Acta Pharm. **66** (2016) 173–189
DOI: 10.1515/acph-2016-0025

Original research paper

Piecewise function parameters as responses of the design of experiment in the development of a pulsatile release chronopharmaceutical system

ANDREEA LOREDANA VONICA-GLIGOR[1,2,3]
IOAN TOMUȚĂ[1]
SORIN E. LEUCUȚA[1]

[1] *Department of Pharmaceutical Technology and Biopharmacy Faculty of Pharmacy University of Medicine and Pharmacy "Iuliu Haţieganu" 40012 – Cluj-Napoca, Romania*

[2] *S.C. Polisno Pharmaceuticals S.R.L. 550201 – Sibiu, Romania*

[3] *Faculty of Medicine, "Lucian Blaga" University of Sibiu, 550169 – Sibiu Romania*

Accepted February 23, 2016
Published online April 8, 2016

The aim of this work was to develop a pulsatile release system with metoprolol for chronotherapeutical use by coating swellable mini-tablets with Eudragit RS. To study the influence of the formulation factors (amount of coating polymer, plasticizer percentage in film coating and swelling agent percentage in mini-tablets), a Box-Behnken design of experiment (DoE) was used. To evaluate the influence of the studied factors on the sigmoid shape of the dissolution profile, piecewise function parameters were used as the responses of DoE. The results show that higher concentrations of coating polymer and higher concentrations of plasticizer polymer led to a thicker and more elastic polymeric film, which led to a delay in drug release. Using the parameters of the piecewise function as DoE responses, an optimum formulation with a sigmoid shape dissolution profile and a 2.5-h lag time followed by rapid drug release were obtained.

Keywords: pulsatile release, chronopharmaceutical system, piecewise function, design of experiment, Eudragit RS, metoprolol

Chronotherapy is the delivery of a drug at the right concentration to the right targeted tissues at the right time to meet the biological rhythm-determined needs (1–3). Blood pressure is characterized by predictable changes over 24 hours in synchrony with the rest–activity cycle. The rate of blood pressure rise at the commencement of diurnal activity is a predictor of the risk of morning stroke and acute coronary syndrome, and it is also hypothesized to be a trigger for myocardial infarction at that time of the day (4, 5). Metoprolol is a cardioselective beta-blocker used in the management of hypertension, angina pectoris cardiac arrhythmias, myocardial infarction and heart failure. Because of its antihypertensive action, a formulation suitable for chronotherapy could be important for the treatment of hypertension (6, 7).

The values of blood pressure vary throughout the day, especially in the morning hours before waking up. Therefore, a chronopharmaceutical formulation could provide

* Correspondence; e-mail: tomutaioan@umfcluj.ro

the necessary quantity of metoprolol in the blood before waking up and would potentially control the increase in blood pressure. The patient would take the chronopharmaceutical formulation with metoprolol at bedtime; after a lag time of 2.5-3 hours, followed by a rapid drug release (1-1.5 hours), the needed metoprolol quantity would be in the blood stream before the early morning hours when blood pressure is most likely to increase, thus providing the necessary control (8, 9).

Chronotherapy can be attained with pulsatile drug delivery systems. Ideally, a pulsatile release system releases the drug rapidly and completely after a predefined lag time of no drug release. The mechanism of reservoir-type pulsatile systems is based on drug release from a drug core (tablet or capsule) after the rupture of the surrounding polymer coating layer caused by the pressure built-up within the system. The pressure necessary to break the coating layer can be achieved with the aid of gas-producing effervescent excipients or by increasing the inner osmotic pressure using swelling agents such as cellulose ethers, polysaccharides or superdisintegrants (10–12).

In vitro drug dissolution has been recognized as an important tool in drug development. Several kinetic equations describe drug release from immediate and modified oral dosage forms (13, 14). Quantitative interpretation of the values obtained from *in vitro* dissolution studies is facilitated by the use of a mathematical equation that translates the dissolution profile into parameters relating to the pharmaceutical characteristics of the dosage form. The drug release profile from a pulsatile release chronopharmaceutical system is characterized by a period of low or no drug release (lag time), followed by a rapid drug release period (pulse release) and then a period after most of the drug has been released. The ideal shape of such a profile is sigmoid and has two turning points: (i) the end of the lag time period – the beginning of the rapid release period, and (ii) the end of the rapid release period (Fig. 1a) (15).

The piecewise function is a function defined by multiple sub-functions, each subfunction applying to a certain interval (domain) of the main function. Piecewise three segment linear function is a linear function that consists of three segments and two

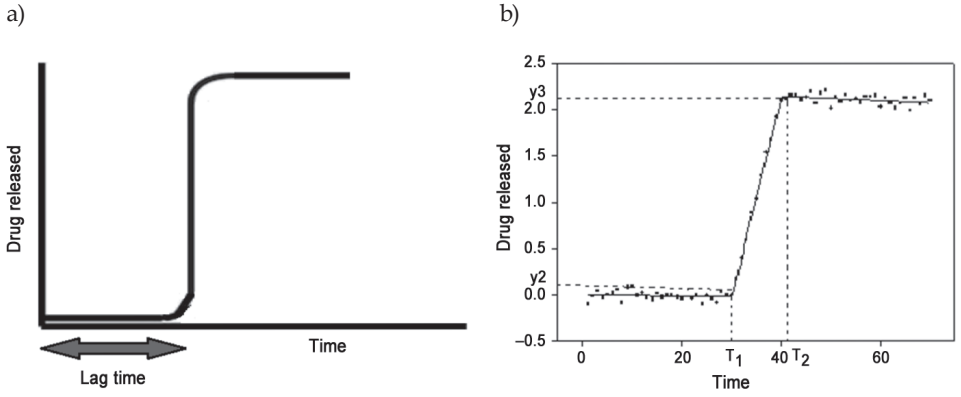

Fig. 1. Drug release from pulsatile drug delivery systems. a) ideal sigmoid release, b) graphical shape of piecewise three segment linear function; y_2 – drug released at the first turning point; y_3 – drug released at the second turning point; t_1 – time at the first turning point; t_2 – time at the second turning point.

turning points. It is described by equation 1 and presents a sigmoid shape shown in Fig. 1b.

$$y = \begin{cases} [y_1(T_1 - t) + y_2(t - t_1)] / (T_1 - t_1) & t_1 \le t \le T_1 \\ [y_2(T_2 - t) + y_3(t - T_1)] / (T_2 - T_1) & T_1 < t \le T_2 \\ [y_3(t_3 - t) + y_4(t - T_2)] / (t_3 - T_2) & T_2 < t \le t_3 \end{cases} \quad \textit{(Eq. 1)}$$

Where: y_1 – minimum value; y_2 – first turning point; y_3 – second turning point;
y_4 – maximum value; t_1 – time at the first turning point;
t_2 – time at the second turning point.

