Acta Poloniae Pharmaceutica – Drug Research, Vol. 70 No. 2 pp. 271–281, 2013 ISSN 0001-6837
Polish Pharmaceutical Society

PHARMACEUTICAL TECHNOLOGY

THE INFLUENCE OF PROCESS VARIABLES OF PREPARATION OF OXYCELLULOSE BEADS ON THEIR DISSOLUTION PROFILE

MARTINA BAJEROVÁ*, JAN MUSELÍK, JAN GAJDZIOK, RUTA MASTEIKOVÁ and KATEŘINA DVOŘÁČKOVÁ

Department of Pharmaceutics, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences Brno, Palackého 1/3, 612 42 Brno, Czech Republic

Abstract: Particles preparation from biodegradable polymers as carriers for the controlled release of drugs has been the focus of many investigations and the subject of a growing field of research in recent years. The aim of this study was to develop and optimize the preparation of oxycellulose beads containing diclofenac sodium as a model drug. Particle size, surface, drug content and encapsulation efficiency were evaluated, drug dissolution profiles were measured and drug release mechanism estimated. The prepared oxycellulose beads were uniform in size with encapsulation efficiency ranging from 53.2 to 74.9%. The lower temperature of the crosslinking solution and its saturation with diclofenac sodium increased the encapsulation efficiency, especially when both parameters were combined. The application of ultrasound had a negative effect on drug encapsulation. The dissolution of diclofenac sodium in pH 1.2 was close to zero as its solubility in this medium is very limited. The drug release in pH 6.8 lasted from 10 to 16 h showing biphasic behavior with a significant lag time. $T_{1/2}$ decreased with increasing encapsulation efficiency and ultrasound application. Diclofenac sodium was released from the prepared oxycellulose particles by diffusion as well as by erosion process; a high correlation was found with zero order kinetics.

Keywords: oxycellulose beads, external gelation, encapsulation efficiency, dissolution profile, lag time

Abbreviatons: DS - diclofenac sodium, EE - encapsulation efficiency, LOQ - limit of quantitation, NaOC - sodium salt of oxycellulose, OC - oxycellulose, PTFE - polytetrafluoroethylene, SEM - scanning electron microscopy

A successful incorporation of a drug into its final dosage form plays an important role in the pharmaceutical research. Innovations in dosage forms and dose delivery systems offer substantial clinical advantages, including reduced dosing frequency and improved patient adherence; minimized fluctuation of drug concentrations and maintenance of blood levels within a desired range; localized drug delivery; and the potential for reduced adverse effects and increased safety. In the ideal case, the dosage form should release the drug at the desired rate and also in a specific body compartment. In the development of new dosage forms, there is a tendency to prefer biodegradable, biocompatible and bioresorbable polymers, which allow the controlled drug release while reducing the side effects (1). One of these perspective polymers is oxycellulose with its ability to degrade at physiological pH (2, 3). In comparison with other cellulose derivatives (4), oxycellulose has not yet been used to a wide extent. At present, it is used as hemostatic agent, biodegradable wound dressing with antibacterial activity, and for prevention of tissue adhesion after surgical operations. It has also been tested as a carrier of drugs, enzymes or proteins (5, 6).

Diclofenac sodium is a well known and widely commercially available synthetic non-steroidal anti-inflammatory drug (7). After oral application it quickly dissolves and reaches maximum concentration after 30 min. The solubility of diclofenac sodium also depends on pH values; in acidic solutions the solubility is lower than 2 μg/mL. Nevertheless, the solubility increases with pHs of above 6.8 (670 μg/mL) (8). This fact explains why *in vitro* dissolution tests have to be performed using buffered solutions with those pHs. Furthermore,

* Corresponding author: e-mail: martina.bajerova@seznam.cz

diclofenac sodium undergoes an intramolecular cyclization under the acidic conditions of gastric juice, which can cause its inactivation, so it is recommended to take it after meals (9). Low biological availability (60%) after oral administration (high percentage of protein binding and pre-systemic metabolism), short biological half-life, narrow therapeutic index, risk of accumulation after repeated application (10) make a suitable candidate for the controlled release dosage form. One possibility is its encapsulation into matrix particles by an external gelation method which is also known as the ionotropic gelation based on the impact of physical (electrostatic) forces and polyelectrolyte complexation with the presence of polyvalent ions. A typical example is extruding sodium alginate dispersion into calcium chloride solution, creating solid gel microparticles (11).

Dissolution studies represent one of the most important characteristics of dosage forms with controlled release of the drug. These studies inform about the amount of a drug dissolved within a particular period of time. Various kinetic models, such as: zero order, first order, Higuchi, Korsmeyer-Peppas and Hixson-Crowell, can be utilized to determine how a drug is released from its dosage form (Tab. 1). Drug release is indicated by the correlation with individual theoretical models as well as by a visual evaluation after the dissolution test.

The aim of this work was to prepare and evaluate oxycellulose-based matrix beads with diclofenac sodium. Particles with controlled drug release were prepared under various processing conditions, and their influence on the dissolution model of a drug was evaluated *in vitro*.

