve analysis of formulation G5 are shown in Figure 3 and the sieve fraction of 100-200 µm (G5 B) was found to be the major one.

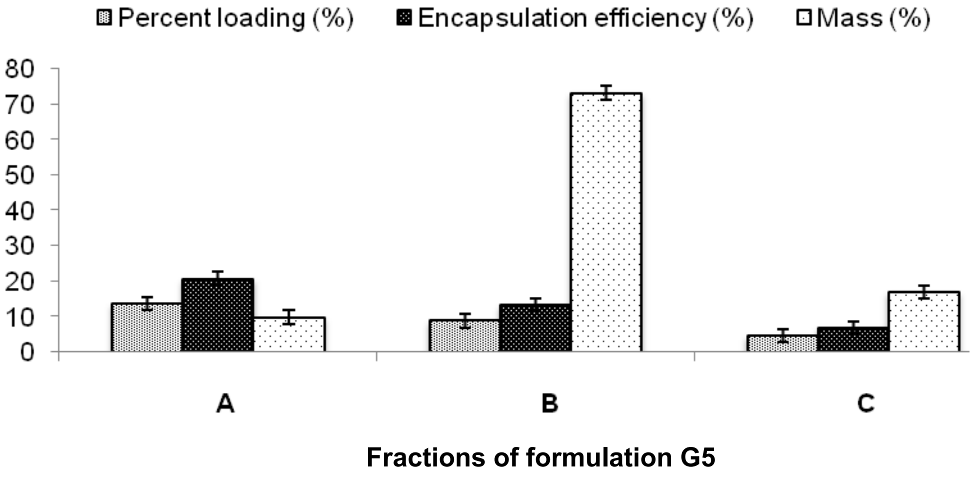

Fig. 3. Characteristics of different fraction of G5 formulation: A (microcapsules above 200 µm), B (between 100 and 200 µm) and C (below 100 µm).

A stability study of formulation G5 has shown that no significant changes in percent of drug loading and encapsulation efficiency were observed (Table 2).

Tab. 2. Storage stability study of CIP loaded PCL microcapsules directly after formulation (T_0 25°C) and after one month storage (T_{30} 25 ± 1°C and T_{30} 37 ± 1°C).

Fraction of formulation G5	Percent loading (%)	Encapsulation efficiency (%)
	T_0 (25 ± 1°C)	
A	13.76	20.63
B	8.87	13.30
C	4.60	6.30
	T_{30} (25 ± 1°C)	
A	13.11	19.66
B	8.54	12.8
C	4.30	6.45
	T_{30} (37 ± 1°C)	
A	12.66	19.00
B	8.32	12.47
C	4.23	6.35

The physical state of CIP, PCL and microcapsules of formulation G5 was determined by differential scanning calorimetry and thermogravimetric studies. DSC thermograms of analyzed samples are shown in Figure 4. The PCL broad endodermic peak is at 62.56°C, which corresponds to the melting point of semi-crystalline PCL. CIP exhibited a sharp endothermic peak at 163.45°C, corresponding to the melting point of pure CIP. However, in the thermogram of CIP loaded PCL microcapsules, DSC did not detect the melting peak for CIP and this fact might suggest that the drug was dispersed in the microcapsules in an amorphous form [24]. TG curve of CIP loaded PCL microcapsules is shown in Figure 5. A one step thermal decomposition between 372.80° and 449.67°C represents 98.3% of the mass loss and indicates that obtained microcapsules exhibit thermal stability and that PCL as a shell material provides a good protection for CIP [25].