The *in vitro* dissolution profiles of a pulsatile release chronopharmaceutical system can be described by the piecewise three segment linear function, where the first segment is the lag time period when the drug is not released or the amount of drug release is low, the second segment is the period of rapid drug release, the third segment is the period after more than 80 % of the drug has been released, and the turning points are the points of the start and the end of the rapid release period. Each turning point is characterized by two parameters, whose values can be used for the quantitative characterization of the release profile of the pulsatile chronopharmaceutical system.

Many papers have reported the development of pulsatile chronopharmaceutical systems (1, 7, 9, 10), in some of which design of experiments was used (2, 13, 20), but so far the use of piecewise function parameters for characterization of the dissolution profile shape has not been reported. The advantage of using piecewise function is precise characterization of the pulsatile release profile by two turning points of the sigmoid shape. Further, each turning point is numerically characterized by two parameters that may be the responses of an experimental design. Thus, in the development of a pulsatile drug delivery system using an experimental design, the parameters of piecewise function may have the target value to obtain the desired sigmoid shape of the dissolution release profile.

In a previous experimental paper, we established that a suitable film forming polymer for obtaining pulsatile release from film-coated swellable metoprolol cores was Eudragit RS, an insoluble, but slowly permeable polymer (17). From these reservoir-type pulsatile systems, rapid release of the drug from the core after a lag time period (the moment when the polymeric film breaks) can be modulated by modifying the thickness (amount) of the film coating, the elasticity of the film coating and the inner pressure generated by core swelling.

The aim of this work was to use a Box Behnken experimental design and piecewise three segment linear function parameters as dependent variables (responses) of the experimental design to study the influence of the film coating polymer (Eudragit RS) percentage, the plasticizer in film coating (triethylcitrate) percentage and the swelling agent in mini-tablet cores (sodium starch glycolate) percentage on the drug release profile in order to develop a mini-tablet type pulsatile release chronopharmaceutical system with an optimized drug release profile.

EXPERIMENTAL

Materials

Metoprolol tartrate (Microsin, Romania) was chosen as a model drug. Lactose monohydrate 800 Mesh (HMS, Holland), microcrystalline cellulose – PH 102 (JRS Pharma, Germany), silicon dioxide – Aerosil (BASF, Germany), talcum (S&D Chemicals, UK) and magnesium stearate (Merck, Germany) were used as excipients in the core tablets. Polyvinylpyrolidone K25 (Merck, Germany) was used as a binder in the granulation step. Sodium starch glycolate (JRS Pharma, Germany) was used as a swelling agent in the core tablets. Mini-tablets were coated with different amounts of the insoluble, but slowly permeable Eudragit RS 30D (Rohm Pharma, Germany) film coating polymer. Triethylcitrate (Merck, Germany) was added as a plasticizer and simeticone (Colorcon, UK) was added as an anti-foaming agent to the film coat.

Apparatus

A fluid bed device Strea 1 (Aeromatic A.G., Switzerland) was used to obtain the granules and to coat mini-tablets. A DIN sieve set (VEB MLW, Germany) and a mass volumetric

Table I. Independent and dependent variables of the Box Behnken design of experiment

Variables	Used levels		
	Low –1	Medium 0	High +1
Independent variables (formulation factors)			
x_1 = film forming polymer percentage (Eudragit RS)	5	11	17
x_2 = swelling agent percentage (sodium starch glycolate)	10	15	20
x_3 = plasticizer percentage (triethylcitrate)	8	12	16
Dependent variables (responses)			
y_1 = cumulative % of metoprolol released after 1.0 h			
y_2 = cumulative % of metoprolol released after 2.0 h			
y_3 = cumulative % of metoprolol released after 2.5 h			
y_4 = cumulative % of metoprolol released after 3.0 h			
y_5 = cumulative % of metoprolol released after 3.5 h			
y_6 = cumulative % of metoprolol released after 4.0 h			
y_7 = cumulative % of metoprolol released after 5.0 h			
y_8 = cumulative % of metoprolol released after 7.0 h			
$y_9 = c_2$ – concentration at the first turning point			
$y_{10} = t_1$ – time at the first turning point			
$y_{11} = c_3$ – concentration at the second turning point			
$y_{12} = t_2$ – time at the second turning point			

test apparatus SVM (Erweka, Germany) were used for particle measurements and for the Carr Index and Haussner ratio determination. The cores were obtained using a tablet press EK-0 (Korsch, Germany) and their hardness was tested using a Monsanto tablet hardness test apparatus (Monsanto, Italy). Dissolution studies were done in a PT-DT7 dissolution apparatus (PharmaTest, Germany) and the *in vitro* dug assay was done using a UV-Vis V530 spectrophotometer (Jasco, Japan).

Design of Experiment (DoE)

A Box Behnken design of experiment with three factors and three levels was used to study the influence of formulation variables on the drug release profiles and piecewise equation parameters. The studied formulation factors (independent variables) were the percentage of polymeric film used for coating (Eudragit RS 30D) – x_1, percentage of the swelling core agent (sodium starch glycolate) – x_2, and percentage of the polymeric film plasticizer (triethylcitrate) – x_3. Dependent variables (responses) were the cumulative percentage of drug release at different time intervals and the piecewise function parameters. The levels of variation of independent variables and the dependent variables are shown in Table I.

The construction of the design of experiment, computation of coefficients, statistical parameters and fitting of the experimental data in order to assess the results were performed using the Modde 10.0 software, Umetrics, Sweden. The data fit was done using the Partial Least Squares (PLS) method.

Granule preparation and evaluation

In order to obtain metoprolol mini-tablets as cores (25 mg metoprolol in 75 mg tablets, or 33.34 %), a preliminary granulation step was necessary. Metoprolol tartrate was mixed with various ratios of sodium starch glycolate (10, 15 and 20 %) and monohydrate lactose (18.99, 13.99 and 8.99 %). The mixtures were transformed into granules using a 10 % polyvinylpyrrolidone aqueous solution as binder in a Strea 1 fluid bed device (Aeromatic, Switzerland). The granulation process was done using the optimized method previously reported (18).