EXPERIMENTAL

Materials

The model drug designed for encapsulation was diclofenac sodium (Amoli Organics, India). The polymer carrier was sodium salt of oxycellulose (Synthesia a.s., Czech Republic). Calcium chloride (Kirsch Pharma, Salzgitter, Germany) was used as the crosslinking agent. The following materials were used in the dissolution studies: artificial gastric juice pH 1.2 containing hydrochloric acid 35% (Merck KGaA, Germany) with sodium chloride (Merck KGaA, Germany) and phosphate buffer pH 6.8 to simulate the environment of the upper part of the small intestine along with hydrogen phosphate dodecahydrate (Merck KGaA, Germany), and potassium dihydrogen phosphate (Merck KGaA, Germany) in purified water. All materials were of

Table 1. Equations for the selected kinetic models.

Zero order kinetic	$M_t/M_\infty = K_0 t$
First order kinetic	$M_t/M_\infty = 1 - e^{-K_1 t}$
Higuchi model	$M_t/M_\infty = K_H \sqrt{t}$
Korsmeyer-Peppas model	$M_t/M_\infty = K_{KP}\, t^n$
Hixson-Crowell model	$(M_\infty)^{1/3} - (M_t)^{1/3} = K_{HC}\, t$

M_t is amount of drug released in time t; M_∞ is the absolute cumulative amount of drug released at infinitive time; K_0, K_1, K_H, K_{HC}, K_{KP} are the zero order, first order, Higuchi, Hixson-Crowell, Korsmeyer-Peppas release constants (12, 13). Release exponent n characterizes the mechanism of drug release (14).

Table 2. Conditions for the beads preparation.

Sample	Crosslinking solution temperature	DS in crosslinking solution	Ultrasound application
BD1	room	no	no
BD2	4°C	no	no
BD3	4°C	no	yes
BD4	4°C	yes	no
BD5	4°C	yes	yes

Ph. Eur. quality, except OC which corresponded to USP XXVI monograph.

Preparation of particles

Particles were prepared by an ionotropic gelation of oxycellulose sodium dispersions (NaOC) containing drug. Dispersions were extruded through a needle with an internal diameter of 0.4 mm at a dropping rate of 0.175 mL/min into 120 mL of crosslinking medium, which was either 1 M $CaCl_2$ with pH value ~ 4 or 1 M $CaCl_2$ saturated by diclofenac sodium. The distance between the edge of the needle and the surface of the medium was adjusted to 0.5 cm. The crosslinking bath was rotated at 4 rpm to prevent aggregation of particle droplets. NaOC dispersions were prepared by dispersing 12 g of NaOC in purified water. Dispersions were heated at 60°C while mixed using a magnetic stirrer Ikamag (RT5 Power, IKA-Werke, Staufen, Germany) at 600 rpm for 10 min. Subsequently, dispersions were homogenized using an Ultra-Turrax (T25 basic, IKA-Werke, Germany) at 13.000 rpm for 5 min. Then, 30 g of fine powdered diclofenac sodium suspension (particles size 5.2 μm) were added and stirred, the volume of dispersion was then adjusted to 100 mL with purified water (drug content 2 g in 100 g of dispersion). Five samples of OC beads were prepared under different conditions. The process variables were: composition of crosslinking solution, its temperature and application of ultrasound during the crosslinking process. The sample BD1 was prepared at room temperature by extruding NaOC dispersion into 1 M $CaCl_2$. The sample BD2 was prepared by extruding NaOC dispersion into cross-linking solution cooled to 4°C. Particles were carried out at the same temperature to accelerate solid structure of formed gel beads (15). The sample BD3 differed from the sample BD2 by use of ultrasound during crosslinking (16). The sample BD4 was prepared by extruding NaOC dispersion into cross-linking solution 1 M $CaCl_2$ cooled to 4°C and saturated by diclofenac sodium. The sample BD5 was treated under the same conditions as sample BD4 and using ultrasound during crosslinking (for preparation conditions see Table 2). Beads (BD1–BD5) were formed immediately and were left in the cross-linking solution for 1 h to harden while gently mixed. Then, they were washed three times with purified water and dried at 30°C in a cabinet drier for 24 h before testing. The preparation of every sample was repeated three times to achieve reproducibility and individual batches were marked by letters a, b and c after the sample number.

Particle size analysis

Diclofenac sodium-loaded oxycellulose beads were analyzed for their size using a stereoscopic microscope (STM 902 Opting, Czech Republic) equipped with a CCD camera (Alphaphot, Nikon, Japan). Particles were visualized under 7× magnifications. Acquired images of 200 randomly chosen beads were stored and subsequently processed using the Ia32 software (Leco Corporation, USA). Equivalent diameter was calculated from the measured values according to equation 1, and expressed as arithmetic mean ± standard deviation.

$$equivalent\ diameter = \sqrt{\frac{4 \times area}{\pi}}\ [\text{mm}] \qquad (1)$$

Scanning electron microscopy

The morphology and surface topography of beads were examined by scanning electron microscopy. The samples were mounted directly onto the SEM sample holder using a double-side sticking tape and were gold spray-coated in a sputter-coater (SCD 005, BalTec Inc., Switzerland) and images were taken using the scanning electron microscope Hitachi S-4300 (Hitachi Scientific Instruments, Japan) at an accelerating voltages of 8.0 kV.

Determining of drug loading and encapsulation efficiency

The drug content was determined by dissolving appropriate amount of particles (~70 mg) in purified water under sonication using an ultrasonic bath Sonorex (RK 52H, Bandelin, Germany) for at least 1 h. Once the particles were completely disintegrated, the samples were filtered and analyzed spectrophotometrically at 276 nm (Lambda 25, Perkin Elmer, USA) for drug content. Drug-loading efficiency was determined from obtained values by using the following equation (Pasparakis and Bouropolos, 2006):

$$Encapsulation\ efficiency = \frac{c_s}{c_t} \times 100\ [\%] \qquad (2)$$

where c_s corresponds to the actual DS content and c_t corresponds to the theoretical drug load. The assay was carried out in triplicate.