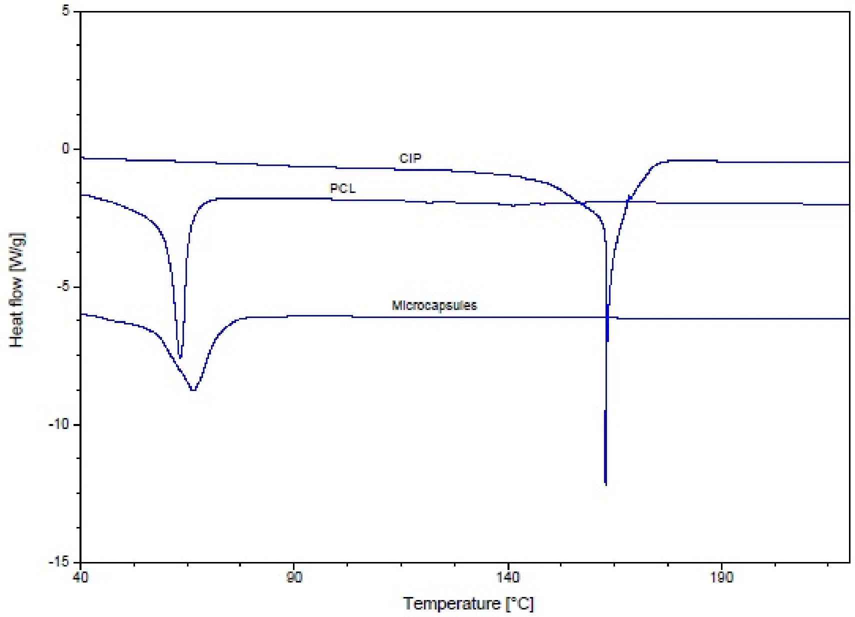

Fig. 4. DSC thermograms of CIP, PCL and CIP loaded PCL microcapsules.

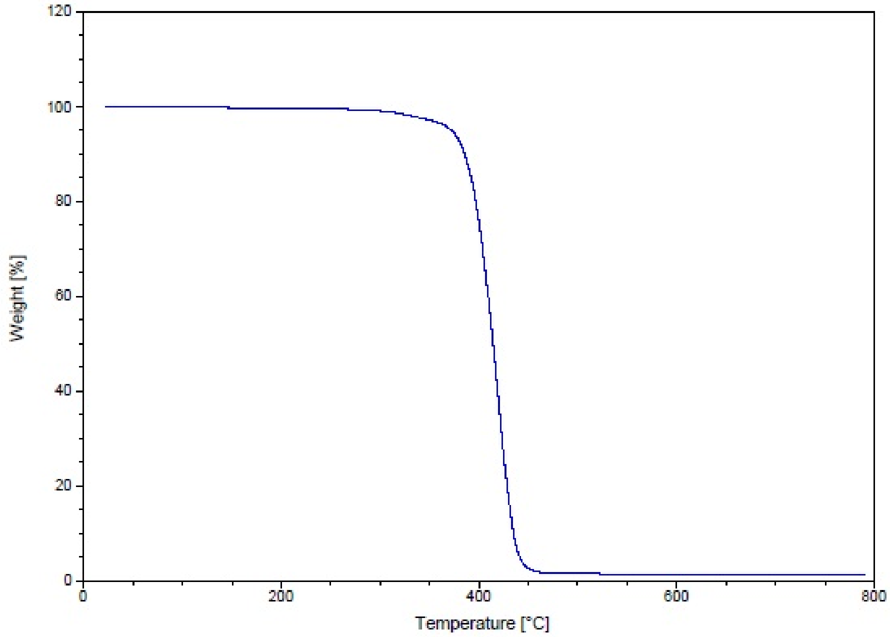

Fig. 5. TG curve of CIP loaded PCL microcapsules.

Figure 6 shows the release profiles of CIP from formulation G5 in 0.1 M HCl at pH 1.2 and phosphate buffer at pH 6.8. In acidic environment CIP release was significantly lower. After 4 h of the study in pH 6.8, the burst release of CIP was observed and CIP was continuously released over 72 hours.