Before tableting, the granules were physically characterized in order to assess the flowability and compressibility properties. To evaluate the Carr index and Hausner ratio, the untapped and tapped densities of the granules were determined according to the well-known methods described in the European Pharmacopoeia (19).

In order to evaluate the size and size distribution, the granules were sieved using a set of sieves with different apertures (100, 200, 300, 400, 500, and 600 mm), and the granules mean diameter (*Md*) and granules poly-dispersion index (P.I.) were calculated.

The mean particle diameter was calculated using the equation:

$$Md = \pm \frac{\sum n_i x_i}{N} \qquad \textit{(Eq. 2)}$$

Md – granule mean diameter,
N – total frequency (in this case 100 %).

P.I. was measured with the equation:

$$P.I. = \pm \frac{\mathrm{SD}}{Md} \times 100 \qquad \textit{(Eq. 3)}$$

Md – granule mean diameter,
SD – standard deviation.

Tablet preparation and tablet coating

Microcrystalline cellulose (33.3 %), magnesium stearate (1 %) and silicon dioxide (1 %) were added to the obtained granules and were compressed using an eccentric press (Korsch EK 0, Germany) equipped with a set of 5 mm biconvex punches. The machine was configured to obtain 75-mg tablets with a crushing strength of 5–9 kg. After the preparation, the tablets were analyzed according to the well-known pharmaceutical procedures from the European Pharmacopeia (17).

The tablets were finally coated in a Wurster configuration fluid bed device (Strea 1 – Aeromatic GEA, Switzerland) with different amounts of Eudragit RS. The composition of the coating suspension was: Eudragit RS 30D (5-11-17 %, according to the experimental design matrix, Table II), triethylcitrate (8-12-19 %, according to the experimental design matrix, Table II), talcum (30 %), titanium dioxide (5 %) and pigment (3 %). To prepare the coating suspension, triethylcitrate was first dispersed in water, then talcum, titanium dioxide and pigment were suspended in water and homogenized (Ultra Turrax); this suspension was poured into the Eudragit RS 30D dispersion under gentle mixing; the obtained mixture was homogenized for 40 minutes before use and then passed through a 0.25-mm sieve; the final mixture containing 22 % solid particles was processed by continuous gentle stirring during the coating process. Technological parameters during the coating process were: gun nozzle 0.8 mm, atomizing air pressure 3.6 atm, spraying rate 10 g/min, inlet air temperature 34–42 °C and outlet air temperature 30–35 °C, fan air 3.6 m^3/min.

Dissolution studies

In vitro dissolution studies were performed in a PharmaTest PT-DT7 device, equipped with paddles (European Pharmacopoeia apparatus 1), employing a rotation speed of 50 rpm. The dissolution medium was 500 mL phosphate buffer (pH = 6.8) at 37 °C. Four tablets were immersed in the dissolution medium, each containing approximately 25 mg metoprolol (100 mg per dissolution vessel – the usual daily dosage of metoprolol). Samples were taken at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5 and 7 h. Each 5-mL sample was immediately passed through a 0.45-μm filter and replaced with the same volume of fresh dissolution medium in order to maintain a constant volume throughout the experiment. The samples were analyzed by UV spectrometry at 275 nm using a validated method. The following parameters were subjected to validation: specificity, linearity, accuracy, precision and stability of metoprolol in dissolution medium). Three determinations were performed for each formulation and the mean and standard deviation were calculated at each dissolution point.

To evaluate the dissolution profile, a piecewise three segment linear function was used (*Eq. 1*). This defines the cumulative expression of fractionized drug release in time as

a time function modelled by three separate equations, representing three linear segments united at their ends. The three segments represent three regions: the time before, during and after rapid release. The first turning point represents the beginning of drug release, after the lag time, and the second turning point represents the end of release (14, 20, 21). Piecewise function parameters were introduced as responses (dependent variables) of the experimental design together with the amount of drug released at defined periods of time (Table I, dependent variables y_9-y_{12}).

RESULTS AND DISCUSSIONS

Influence of formulation factors on granule and tablet characteristics

Mass uniformity of mini-tablets can be assured if the powders for tableting fill the die uniformly. This can be done only if granules have good flow properties and low size distribution variability. According to the obtained results, all the granules had good flow properties, irrespective of the percentage of sodium starch glycolate used. The Carr index was between 5.3 and 11.18, corresponding to an excellent flow (free granule flow). Extreme values appeared when 20 % sodium starch glycolate was used. The Hausner ratio had very low variability, with values between 1.07 and 1.13, all lower than 1.2, corresponding to an easy flow. The mean particle diameter had values between 234.77 and 313.4 μm, and the variation coefficient had values between 39.239 and 49.578, so there were no big differences between particle sizes.

As mini-tablets will later be coated in a fluid bed, they should have very good pharmaceutical properties. All the formulations were analyzed according to the European Pharmacopoeia methods for average mass and mass uniformity, resistance to crushing, disintegration time, and friability. According to the obtained results, all the prepared formulations of metoprolol mini-tablets showed low weight variability (lower than 5 %), desirable drug content (25.15 mg/tablets), good resistance to crushing (between 40 and 50 N), good disintegration time (maximum 3 minutes) and very good friability (0 %).

The different amounts of sodium starch glycolate used did not influence the pharmaceutical properties of the granules (flow properties, particle mean diameter and variation coefficient) and the tablets (mass uniformity, resistance to crushing, time of disintegration and friability).

Thus, all the mini-tablet batches had very good pharmaceutical properties, according to the European Pharmacopeia requirements, and were suitable for coating in the fluid bed device.

Design of experiment analysis. Summary of fit

The results matrix is shown in Table II. The results show that the amount of metoprolol released (y_1-y_{12} responses) depended on the formulation factors studied in the experimental design.