***In vitro* release studies**

The *in vitro* release studies of drug-loaded beads were carried out using 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 either artificial gastric juice without pepsin with

pH 1.2 or phosphate buffer of pH 6.8 both kept at 37.0 ± 0.5°C. Samples were analyzed using an UV spectrophotometer (Lambda 25, Perkin Elmer, USA) at 276 nm. The samples for dissolution test were weighted with respect to their encapsulation efficiency, so that 10 mg of diclofenac sodium were in each sample. Dissolution test was carried out with six samples of each batch and results were expressed as average values and standard deviations. The dissolution data were applied to selected kinetic models to find out the drug release kinetics. The lag time and mean dissolution time $t_{50\%}$, which express the time in which 50 % of drug is released, were calculated (17).

Evaluation of the dissolution profiles similarity

The similarity factor f_2 between individual batches of the same sample was calculated to evaluate the reproducibility of the method and to assess the impact of conditions on mean dissolution profiles and individual samples (18, 19). The dissolution profile of the sample BD1 was used as the reference. The similarity factor values f_2 were calculated as follows (20):

$$f_2 = 50 \times \log\{[1 + 1\ (1/n)\Sigma_{i=1}^{n}|R_i - T_i|^2]^{-0.5} \times 100\} \quad (3)$$

where R_i and T_i mean the amount of drug in the reference (R) and tested (T) samples (in percents) released within the given time interval, and n means number of samplings.

Due to the incomplete release of the drug from some particles samples, the maximum limit was considered 70% of the released drug.

Determination of calcium content in remnants shells

After dissolution test, the remnants shells of beads were removed from the vessels and analyzed for calcium content using atomic absorption spectrometer. Before testing, all samples were mineralized in the microwave system Speedwave MWS-3+ (Berghof, Germany) with the maximum microwave generator output 1.45 kW. Twenty (± 1) mg of samples were placed into the do PTFE dish to mineralize, and 5 mL 65% HNO_3 (p.a.) and 2 mL 30% H_2O_2 (p.a.) were added. The final transparent mineralizate was cooled, placed into a measuring flask (25 mL) and replenished to the defined volume by redistilled water. The atomic absorption spectrometer SensAA (GBC, Australia) with acetylene-air flame was then used for its determination. Measuring was carried out at 422.7 nm, spectral slit width 0.5 nm, and hol-

Table 3. Size of beads, drug content and encapsulation efficiency.

Batch	Eq. diameter [mm] [a]	DS content [b, c]	EE [%] [c]
BD1a	1.54 ± 0.13	7.41 ± 0.59	51.9 ± 4.1
BD1b	1.47 ± 0.16	7.53 ± 0.39	52.7 ± 2.7
BD1c	1.58 ± 0.12	7.83 ± 0.06	54.8 ± 0.4
BD2a	1.55 ± 0.17	8.16 ± 0.19	57.1 ± 8.4
BD2b	1.60 ± 0.21	8.11 ± 0.35	56.8 ± 2.5
BD2c	1.45 ± 0.17	8.53 ± 0.41	59.7 ± 2.9
BD3a	1.46 ± 0.16	< 0.34	< 2.4
BD3b	1.57 ± 0.22	< 0.34	< 2.4
BD3c	1.47 ± 0.11	< 0.34	< 2.4
BD4a	1.51 ± 0.12	10.68 ± 0.23	74.8 ± 1.6
BD4b	1.56 ± 0.19	10.67 ± 0.18	74.7 ± 1.3
BD4c	1.50 ± 0.13	10.74 ± 0.40	75.2 ± 2.8
BD5a	1.50 ± 0.14	10.46 ± 0.06	66.3 ± 6.2
BD5b	1.56 ± 0.20	10.47 ± 0.62	62.8 ± 3.7
BD5c	1.59 ± 0.14	10.42 ± 0.49	62.5 ± 3.0

[a] n = 200, [b] DS content in mg/100 mg beads, [c] n = 3; (Note: theoretical drug content in all beads was 14.29 mg/100 mg particles).

low cathode lamp operating at 3 mA. The calibration was executed in the range 0-20 mg/L Ca. Prior to the analysis, samples of mineralizates were 5× diluted. Results were statistically compared using Microsoft Excel (Microsoft, USA) and the one-way variance analysis (ANOVA) at the significance level $\alpha = 0.1$.

RESULTS AND DISCUSSION

Morphology, drug loading, encapsulation efficiency and particle size analysis

Morphology of prepared particles can be observed in Figure 1. The surface of particles was rough and covered by recrystallized drug (Fig. 1e). The equivalent diameter of the prepared beads, DS content and EE values are shown in Table 3. Morphology was not significantly influenced neither by encapsulation efficiency nor processing parameters with exception of sample BD5 (see later). The equivalent diameter of obtained particles was very uniform varying between 1.45-1.60 mm, DS content was found from 7.41 to 10.74%. Encapsulation efficiency of the prepared beads ranged between 51.9–75.2%. Particles of the sample BD3 with the DS content below the limit of measurement (LOQ = 2.4 μg/mL) were the exception. This finding is due to ultrasound application causing fast drug release even before the solid structure formed. In the previously published experiments (16), it was assumed that ultrasound could have a positive impact on the crosslinking process. This presumption was not confirmed and the application