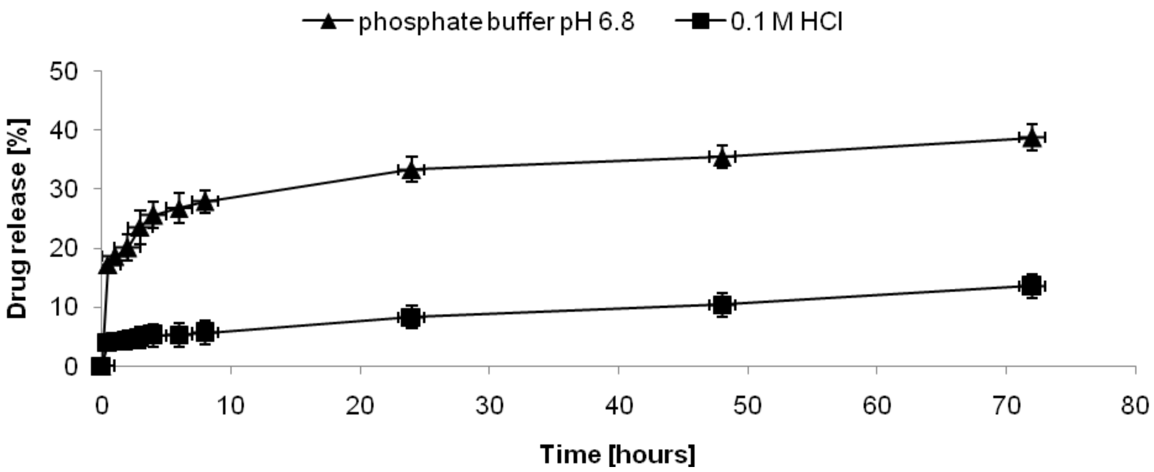

Fig. 6. Release profiles of CIP from formulation G5 in phosphate buffer pH 6.8 and in 0.1 M HCl at pH 1.2.

In vitro release of CIP from various fractions of formulation G5 in phosphate buffer pH 6.8 is shown in Figure 7.

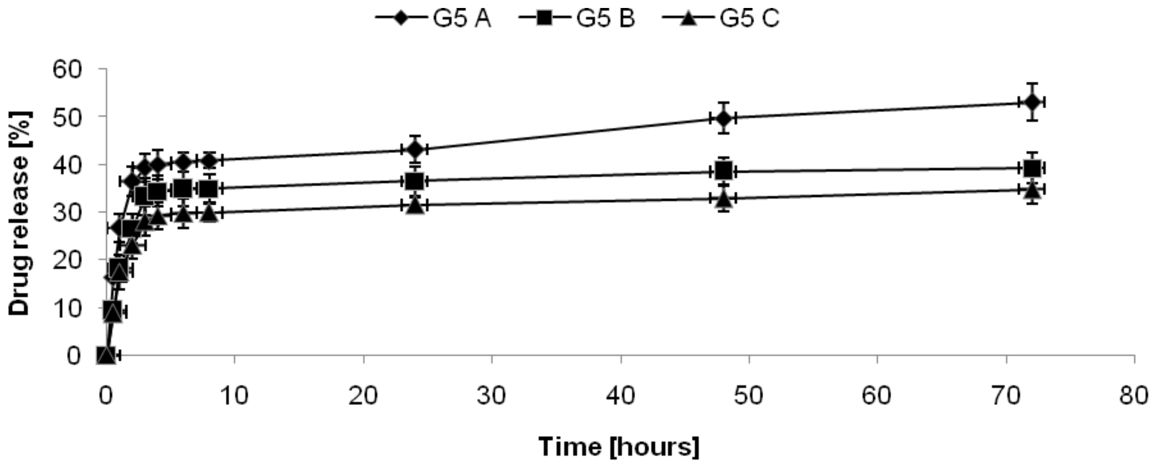

Fig. 7. Release profiles of CIP from different fractions of formulation G5: A (microcapsules above 200 μm), B (between 100 and 200 μm) and C (below 100 μm) in phosphate buffer pH 6.8.

After 24 h of the study the percentage of drug released from the fractions A, B and C were found to be 43.19%, 36.53%, 31.54% and after 72 h – 53.10%, 39.31% and 34.85%, respectively. It was observed that particle size had significant effect on the *in vitro* release behaviour of CIP from microcapsules – the drug release from larger microcapsules was definitely higher probably due to their larger surface area.