To check the experimental design validity, the following statistical parameters were determined: R^2, Q^2 and ANOVA. R^2 represents the fraction of variation of the response explained by the model while Q^2 represents the fraction of variation of the response that

Table II. Matrix of the design of experiment and the results matrix

Experiment name	x_1	x_2	x_3	y_1	y_2	y_3	y_4
N1	5	10	12	8.38	45.17	56.74	66.88
N2	17	10	12	1.87	2.01	1.98	2.55
N3	5	20	12	53.86	97.89	98.04	100.55
N4	17	20	12	3.62	3.58	3.75	3.80
N5	5	15	8	81.21	95.90	97.36	99.92
N6	17	15	8	4.39	4.72	6.29	21.00
N7	5	15	16	3.74	5.19	7.13	10.44
N8	17	15	16	0.18	0.22	0.23	1.92
N9	11	10	8	12.22	39.45	82.08	93.61
N10	11	20	8	4.32	23.89	45.16	68.19
N11	11	10	16	2.68	1.47	1.55	1.24
N12	11	20	16	1.85	2.13	1.78	1.89
N13	11	15	12	0.69	0.92	0.92	4.87
N14	11	15	12	1.64	2.02	1.97	2.65

	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}
N1	76.79	80.73	93.58	101.53	8.52	1.02	77.21	3.22
N2	2.35	2.39	2.71	3.37	1.86	6.23	2.97	6.91
N3	100.52	99.40	99.47	95.70	35.71	0.28	100.5	2.14
N4	3.69	3.98	5.20	10.12	1.35	6.11	4.75	6.84
N5	99.76	99.54	100.43	98.75	10.17	0.33	96.37	1.11
N6	45.72	84.41	102.35	105.25	6.67	2.81	99.55	4.27
N7	14.39	18.68	26.97	43.78	7.32	2.69	39.92	6.42
N8	0.90	0.12	1.14	0.47	0.38	7.21	1.06	7.08
N9	97.75	101.62	101.54	100.54	13.02	0.81	97.11	2.67
N10	87.48	94.58	100.20	99.78	8.32	1.63	95.60	3.67
N11	1.31	1.23	1.52	1.75	1.76	7.00	1.17	7.56
N12	2.21	2.81	2.56	4.01	2.18	6.34	1.85	6.71
N13	2.01	2.96	1.83	2.33	1.07	6.78	3.15	7.03
N14	2.10	1.98	2.11	2.89	1.02	5.69	2.42	7.41

x_1–x_3 – independent variables (formulation factors), according to Table I.
y_1–y_{12} – dependent variables (responses), according to Table I.

can be predicted by the model. Both R^2 and Q^2 values are numbers (usually between 0 and 1) and values close to 1 indicate a good model with excellent predictive power (22). The values of R^2 were over 0.8 for all results, except for y_1, when it was close to 0.8. The values of Q^2 were over 0.6 for y_2, y_3, y_4, y_5, y_6, y_7, y_8, y_{10} and y_{11}, and between 0.1 and 0.6 for y_1, y_9

and y_{12}. ANOVA (analysis of variance) test results were good for almost all the answers studied: p for the model was less than 0.05 for all experiments; p for error was more than 0.05 for all experiments. In conclusion, the results fit well with the y_2, y_3, y_4, y_5, y_7, y_8, and y_{11} responses and are satisfactory for the y_1, y_6, y_9, y_{10} and y_{12} responses.

Design of experiment analysis. In vitro *drug release*

Results of the *in vitro* release of metoprolol from the developed film coated mini-tablet type chronopharmaceutical system at different dissolution time intervals are shown in Fig. 2. Equation coefficients, used to fit the experimental data with the chosen model, represent the influence of the studied factors and of the interaction between the studied factors on the responses. Values of the coefficients (x_1, x_2, and x_3) are related to the effects of these variables on the corresponding responses (y_1-y_8). Coefficients with more than one factor term represent interaction terms, and coefficients with higher order terms indicate the quadratic (non-linear) nature of the relationship. Two and three dimensional plots were formed, based on the model, for the measured responses, in order to assess the chance of response surface. Also, the relationship between formulation factors (independent variables) and responses (dependent variables) can be further understood from these plots (23, 24).

The influence of formulation factors on the responses y_1, y_3, y_5 and y_8 is presented as scaled and centered coefficients and as the response surface plot in Fig. 3. The same was found for other responses as the cumulative percent of metoprolol released (y_2, y_4, y_6, y_7).

Results of the coefficient data analysis indicate that the amount of film forming polymer (x_1) and plasticizer (x_3) had the strongest influence on drug release. The amount of film

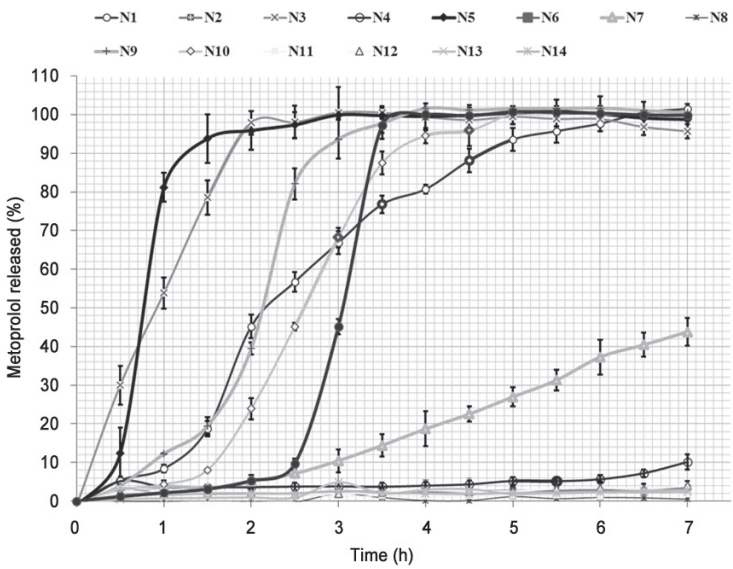

Fig. 2. Amount of metoprolol released at different dissolution time points. N_1 – N_{14} – experiment name according to the experimental design matrix of (Table II).