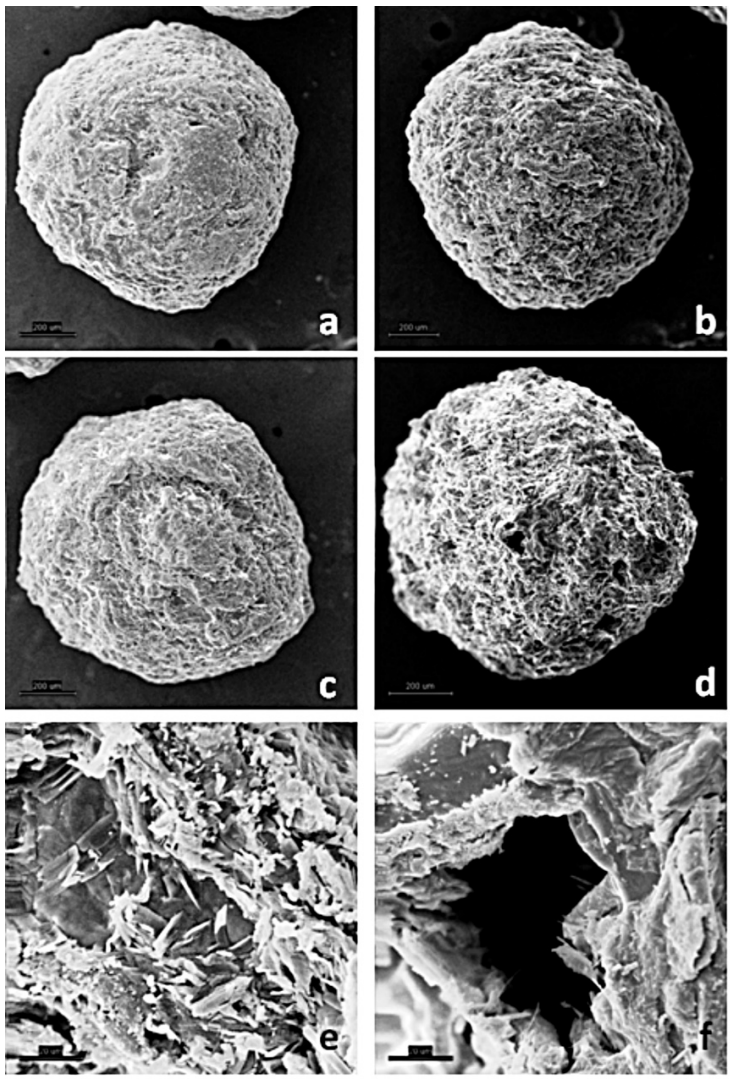

Figure 1. SEM photographs of surface of beads (a) sample BD1, (b) sample BD2, (c, e), sample BD4, (d, f) sample BD5

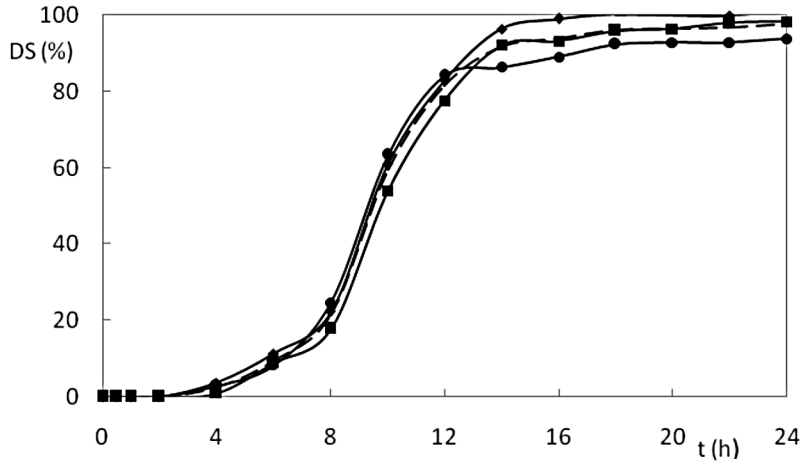

Figure 2. Amount of DS released from matrix beads of the sample BD1 in the dissolution medium with pH value 6.8; ● batch BD1a, ♦ batch BD1b, ■ batch BD1c, – - average value of dissolution data

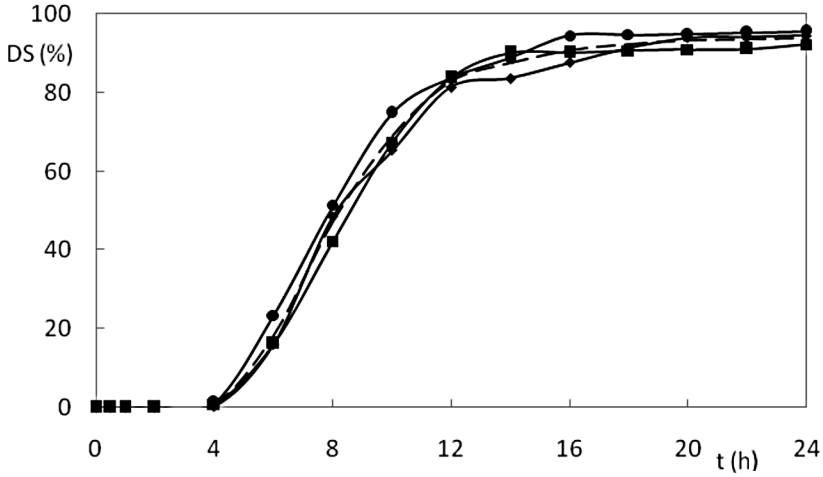

Figure 3. Amount of DS released from matrix beads of the sample BD2 in the dissolution medium with pH value 6.8; ● batch BD2a, ♦ batch BD2b, ■ batch BD2c, – - average value of dissolution data

Table 4. Standard deviations of the amount of DS released from beads within the dissolution test in the dissolution medium with pH value 6.8.