CIP release from microcapsules is a result of diffusion of the drug rather than by degradation, which is very slow in an aqueous medium [26]. According to Korsmeyer-

Peppas model, *n* is the release exponent and provides information about mechanism of the drug release from polymeric of different geometry. When *n* takes a value of 0.43 for sphere shape, the drug diffuses through polymer following Fickian diffusion mechanism. The obtained values of *n* for all examined formulations were found to be less than 0.43 (the range from 0.039 to 0.067, Table 3).

Tab. 3. Release models of microcapsules formulation G5 and its different fractions.

Formulation	Dissolution medium (pH)	Zero order kinetics		First order kinetics		Highuchi model		Korsmeyer-Peppas model		
		R^2	K_0	R^2	K_I	R^2	K_H	R^2	K_{KP}	*n*
G5	1.2	0.987	0.128	0.989	0.009	0.981	1.182	0.995	27.353	0.067
	6.8	0.780	0.264	0.814	0.002	0.915	2.638	0.843	5.272	0.041
G5 A	6.8	0.532	0.316	0.631	0.004	0.668	3.255	0.505	3.622	0.039
G5 B	6.8	0.315	0.223	0.358	0.002	0.471	2.516	0.356	4.864	0.044
G5 C	6.8	0.361	0.197	0.389	0.002	0.517	2.173	0.382	5.483	0.042

This fact shows that CIP release is a combination of diffusion processes: through matrix and through water-filled pores [27]. Analysis of data obtained from the *in vitro* CIP release according to different kinetics and models is given in Table III. It was found that in all formulations the first order plot in all formulations characterized the higher values of correlation coefficient (R^2) and higher linearity than plot of zero order kinetics. It describes the drug release from the systems in which the release rate is concentration dependent. In Highuchi model straight line obtained from the plot indicates that the drug release is based on a diffusion process. The best linearity was found in Highuchi plot and in Korsmeyer-Peppas model at pH 6.8. This result indicates that CIP release from obtained PCL microcapsules is based on Fickian diffusion [28-30]. The *in vivo* conditions, however, differ greatly between the *in vitro* in terms of both pH and the presence of enzymes. PCL degradation after oral administration is a bulk and autocatalyzed process, which can be divided into two stages. The first step includes nonenzymatic hydrolytic cleavage of ester linkages and it is autocatalyzed by the carboxylic acid end groups of the polymer. In the second phase, molecular weight of the polymer is decreased and this process depends mainly on the particle size [6]. Enzymes, i.e. esterases (produced by the liver) and lipases accelerate hydrolysis of the ester linkages. This process is the main way of polycaprolactone degradation *in vivo* and leads to the increase of the drug release from microcapsules. To summarize, the obtained results suggest that PCL is a suitable polymer for developing microcapsules for prolonged delivery of CIP. In view of the biodegradable nature of PCL, these microcapsules might be used as carriers for oral drug delivery of CIP, but further study is needed.

Experimental part

Materials

Ciprofloxacin hydrochloride was a gift from Polfa (Warszawa, Poland). Poly-ε-caprolactone (PCL) (MW 45 000), polyvinyl alcohol (Mowiol 40-88, MW 205 000) and polysorbate 80 was purchased from Sigma Aldrich (Steinheim, Germany). Potassium dihydrogen phosphate, sodium hydroxide, hydrochloric acid were obtained from Chempur (Piekary Śląskie, Poland). Dichloromethane was purchased from Witko

(Łódź, Poland). Water was distilled and passed through a reverse osmosis system Milli-Q Reagent Water System (Billerica, USA).

Preparation of microcapsules

PCL (0.8 g) was dissolved in 10 ml of dichloromethane (DCM) and then different amounts (0.1-1.6 g) of CIP were added and dispersed by magnetic stirrer (IKA RH basic 2, Breisgau, Germany) or homogenizer (Heidolph Silent Crusher M, Schwabach, Germany). This dispersion was poured into 250 ml of purified water containing 0.25% (w/v) polyvinyl alcohol (PVA) at pH 5.5 or 12.0. An o/w emulsion was prepared and DCM was removed with a mechanic stirrer (IKA RW20 Digital, Breisgau, Germany) at 500 rpm at room temperature for 3 h. After decantation, the microcapsules were collected by filtration through 0.45 μm filters, washed three times with purified water and air-dried for 24 h at room temperature [31].