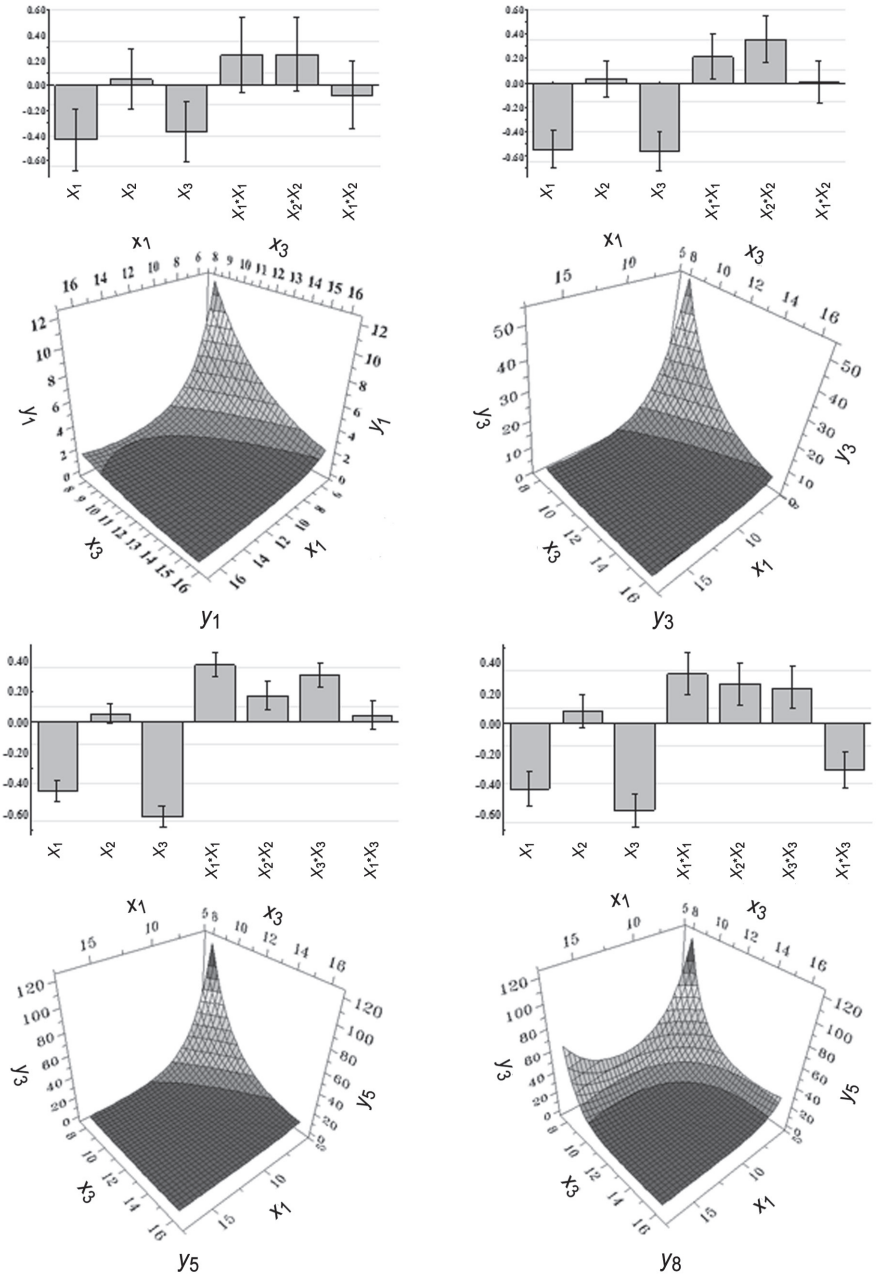

Fig. 3. Influence of the formulation factors on *in vitro* drug release (y_1 – y_8 responses). x_1 – film forming polymer percentage (Eudragit RS); x_2 – swelling agent percentage (sodium starch glycolate); x_3 – plasticizer percentage; y_1, y_3, y_5, y_8, – dependent variables (responses), according to Table I.

forming polymer determines the thickness of the water permeable film. Water enters inside the nucleus making it swell because of the disintegrant (sodium starch glycolate), which leads to breaking of the polymer film. Addition of the plasticizer leads to formation of a more elastic film, which is more resistant to deformation, and thus harder to break. Drug release decreases with the increase of the amounts of film forming polymer and plasticizer. The more polymer is added, the thicker and harder to break is the film. The more plasticizer is added, the more elastic and harder to break is the film. The influence of these two factors increases with time, being smaller at the beginning of the process and then growing as time passes. This influence is small at the beginning because the nucleus swells independently of film thickness at the beginning of the release process. The influence grows with time because the breaking of the film depends on its thickness. Systems involving Eudragit L (25) or a blend of Eudragit RL/RS (26) as coating polymers exhibited a similar release mechanism.

However, the amount of sodium starch glycolate in the nucleus (x_2) did not have a strong influence on the release. This can be explained by the fact that, even though different amounts of sodium starch glycolate were added before the granulation step (10, 15, 20 %), a large amount of microcrystalline cellulose was added after this step. This excipient has a good disintegration property; higher amounts of microcrystalline cellulose increased the disintegration force of the cores, leading to smaller differences between the mixtures with these amounts of sodium starch glycolate (27). Studies have shown that the lag time usually depends on the amount of sodium starch glycolate swelling polymer (28).

Design of experiment analysis. Piecewise equation parameters

In order to analyze the influence of formulation factors on drug release, the piecewise function parameters were introduced as responses (y_9 – concentration at the first turning point, c_2; y_{10} – time at the first turning point, t_1; y_{11} – concentration at the second turning point, c_3; y_{12} – time at the second turning point, t_2) in the experimental design. Coefficients of the equation used to fit the experimental data with the chosen model for profile release evaluation are presented as scaled and centered coefficients and as the response plot surface in Fig. 4.

y_9 represents the concentration at the first turning point (c_2), which is the concentration at the beginning of drug release. When the polymer amount (x_1) increases, the drug concentration at the first turning point decreases because the film obtained is thicker, harder to break, thus releasing a smaller amount of metoprolol. When the amount of plasticizer (x_3) increases, drug concentration at the first turning point decreases because the film obtained is more elastic, harder to break, thus releasing a smaller amount of metoprolol. It can be seen that the influence of these two formulation factors is not linear (x_1x_1 and x_3x_3 have different influence than x_1 and x_3); this can be seen in Fig. 4. y_9. When using small amounts of polymer and plasticizer, a thin and brittle film is formed, easy to break, allowing greater drug release. When using large amounts of polymer and small amounts of plasticizer, the film formed is thick, but easy to break because it is brittle, and when using small amounts of polymer and large amounts of plasticizer, the film formed is elastic, but thin and relatively easy to break. These are the three maximum points of drug concentration, which can be seen in Fig. 4. The amount of sodium starch glycolate (x_2) has no important influence on drug concentration at the first turning point.