	Standard deviations (%) at time intervals (min)									
Sample	30	60	120	240	360	480	600	720	840	960
BD1	0.00	0.00	0.00	1.38	1.54	3.48	4.86	3.48	5.05	5.03
BD2	0.00	0.00	0.00	0.51	4.14	4.68	5.00	1.32	3.37	3.46
BD4	0.00	0.00	0.33	2.01	7.42	9.70	8.09	5.25	3.91	2.95
BD5	0.00	0.73	2.15	0.87	3.10	4.36	3.86	3.71	3.16	3.25

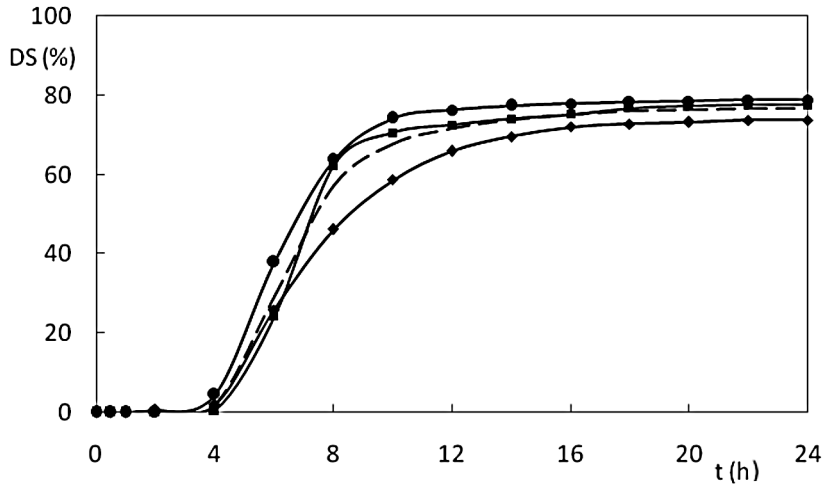

Figure 4. Amount of DS released from matrix beads of the sample BD4 in the dissolution medium with pH value 6.8; ● batch BD4a, ♦ batch BD4b, ■ batch BD4c, – - average value of dissolution data

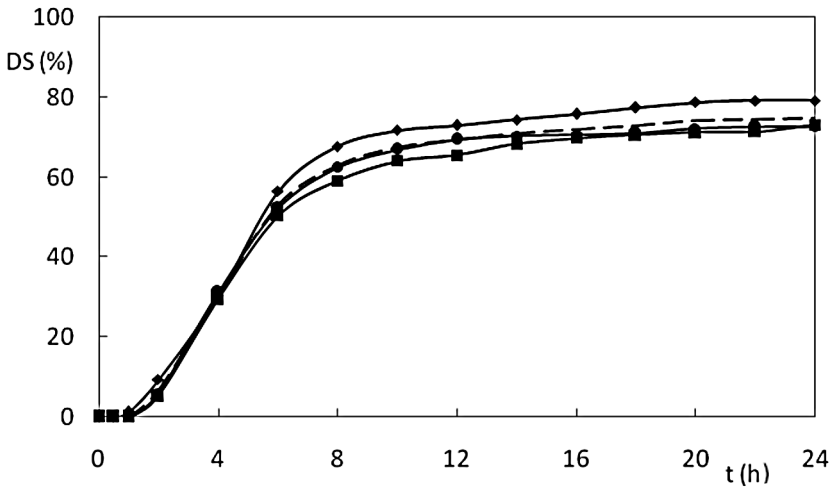

Figure 5. Amount of DS released from matrix beads of the sample BD5 in the dissolution medium with pH value 6.8; ● batch BD5a, ♦ batch BD5b, ■ batch BD5c, – - average value of dissolution data

of ultrasound enhanced the drug release into the crosslinking solution (21). When a low temperature (4^{o}C) of crosslinking solution was used (samples BD1 and BD2) EE increased (+ 4.7%) due to DS lower solubility in cold solution. The lower temperature resulted in the lower solubility of DS and consequently in the slower leakage of the drug from the formed particles into the crosslinking solution (22, 23). The highest encapsulation efficiency was achieved in the sample BD4 using crosslinking solution saturated with a drug at lower temperature (average value 74.9%). EE increased compared to sample BD1 (+ 21.8%), and to sample BD2 (+ 17.1%). Relatively high encapsulation efficiency can be explained by the lower drug solubility in the crosslinking solution and mainly by the decrease of the concentration gradient between beads and the crosslinking solution (Fick's 1st Law). When ultrasound was applied under these conditions (sample BD5), the only process variable, EE decreased (-11.1%) in comparison to the sample BD4. Furthermore, the surface of prepared particles was