Characterization of CIP encapsulated microcapsules

Morphology and particle size distribution

Measurements of the particle size, mean diameter and shape of microcapsules were performed using an optical microscope (Motic BA400, Wetzlar, Germany). The particle size for each formulation was calculated as the average value of the size of 50 dried microcapsules. The morphology of the microcapsules was additionally examinated by a scanning electron microscope (SEM) (Hitachi S4200, Tokyo, Japan).

Production yield

The percentage yield of CIP in the PCL microcapsules was determined by using the formula [32]:

$$Y = W_m / W_t \times 100 \qquad (1)$$

where Y – percentage production yield, W_m – weight of microcapsules and W_t – theoretical weight of drug and polymer.

Determination of CIP loading and encapsulation efficiency

The drug loading in the microcapsules was determined by dissolving an accurately weighted amount of microcapsules (20 mg) in DCM and then diluting the solution with 0.1 M HCl (20 ml). DCM was removed by agitating the solution by magnetic stirrer for 24 h at 150 rpm. Polymer was removed by using 0.45 μm filter. After filtration the absorbance of CIP at 277 nm was measured using a UV–VIS spectrophotometer (Hitachi U-1800, Tokyo, Japan). The measured absorbance was then converted to the amount of CIP by preparing standard calibration curve, which the correlation coefficient R^2 was 0.999. Each sample was analyzed in triplicate. The results are expressed as the amount of CIP in 100 mg of microcapsules.

The percentage of drug loading in microcapsules was calculated by using the following equation [33, 34]:

$$L = Q_m / W_m \times 100 \qquad (2)$$

where L – percentage of drug loading, Q_m – drug loaded in the microcapsules and W_m – weight of the microcapsules. The percentage of encapsulation efficiency was determined by using the following formula:

$$EE = Q_a / Q_t \times 100 \quad (3)$$

where EE – percentage of encapsulation efficiency, Q_a – actual drug content, Q_t – theoretical drug content.

Sieve analysis

Microcapsules of formulation G5 with the highest drug loading were separated by sieve analysis using standard sieves (Ø 100 and 200 µm) and then weight percent retained on different sieves, size range of microcapsules, drug loading and encapsulation efficiency were calculated.

Storage stability

The stability of encapsulated CIP in PCL microcapsules during one month storage at 25 ± 1°C and at 37 ± 1°C was evaluated. After this time morphology, particle size, drug loading and encapsulation efficiency was determined.

Thermal analysis

Differential scanning calorimetric (DSC) analysis of CIP, PCL and formulation G5 of microcapsules was performed using a DSC Q2000 (TA Instruments, New Castle, Delaware, USA). Each sample was weighted (5 mg) and heated in sealed aluminium pans from 25 °C to 300 °C at scanning rate of 10°C/min under nitrogen flow of 20 ml/min.

Thermogravimetric studies (TG) of CIP loaded PCL microcapsules was performed using a TGA Q50 (TA Instruments, New Castle, Delaware, USA). The sample was placed in an aluminium pan and heated from 25°C to 800°C under a nitrogen flow of 20 ml/min.

In vitro drug release

CIP release profiles from formulation G5 and different fractions of this formulation were obtained according to the modified USP method using dissolution paddle apparatus (Erweka, Munich, Germany) [35]. Microcapsules were suspended in 500 ml of the release medium and then stirred at 75 rpm at 37 ± 1^{o}C for 72 h. Samples (5 ml) were withdrawn and filtered through 0.2 µm CA Millipore filters (Billerica, USA) at predetermined time intervals and replaced with fresh dissolution medium. Samples were analyzed spectrophotometrically and concentration value was determined from standard calibration curve, which the correlation coefficient (R^2) was 0.999. The studies were carried out in triplicate. CIP release was conducted from formulation G5 in 0.1 M HCl at pH 1.2 (to imitate the stomach pH) or in the phosphate buffer at pH 6.8 (as an intestinal pH) containing 1% w/v polysorbate 80 to maintain the *sink* condition.