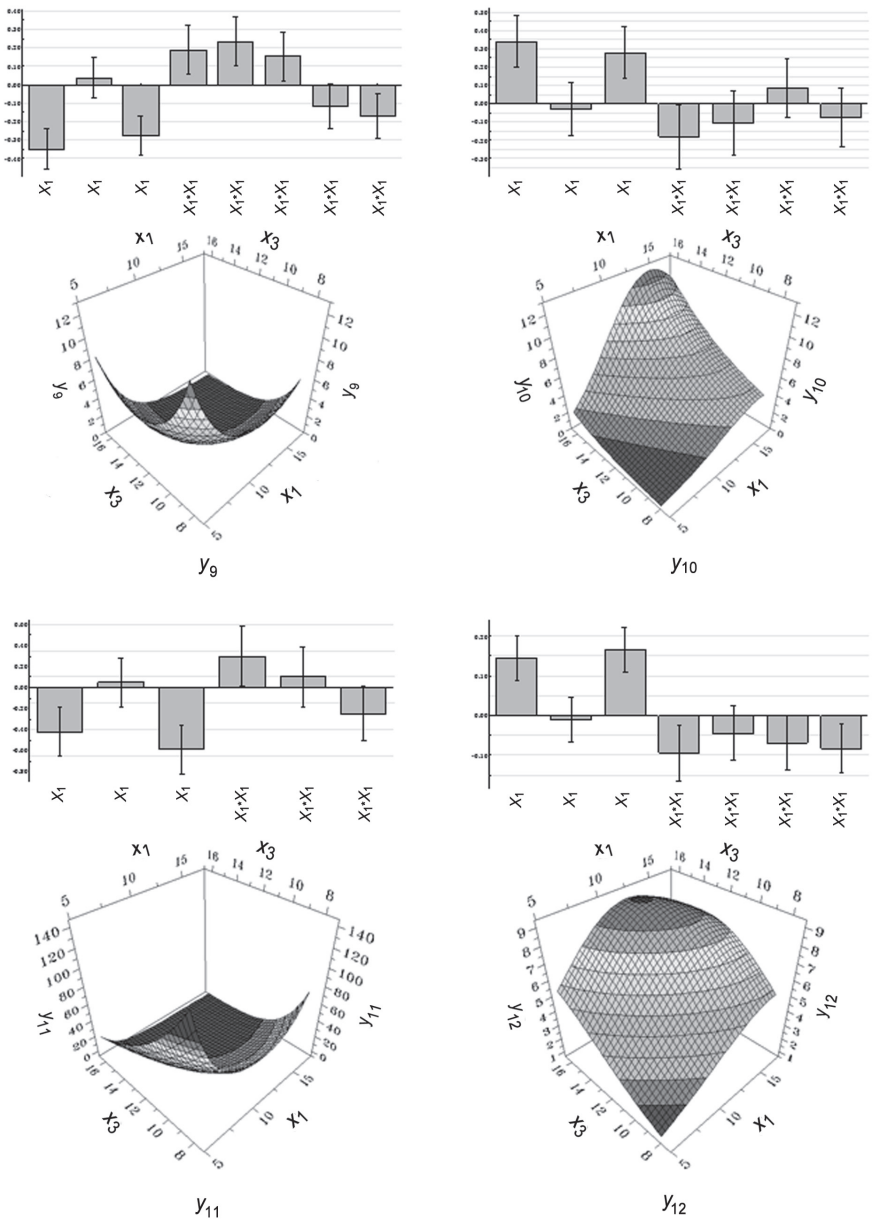

Fig. 4. Influence of the formulation factors on piecewise parameters (y_9–y_{12} responses).

x_1 – film forming polymer percentage (Eudragit RS); x_2 – swelling agent percentage (sodium starch glycolate); x_3 – plasticizer percentage y_9–c_2, concentration at the first turning point; y_{10}–t_1, time at the first turning point; y_{11}–c_3, concentration at the second turningz point; y_{12}–t_2, time at the second turning point.

y_{10} represents the time at the first turning point (t_1), which is the moment when drug release begins. The time at the first turning point increases when the polymer amount increases, and also when the plasticizer amount increases, because the film formed is thick or elastic, harder to break, delaying the drug release. It can be seen that the influence of these two formulation factors is not linear (x_1x_1 and x_3x_3 have a different influence than x_1 and x_3), as can be seen in Fig. 4. y_{10}. There is a maximum of time at the first turning point, which is reached with a pretty large amount of polymer and with the maximum amount of plasticizer, when the film is very thick and elastic, thus very hard to break. However, there is a small decrease in time when increasing the polymer amount to maximum. Sodium starch glycolate (x_2) does not have a significant influence on time at the first turning point.

y_{11} represents the concentration at the second turning point (c_3), which is the maximum amount of drug released. When the polymer amount increases, the drug concentration at the second turning point decreases, because the film obtained is thicker, harder to break, thus releasing a smaller amount of metoprolol. When the amount of plasticizer increases, drug concentration at the second turning point decreases because the film obtained is more elastic, harder to break, thus releasing a smaller amount of metoprolol. It can be seen that the influence of these two formulation factors is not linear (x_1x_1 and x_3x_3 have a different influence than x_1 and x_3), as can be seen in Fig. 4. y_{11}. When using small amounts of polymer and plasticizer, a thin and brittle film is formed, easy to break, allowing a greater drug release. When using large amounts of polymer and small amounts of plasticizer, the film formed is thick, but easy to break because it is brittle, and when using small amounts of polymer and large amounts of plasticizer, the film formed is elastic, but thin and relatively easy to break. These are the three maximum points of drug concentration, which can be seen in Fig. 4, surface response plot for y_{11} response. The amount of sodium starch glycolate (x_2) has no important influence on drug concentration at the second turning point.

y_{12} represents the time at the second turning point (t_2), which is the moment when drug release reaches its maximum. The time at the second turning point increases when the polymer amount increases, and also when the plasticizer amount increases, because the film formed is thick or elastic, harder to break, delaying the moment when drug release ends. It can be seen that the influence of these two formulation factors is not linear (x_1x_1 and x_3x_3 have a different influence than x_1 and x_3), and this can be seen in Fig. 4.$y_{12.}$ There is maximum time at the second turning point, which is reached with quite a large amount of polymer and with the maximum amount of plasticizer, when the film is very thick and elastic, thus very hard to break. However, there is a small decrease in time when increasing the polymer amount to the maximum. Sodium starch glycolate (x_2) has no significant influence on the time at the second turning point.