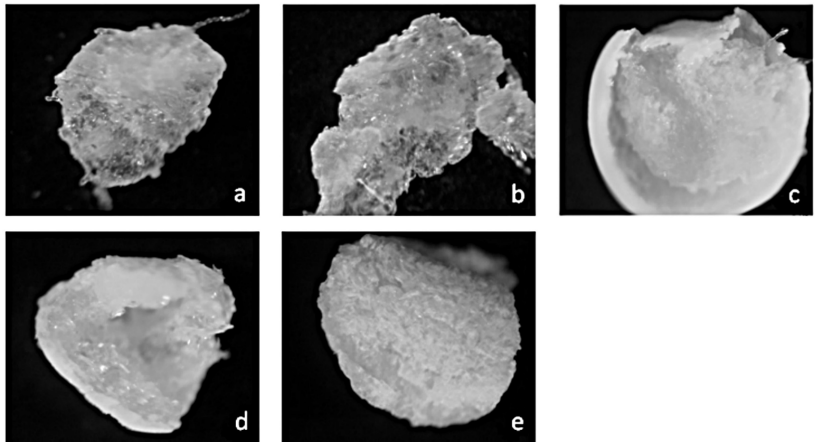

Figure 6. Photographs of beads after the 24-h dissolution test in phosphate buffer with pH value 6.8 (a-d) and artificial gastric juice with pH value 1.2 (e) obtained by stereoscopic microscope; (a) sample BD1, (b) sample BD2, (c) sample BD4, (d) sample BD5, (e) sample BD1 – particle cross-section

Table 5. Similarity factors f_2 calculated between dissolution profiles of the prepared particle batches.

Batch	Similarity factor f_2
BD1a : BD1b	72.62
BD1b : BD1c	74.34
BD1c : BD1a	69.82
BD2a : BD2b	74.31
BD2b : BD2c	72.30
BD2c : BD2a	67.89
BD4a : BD4b	52.41
BD4b : BD4c	57.41
BD4c : BD4a	66.83
BD5a : BD5b	65.06
BD5b : BD5c	60.13
BD5c : BD5a	78.30

disrupted leading to the holes creation, through which the drug leaked out of the beads (Fig. 1f). However, the EE decrease was not as significant as in BD3 due to the positive effect of the saturated concentration of DS in the crosslinking solution which reduced the drug leakage from beads. From these results it is obvious that it was necessary to use saturated crosslinking solutions when ultrasound was applied within particles preparation (24).

In vitro release studies and dissolution profile similarity

Dissolution studies were carried out in two dissolution media with various pH values, artificial gastric juice of pH 1.2 and phosphate buffer of pH 6.8. Within 2 h lasting dissolution test in acidic medium no amount of DS from all studied samples was released due to DS insolubility under these conditions (14). Tested beads did not disintegrate within this test what is a good presumption for their successful passage through stomach (data not presented as the values were close to zero during all the tested period). The drug release from particles was extended in phosphate buffer of pH 6.8 and lasted from 10 to 16 h. Obtained dissolution data are shown in Figures 2–5. Table 4 presents standard deviations of average values of released drug to provide better understanding of the figures.

The similarity factor f_2, lag time and parameter $t_{50\%}$ were selected as crucial parameters for evaluation of influence of process conditions on the DS dissolution profile from beads. The lag time expresses the time during which the drug does not release from its dosage form (25). Parameter $t_{50\%}$ expresses the time in which 50% of the drug is released (26). The similarity analysis factor (Table 5) for the DS dissolution data from three batches of the same particles sample suggested that all the dissolution curves were similar (where $f_2 > 50$) which determined a good reproducibility of the applied methods. Similarity factor f_2 between three batches of the sample BD1 reached values 69.82–74.34; sample BD2 67.89–74.31; sample BD4 52.41–66.83, and sample BD5 60.13–78.30 (Table 5).

Similarity factor f_2 was also applied on the dissolution data between each sample. From the dissolution tests and similarity factors f_2 (Table 6) calculated between samples BD1, BD2, BD4 and BD5 it is obvious that the applied process variables influ-

Table 6. Parameters of dissolution profiles and similarity factors f_2 between dissolution profiles of beads samples prepared under various processing conditions.

Sample	Similarity factor f_2				$t_{1/2}$ [min]	Lag time [h]
	BD1	BD2	BD4	BD5		
BD1	-	40.47	31.25	25.44	557	4
BD2	40.47	-	49.16	-	480	4
BD4	31.25	49.16	-	43.33	434	4
BD5	25.44	-	43.33	-	333	1

Table 7. Calcium content in remnants shells.