Mathematical modeling of drug release profile

The amount of released CIP from the microcapsules were fitted to zero order kinetics, first order kinetics, Higuchi model and Korsmeyer – Peppas model to characterized mechanism of the drug release. Zero order kinetic describes the system, in which the drug release rate is independent of its concentration:

$$Q_t = Q_o + K_o t \quad (4)$$

where Q_t – amount of drug dissolved in time t, Q_o – initial amount of drug in the solution, and K_o – the zero order release constant.

First order kinetic describes the drug release from the systems in which the release rate is concentration dependent:

$$\log Q_t = \log Q_o + kt/ 2.303 \quad (5)$$

where Q_t – the amount of drug released in time t, Q_o – the initial amount of drug in the solution and kt – the first order release constant.

Higuchi model describes the mechanism of the drug release from modified pharmaceutical form:

$$Q = k_H t^{1/2} \quad (6)$$

where Q – cumulative amounts of drug release at time t and k_H – the Higuchi dissolution constant reflection formulation characteristics.

Korsmeyer-Peppas model describes the drug release from the polymeric system which release deviates from Fickian diffusion, as expressed by the following equation:

$$M_t / M_\infty = kt^n \quad (7)$$

where M_t and M_∞ – amounts of drug release at time t and infinite time, k is the constant incorporating structural and geometrical characteristics and n – diffusional release exponent used to interpretation of diffusional release mechanism [28-30].

Statistical analysis

Quantity variables were expressed as the mean and standard deviation. Statistical analysis was performed using nonparametric Kruskal-Wallis test and conducted by using STATISTICA 10 software. Differences between groups were considered to be significantly at $p < 0.05$.

References

[1] Jyothi, N. V.; Prasanna, P. M.; Sakarkar, S. N.; Prabha, K. S.; Ramaiah, P. S.; Srawan, G. Y. J. *Microencapsulation* **2010**, 27, 187.
[2] Ghosh, S. K. *In Functional coatings by polymer microencapsulation*, Ghosh, S. K. (Ed.); Wiley: New York, **2006**; pp. 12.
[3] Venkatesan, P.; Manavalan, R.; Valliappan, K. *J. Pharm. Sci. Res.* **2009**, 1, 26.
[4] Bansode, S. S.; Banarjee, S. K.; Gaikwad, D. D.; Jadhav, S. L.; Thorat, R. M. *Int. J. Pharm. Sci. Rev. Res.* **2010**, 1, 38.
[5] Yeo, Y.; Baek, N. *Biotechnol. Bioprocess Eng.* **2001**, 6, 213.
[6] Sinha, V. R.; Bansal, K.; Kaushik, R.; Kumria, R.; Trehan, A. *Int. J. Pharm.* **2004**, 278, 1.
[7] Pillai, O.; Panchagnula, R. *Curr. Opin. Chem. Biol.* **2001**, 5, 447.
[8] Jones, M. N.; Song, Y. H.; Kaszuba, M.; Reboira, M. D. *J. Drug Targeting* **1997**, 5, 25.
[9] DiTtizio, V.; Karlgard, C.; Lilge, L.; Khoury, A. E.; Mittelman, M. W.; DiCosmo, F. *J. Biomed. Mater. Res.* **2000**, 51, 96.