Optimum formula determination

For a pulsatile release chronopharmaceutical system, the dissolution profile of the experimental formulation should be compared with the ideal shape of a pulsatile release chronopharmaceutical system. This dissolution profile should have a sigmoid shape and two turning points (Fig. 1) and may be characterized by the parameters of the piecewise three segment linear function.

In order to obtain such a dissolution profile, the requirements during the optimization step were to minimize the concentration at the first turning point (y_9), to obtain a target of a minimum of 2.5 h for the time at the first turning point (y_{10}), to maximize the concentra-

tion at the second turning point (y_{11}) and to minimize the time at the second turning point (y_{12}). The optimum formula was calculated using the optimization module from Modde 10.0 software. The level of the formulation factor for the optimum formula and the results of the optimum formula are shown in Table III. As seen in the last column of Table III,

Table III. The optimum formula

Independent variables (formulation factors)		Value level	
x_1	film forming polymer percentage (Eudragit RS)	13.21	
x_2	swelling agent percentage (sodium starch glycolate)	17.90	
x_3	plasticizer percentage (triethylcitrate)	8.00	
Dependent variables (responses)		Theoretical (predicted)	Practical (obtained)
y_1	Cumulative % of metoprolol released after 1.0 h	4.040	4.13
y_2	Cumulative % of metoprolol released after 2.0 h	8.050	8.26
y_3	Cumulative % of metoprolol released after 2.5 h	11.111	20.68
y_4	Cumulative % of metoprolol released after 3.0 h	23.385	45.17
y_5	Cumulative % of metoprolol released after 3.5 h	46.513	97.23
y_6	Cumulative % of metoprolol released after 4.0 h	89.246	100.00
y_7	Cumulative % of metoprolol released after 5.0 h	90.274	100.73
y_8	Cumulative % of metoprolol released after 7.0 h	101.263	99.83
y_9	c_2 – concentration at the first turning point	10.271	15.68
y_{10}	t_1 – time at the first turning point	2.699	2.53
y_{12}	c_3 – concentration at the second turning point	91.324	100.24
y_{13}	t_2 – time at the second turning point	3.941	3.53

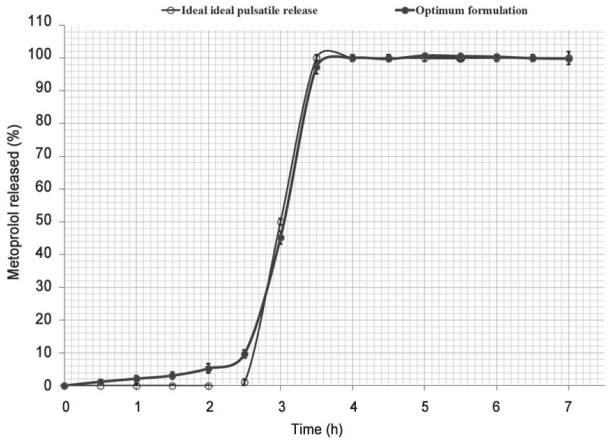

Fig. 5. Drug release from the optimum formula in comparison with ideal pulsatile drug delivery.

practical results obtained with the optimum formula are relatively close to the value predicted *via* experimental design. The ideal shape of the dissolution profile of a pulsatile release chronopharmaceutical system that has a lag time of 2.5 h followed by rapid release (maximum one h) is a sigmoid shape with two turning points at 2.5 and 3.5 h. This profile is very similar to the dissolution profile of the optimum formulation (Fig. 5).

The differences are only in the first part of the dissolution profile, but the percentage of drug released until the first turning point (2.5 h) is less than 10 %. Thus, using the optimization module from Modde 10.0 software and piecewise function parameters as desired responses, the theoretical optimum formula was determined. Moreover, the dissolution profile of the optimal formulation has a sigmoid shape and a lag time of 2.5 h, followed by rapid drug release (in one h).

CONCLUSIONS

In this work, a Box Behnken DoE and piecewise three segment linear function parameters were used to develop a pulsatile release chronopharmaceutical system made up of mini-tablets with an increased capacity to swell, coated with an insoluble and slowly permeable but breakable polymeric film. Among the studied factors (amount of coating polymer, percentage of core swelling agent and percentage of film plasticizer in polymeric film), two had a great influence on the release profiles: the amount of film forming polymer and the percentage of plasticizer added. Increasing the amount of film forming polymer leads to a thicker film, harder to break, leading to delayed drug release, or in case of large values, to stopping the drug release altogether. Similarly, increasing the percentage of plasticizer leads to formation of a more elastic film, less brittle, which causes a delay of drug release or even stopping of the drug release at higher concentrations. Using piecewise function parameters as DoE responses, an optimal formulation with a sigmoid shape dissolution release profile and a lag time of 2.5 h, followed by rapid drug release, was obtained.

This work illustrates the possibility of modulating the drug release profile from the pulsatile release chronopharmaceutical system by using DoE to study the influence of formulation factors on the *in vitro* dissolution behavior and piecewise three segment linear function parameters as responses for dissolution release profile shape characterization. The results of this study may be exploited for further design of novel pulsatile drug release pharmaceutical systems.

REFERENCES

1. A. E. Yassin, A. H. Aodah, S. Al-Suwayeh and E. I. Taha, Theophylline colon specific tablets for chronotherapeutic treatment of nocturnal asthma, *Pharm. Dev. Technol.* **17** (2012) 712–718; DOI: 10.3109/10837450.2011.572896.
2. K. P. Pagar and P. R. Vavia, Felodipine β-cyclodextrin complex as an active core for time delayed chronotherapeutic treatment of hypertension, *Acta Pharm.* **62** (2012) 395–410. DOI: 10.2478/v10007-012-0023-0.
3. V. D. Kadam and S. G. Gattani, Formulation and evaluation of a pulsatile drug delivery system using time- and pH-dependent polymers, *Pharm. Dev. Technol.* **15** (2010) 57–63; DOI: 10.3109/10837450902980254.