Sample	Calcium content [mg/g]
BD1	29 ± 4
BD2	30 ± 5
BD4	47 ± 6
BD5	40 ± 5

enced the release of DS from matrix beads. The dissolution profile for the sample BD1 (Fig. 3) showed 4-h lasting lag time; half of the drug was released from prepared beads in 557 min. The sample BD2, prepared under lower temperature of the crosslinking solution (4°C), had the same lag time (4 h). However, the drug release from sample BD2 was faster and half of the incorporated drug was released in 480 min. This acceleration can be explained by the higher encapsulation efficiency of sample BD2. Higher DS concentration inside particles resulted in higher concentration gradient between the particles and dissolution medium (27). Saturation of the crosslinking solution by DS increased encapsulation efficiency in the sample BD4. Therefore, concentration gradient was increased, which led to faster drug release from beads of sample BD4 (Fig. 4) (maintaining the same 4-h lasting lag time). The time in which half of the drug released from the sample BD4, was reduced to 434 min. The sample BD5, prepared under lower temperature, with drug-saturated crosslinking solution and using of ultrasound, had the lowest $t_{50\%}$ and took value of 333 min. Compared to the other samples, the lag time was significantly shorter (1 h) (Fig. 5). This was caused by the use of ultrasound and erosion of the surface structure of beads during the preparation. To determine the similarity between the obtained drug release profiles, f_2 factor was calculated. Similarity factor f_2 between the samples BD1 and BD2 reached 40.47. This value indicates that the temperature decrease was a significant process parameter. Higher encapsulation efficiency achieved under the given conditions has a significant influence on the dissolution profile and resulted in the faster drug release. The similarity factor f_2 between samples BD1 and BD4 which differed in two process parameters (BD4 – saturation of the crosslinking solution by DS and temperature 4°C) reached the value of 31.25 and proved that the dissolution profiles were not similar. The combination of these process variables had a significant influence on EE and consequently on the DS dissolution profile. The lowest f_2 was obtained when samples BD1 and BD5 dissolution profiles were compared (f_2 = 25.44). The use of ultrasound and shorter lag time played a significant role. The dissolution profiles of BD2 and BD4, which only differed by the crosslinking solution saturation, were not similar to each other, as confirmed by the similarity factor (f_2 = 49.16). The crosslinking solution saturation had significant influence on the drug dissolution profile of the drug which was not completely released (during the 24-h test approximately 77% of the drug was released). This was caused by formation of insoluble diclofenac calcium on the surface of prepared particles, in this case of sample BD4 (28), which remained in the surface layer even after the dissolution test. The same insoluble surface layer appeared in BD5, which was prepared under the same process conditions with additional use of ultrasound within the crosslinking (see below). In this case the drug was also not completely released (during the 24-h only 75% was released).

Determination of calcium content in remnants shells

After the 24-h dissolution test, the tested beads were taken out and evaluated visually by the stereoscopic microscope. Particles were not compact; only shells of various size and white color intensity were found (Fig. 6a-d). It was assumed that white color in

Table 8. Fittings of DS release data to different kinetic equations.

MODEL	Zero order	First order	Higuchi	Korsmeyer-Peppas		Hixson-Crowell
Sample	K_0 (h^{-1}) (CI) **R^2**	K_1 (h^{-1}) (CI) **R^2**	K_H ($h^{-1/2}$) (CI) **R^2**	K_{KP} (h^{-n}) (CI) **R^2**	n (CI)	K_{HC} ($h^{-1/3}$) (CI) **R^2**
BD1	9.8 (4.7-14.8) **0.9333**	0.38 (0.21-0.55) **0.9677**	51 (17-86) **0.8928**	0.03 (0.01-0.11) **0.9758**	1.6 (0.8-2.4)	0.016 (0.008-0.025) **0.9258**
BD2	10.4 (8.8-12.0) **0.9907**	* 0.31 (-0.10-0.73) **0.9084**	58 (49-67) **0.9913**	0.10 (0.04-0.26) **0.9808**	1.0 (0.5-1.6)	0.017 (0.014-0.019) **0.9905**
BD4	11.6 (6.7-16.7) **0.9773**	* 0.35 (-0.45-1.15) **0.8882**	58 (36-81) **0.9842**	0.04 (0.00-0.71) **0.9624**	1.6 (-0.2-3.4)	0.018 (0.009-0.027) **0.9714**
BD5	9.9 (5.0-14.7) **0.9814**	* 0.26 (-0.17-0.68) **0.9142**	41 (31-52) **0.9928**	0.08 (0.03-0.22) **0.9843**	1.1 (0.5-1.7)	0.017 (0.008-0.023) **0.9827**

R^2 - determination coeffiecient, CI - confidence interval.

the samples BD4 and BD5 indicated insoluble diclofenac calcium, which was not released from the surface structures during the dissolution test. Atomic absorption spectrometry was used to detect the total calcium content in empty shells (Table 7). Results indicated that the highest calcium content 47 ± 6 mg/g was in sample BD4. This was caused by higher concentration of diclofenac sodium in the crosslinking solution, where competition for calcium ions between DS and NaOC occurred. It led to creation of insoluble diclofenac calcium (29). The similar phenomenon occurred in the sample BD5, where the calcium content was 40 ± 5 mg/g. The lower content than in BD4 was probably caused by using of ultrasound during the crosslinking. However, the calcium content decrease was not statistically significant. The sample BD2, where the lower temperature was applied during the crosslinking, contained 30 ± 5 mg/g. The lowest calcium content was in the sample BD1 – 29 ± 4 mg/g. There was no statistically significant difference in the calcium content between samples BD1 and BD2.

Release kinetics

Table 8 summarizes results for the evaluation of the DS release mechanism from oxycellulose beads. The samples BD2, BD4 and BD5 correlated with zero order kinetics ($R^2 > 0.97$). The correlation with zero order kinetic represents an ideal mechanism for the drug liberation. The exception was the sample BD1 where determination coefficient was lower (~ 0.93). Dissolution data of the samples BD2, BD4 and BD5 did not correspond with the first-order kinetic model ($R^2 < 0.91$) except sample BD1 which was confirmed by high value of determination coefficient ($R^2 > 0.97$). The low correlation of the sample BD1 with all kinetic models was probably caused by the biphasic drug release, i.e., two rate trends can be detected on the dissolution curve (Fig. 2) (30). In the samples BD2, BD4 and BD5 the determination coefficient for Higuchi model ranged between 0.98-0.99. These results indicated diffusion release of DS from beads. R^2 values for Korsmeyer-Peppas model ranged in all samples between 0.96-0.98. The release mechanism can be determined from the exponent *n* value. Nevertheless, the exact mechanism release could not be predicted in this case due to the large confidence interval of the exponent *n*. The determination coefficient R^2 values for Hixson-Crowell model were for the samples BD2, BD4 and BD5 relatively high (0.97-0.99), which indicated influence of erosion on the drug release. DS was probably released from samples BD2, BD4 and BD5 by combination of diffusion and by erosion process.