[10] Takei, T.; Yoshida, M.; Hatate, Y.; Shiomori, K.; Kiyoyama, S. *J. Biosci. Bioeng.* **2008**, 106, 268.
[11] Gunatillake, P. A.; Adhikari, R. *Eur. Cells Mater.* **2003**, 5, 1.
[12] Sun, H.; Mei, L.; Song, C.; Cui, X.; Wang, P. *Biomaterials* **2006**, 27, 1735.
[13] Yeo, A.; Cheok, C.; Teoh, S. H.; Zhang, Z. Y.; Buser, D.; Bosshardt, D. D. *Clinical Oral Implants Research* **2011**, 00, 1.
[14] Kedzierewicz, F.; Thouvenot, P.; Monot, I.; Hoffman, M.; Maincent, P. *J. Biomed. Mater. Res.* **1998**, 3, 588.
[15] Calvo, P.; Vila-Jato, J. L.; Alonso, M. J. *J. Pharm. Sci.* **1996**, 85, 530.
[16] Torniainen, K.; Tammilehto, S.; Ulvi, V. *Int. J. Pharm.* **1996**, 132, 53.
[17] Olivera, M. E.; Manzo, R. H.; Junginger, H. E.; Midha, K. K.; Shah, V. P.; Stavchansky, S.; Dressman, J. B.; Barends, D. M. *J. Pharm. Sci.* **2011**, 100, 22.
[18] Drug Bank Open Data Drug & Drug Target Database, http://www.drugbank.ca/drugs/DB00537. Accessed June 15, **2012**.
[19] Sachan, K. N.; Singh, B.; Rao, K. R. *Malays. J. Pharm. Sci.* **2006**, 4, 65.
[20] Stahlmann, R.; Lode, H. *In The Quinolones; Andriole, V. T.* (Ed.), Academic Press: San Diego, **2000**; pp. 397.
[21] *The European Directorate for the Quality of Medicines & HealthCare*, European Pharmacopeia 6th edition, **2007**; pp. 1550.
[22] Kissel, T.; Maretschek, S.; Packhauser, C.; Schnieders, J.; Seidel, N. *In Microencapsulation. Methods and industrial applications;* S. Benita (Ed.), CRC Press Taylor&Francis Group: New York, **2006**; pp. 99.
[23] Yang, Y.-Y.; Chung, T.-S.; Bai, X.-L.; Chan, W.-K. *Chem. Eng. Sci.* **2000**, 55, 2223.
[24] Pokharkar, V. B.; Mandpe, L. P.; Padamwar, M. N.; Ambike, A. A.; Mahadik, K. R.; Paradkar, A. *Powder Technol.* **2006**, 167, 20.
[25] Yuan, L.; Liang, G. Z.; Xie, J. Q.; Li, L.; Guo, J. *J. Mater. Sci.* **2007**, 42, 4390.
[26] Ha, J.-H.; Kim, S.-H.; Han, S.-Y.; Sung, Y.-K.; Lee, Y.-M.; Kang, I.-K.; Cho, Ch.-S. *J. Controlled Release* **1997**, 49, 253.
[27] Tarvainen, T.; Karjalainen, T.; Malin, M.; Peräkorpi, K.; Tuominen, J.; Seppälä, J.; Järvinen, K. *Eur. J. Pharm. Sci.* **2002**, 16, 323.
[28] Schliecker, G.; Schmidt, C.; Fuchs, S.; Ehinger, A.; Sandow, J.; Kissel, T. *J Controlled Release* **2004**, 94, 25.
[29] Costa, P.; Sousa Lobo, J. M. *Eur. J. Pharm. Sci.* **2001**, 13, 123.
[30] Pradhan, R.; Budhathoki, U.; Thapa, P. *Kathmandu University Journal of Science, Engineering and Technology* **2008**, 1, 55.
[31] Hombreiro Perez, M.; Zinutti, C.; Lamprecht, A. *J. Controlled Release* **2000**, 65, 429.
[32] El-Kamel, A.; Al-Shora, D. H.; El-Sayed, Y. M. *J. Microencapsulation* **2006**, 23, 389.
[33] Jeong, Y. I.; Na, H. S.; Seo, D. H.; Kim, D. G.; Lee, H. C.; Jang, M. K.; Na, S. K.; Roh, S. H.; Kim, S. I.; Nah, J. W. *Int. J. Pharm.* **2008**, 352, 317.
[34] Ranjha, N. M.; Khan, K. I.; Naseem, S. J. *Sol-Gel Sci. Technol.* **2009**, 50, 281.
[35] *The United States Pharmacopeia 35 National Formulatory 30*. The United States Pharmacopeial Convention: Rockville, **2012**; Vol. 1, pp. 264-271.