4. R. C. Hermida, D. E. Ayala, C. Calvo, F. Portaluppi and M. H. Smolensky, Chronotherapy of hypertension: administration-time-dependent effects of treatment on the circadian pattern of blood pressure, *Adv. Drug Deliv. Rev.* **59** (2007) 923–39; DOI: 10.1016/j.addr.2006.09.021
5. F. Portaluppi, R. Tiseo, M. H. Smolensky, R. C. Hermida, D. E. Ayala and F. Fabbian, Circadian rhythms and cardiovascular health, *Sleep Med. Rev.* **16(2)** (2012) 151–166; DOI: 10.1016/j.smrv.2011.04.003.
6. A. El Sayeh, F. Abou el Ela, E. H. Ibrahim and A. A. Allam, Bucco-adhesive tablets containing metoprolol tartarate: formulation, in vitro and in vivo characterization, *J. Drug Del. Sci. Tech.* **23** (2013) 171–179; DOI: 10.1016/S1773-2247(13)50026-8.
7. H. Ravishankar, P. Patil, A. Samel, H. U. Petereit, R. Lizio and J. Iyer-Chavan, Modulated release metoprolol succinate formulation based on ionic interactions: In vivo proof of concept, *J. Control. Release* **111** (2006) 65–72; DOI: 10.1016/j.jconrel.2005.12.007.
8. R. C. Hermida, D. E. Ayala and F. Portaluppi, Circadian variation of blood pressure: The basis for the chronotherapy of hypertension, *Adv. Drug Deliv. Rev.* **59** (2007) 904–922; DOI: 10.1016/j.addr.2006.08.003.
9. D. A. Taylor and A. A. Abdel-Rahman, Novel strategies and targets for the management of hypertension, *Adv. Pharmacol.* **57** (2009) 291–345; DOI: 10.1016/S1054-3589(08)57008-6.
10. T. Bussemer and R. A. Bodmeier, *Drug Delivery Pulsatile Systems*, in *Encyclopaedia of Pharmaceutical Technology* (Ed. J. Swarbrick) 3rd ed., Informa Healthcare, New York 2007, pp. 1287–1297.
11. A. Maroni, L. Zema, G. Loreti, L. Palugan and A. Gazzaniga, Film coatings for oral pulsatile release, *Int. J. Pharm.* **457** (2013) 362–371; DOI: 10.1016/j.ijpharm.2013.03.010.
12. L. Palugan, M. Cerea, L. Zema, A. Gazzaniga and A Maroni, Coated pellets for oral colon delivery, *J. Drug Del. Sci. Tech.* **25** (2015) 1–15; DOI: 10.1016/j.jddst.2014.12.003
13. Y. Zhang, M. Huo, J. Zhou, A. Zou, Li W, Yao C and Xie S, DDSolver: an add-in program for modeling and comparison of drug dissolution profiles, *AAPS J.* **12** (2010) 263–71; DOI: 10.1208/s12248-010-9185-1.
14. S. Dash, P. N. Murthy, L. Nath and P. Chowdhury, Kinetic modeling on drug release from controlled drug delivery systems, *Acta Pol. Pharm.* **67** (2010) 217–223.
15. Y. Liu, S. Liu, Q. Dai, Design and evaluation of pH-independent pulsatile release pellets containing isosorbide-5-mononitrate, *Chem. Pharm. Bull.* **7** (2009) 55–60; DOI: 10.1248/cpb.57.55.
16. Sigma Plot 11, *Statistical User Guide Manual*, Systat Software Inc, 2008.
17. A. L. Vonica, I. Tomuță and S. E. Leucuța, Experimental design for the screening of three types of coating polymers for metoprolol minitablets with pulsatile release, *Farmacia* **59** (2011) 742–749.
18. I. Tomuță, C. Alecu, L. L. Rus and S. E. Leucuța, Optimization of fluid bed formulations of metoprolol granules and tablets using an experimental design, *Drug Dev. Ind. Pharm.* **35** (2009) 1072–1081; DOI: 10.1080/03639040902774149.
19. *European Pharmacopoeia* 8.2. Online Edition 2014.
20. S. Varigonda, T. T. Georgiou, R. A. Siegel and P. Daoutidis, Optimal periodic control of a drug delivery system, *Comput. Chem. Eng.* **32** (2008) 2256–2262; DOI: 10.1016/j.compchemeng.2007.11.003.
21. H. Wang, J. Li and Y. Kuang, Mathematical modeling and quantitative analysis of insulin therapies, *Math. Biosci.* **210** (2007) 17–33; DOI: 10.1016/j.mbs.2007.05.008.
22. L. Eriksson, E. Johansson, N. Kettaneh-Wold, C. Wikström and S. Wold, *Design of Experiments, Principles and Applications,* 3rd ed., Umetrics Academy, Umea 2008.
23. MODDE 10. *Software for Design of Experiments and Optimization: User's Guide and Tutorial*. Umetrics Academy, Umea 2014.
24. I. Tomuță and S.E. Leucuta, Development and in vitro evaluation of multiparticulate sustained release carbamazepine formulation, *Acta Pol. Pharm.* **69** (2012) 951–964.

25. S. F. Hung, C. M. Hsieh, Y. C. Chen, C. M. Lin, H. O. Ho and M. T. Sheu, Formulation and process optimization of multiparticulate pulsatile system delivered by osmotic pressure-activated rupturable membrane, *Int. J. Pharm.* **480** (2015) 15–26; DOI: 10.1016/j.ijpharm.2015.01.006.
26. M. Desai, R. R. Jivani, L. D. Patel, N. P. Jivani and B. Sonagara, Development of time controlled chronomodulated tablet with swelling and rupturable layers: Optimization of factors influencing lag-time and drug release, *Int. J. Pharm. Investig.* **2** (2012) 208–212; DOI: 10.4103/2230-973*x*.107005.
27. S. Sungthongjeen, S. Puttipipatkhachorn, O. Paeratakul, A. Dashevsky and R. Bodmeier, Development of pulsatile release tablets with swelling and rupturable layers, *J. Control. Release* **95** (2004) 147–159; DOI: 10.1016/j.jconrel..2003.10.023.
28. T. Bussemer, N. A. Peppas and R. Bodmeier, Evaluation of the swelling, hydration and rupturing properties of the swelling layer of a rupturable pulsatile drug delivery system, *Eur. J. Pharm. Biopharm.* **56** (2003) 261–270; DOI: 10.1016/S0939-6411(03)00070-5.