CONCLUSION

The obtained results showed that the saturation of the crosslinking solution led to increase of encapsulation efficiency; however, the entire amount of the incorporated drug did not release during the dissolution test and remained in the form of insoluble diclofenac calcium in external layer of prepared beads. The use of ultrasound within the particles crosslinking represented a significant process variable, because it led to surface disruption of the sur-

face structure of beads and significantly shortened the drug release lag time. The use of ultrasound within the particles formation is possible only when the drug-saturated crosslinking solution was used.

Acknowledgments

This experimental work was realized by support of IGA VFU Brno Czech Republic, project 15/2010/FaF and project MPO K-103/2006/FaF.

REFERENCES

1. Manjanna K.M., Kumar Pramod T.M., Shivakumar B.: Drug Invention Today 1, 98 (2009).
2. Dimitrijevich S. D., Tatarko M., Gracy R.W.: Carbohyd. Res. 195, 247 (1990).
3. Levy M.L.; Day J.D., Fukushima T., Batjer H.H., Gamache, F.W.: Neurosurgery 41, 701 (1997).
4. Dvořáčková K.: Chem. Listy 103, 66 (2009).
5. Schonauer C., Tessitore E., Barbagallo G., Albanese V., Moraci, A.: Eur. Spine J. 13 (Suppl. 1), S89 (2004).
6. Mášová L, Ryšavá J, Křížová P, Suttnar J, Salaj P, Dyr JE, Homola J, Dostálek J, Myška K, Pecka M.: Sbor. Lek. 104, 231 (2003).
7. Tudja P., Khan, M.Z.I., Meštrović E., Horvat M., Golja P.: Chem. Pharm. Bull. 49, 1245 (2001).
8. Kincl M., Vrecer F., Veber M.: Anal. Chim. Acta 502, 107 (2004).
9. Palomo M.E., Ballesteros M.P., Frutos P.: J. Pharm. Biomed. Anal. 21, 83 (1999).
10. Bartolomei M., Bertocchi P., Antoniella E., Rodomonte A.: J. Pharm. Biomed. Anal. 40, 1105 (2006).
11. Yua C.-Y., Zhanga X.-Ch., Zhoua F.-Z., Zhanga X.-Z., Chenga S.-X.: Int. J. Pharm. 357, 15 (2008).
12. Avachat A., Kotwal V.: AAPS PharmSciTech. 8, E1 (2007).
13. Khan F., Millat S., Razzak I., Khan Z.R., Azam K.R., Sadat S.M.A., Reza S.: J. Pharm. Sci. 7, 65 (2008).
14. Dvořáčková K., Rabišková M., Masteiková R., Muselík J., Krejčová K.: Drug Dev. Ind. Pharm. 35, 930 (2009).
15. Fonkwea L.G., Narsimhana G., Chab A.S: Food Hydrocolloids 17, 87 (2003).
16. Catro M.D.L., Priego-Capote F.: Talanta 72, 321 (2007).
17. Dvořáčková K., Rabišková M., Masteiková R., Okáčová L.: Česka Slov. Farm. 57, 269 (2008).
18. Adams E., De Maesschalck R., De Spiegeleer B., Vander Heyden Y., Smeyers-Verbeke J., Massart D.L.: Int. J. Pharm. 212, 41 (2001).
19. Vetchý D., Vetchá M., Rabišková M., Gryczová E., Bartošíková L.: Medicina (Kaunas) 43, 326 (2007).
20. Costa P., Lobo J.M.S.: Eur. J. Pharm. Sci. 13, 123 (2001).
21. Saravanana M., Bhaskara K., Maharajanb G., Pillaic K.S.: Int. J. Pharm. 283, 71 (2004).
22. Bodek K.H.: Acta Pol. Pharm. Drug Res. 57, 431 (2000).
23. Avdeef A., Berger C.M., Brownel Ch.: Pharm. Res. 17, 85 (2000).
24. Bajerová M., Dvořáčková K., Gajdziok J., Masteiková R.: Česka Slov. Farm. 59, 67 (2010).
25. Kao Ch.-Ch., Chen S.-Ch., Sheu M.-S.: J. Control. Release 44, 263 (1997).
26. El-Gibaly I.: Int. J. Pharm. 232, 199 (2002).
27. Tan J.P.K., Zenga A.Q.F., Changa Ch.C., Tamb K.C.: Int. J. Pharm. 357, 305 (2008).
28. Fini A., Fazio G., Roseti F., Holgado A., Iruín A., Alvarez-Fuentes J.: J. Pharm. Sci. 94, 2416 (2005).
29. Gonzáles-Rodríguez M.L., Halgado M.A., Sánchez-Lafuente C., Rabasco A.M., Fini A.: Int. J. Pharm. 232, 225 (2002).
30. Ofori-Kwakye K., Fell J.T.: Int. J. Pharm. 250, 431 (2003).

Received: 13. 10. 2010