NASTAVNO-NAUČNOM VEĆU FARMACEUTSKOG FAKULTETA
UNIVERZITETA U BEOGRADU
TO THE ACADEMIC COUNCIL OF THE FACULTY OF PHARMACY – UNIVERSITY OF BELGRADE

KOMISIJI ZA POSLEDIPLOMSKE STUDIJE
TO THE COMMITTEE FOR POSTGRADUATE STUDIES

Na sednici Nastavno-naučnog veća Farmaceutskog fakulteta, Univerziteta u Beogradu, održanoj 28.04.2016. godine imenovani su članovi Komisije za ocenu i odbranu završene doktorske disertacije kandidata Jovane Kovačević, magistra farmacije, pod naslovom:

The Academic Council of the Faculty of Pharmacy, University of Belgrade on the meeting held on 28/04/2016 has nominated the Committee for evaluation and defense of doctoral dissertation of Jovana Kovačević, master of pharmacy entitled:

Farmaceutski razvoj gastrorezistentnih peleta uz primenu koncepta dizajna kvaliteta i tehnika veštačke inteligencije

Farmaceutical development of enteric-coated pellets by application of quality by design concept and artificial intelligence techniques

Komisija u sastavu/*Committee consisting of*:

1. Dr sc Svetlana Ibrić, mentor rada, redovni profesor, Univerzitet u Beogradu-Farmaceutski fakultet/ *supervisor, full professor, University of Belgrade-Faculty of Pharmacy*
2. Dr sc Jelena Đuriš, docent, Univerzitet u Beogradu-Farmaceutski fakultet/*assistant professor, University of Belgrade-Faculty of Pharmacy*
3. Dr sc Tijana Miletić, naučni saradnik, Hemofarm a.d. Vršac/*scientific associate Hemofarm a.d. Vršac*
4. Doc dr Aleksandar Kovačević, Fakultet tehničkih nauka, Univerzitet u Novom Sadu/ *assistant professor, University of Novi Sad-Faculty of Technical Sciences*

5. Prof dr Peter Klainebudde, redovni profesor, Institut za farmaciju i biofarmaciju, Hajnrih Hajne univerzitet u Dizeldorfu/ *full professor, Institute of Pharmaceutics and Biopharmaceutics, Heinrich-Heine-University, Duesseldorf, Germany*

pregledala je priloženu disertaciju i podnosi Nastavno-naučnom veću Farmaceutskog fakulteta Univerziteta u Beogradu sledeći/

based on detailed review of the submitted dissertation, presents to the Academic Council of the Faculty of Pharmacy-University of Belgrade the following

IZVEŠTAJ/*REPORT*

A. PRIKAZ SADRŽAJA DOKTORSKE DISERTACIJE/*THE CONTENT OF THE DOCTORAL DISSERTATION*

Doktorska disertacija kandidata magistra farmacije Jovane Kovačević, pod nazivom ***„ Farmaceutski razvoj gastrorezistentnih peleta uz primenu koncepta dizajna kvaliteta i tehnika veštačke inteligencije”***, napisana je na 164 strane i sadrži pet poglavlja: Uvod, Ciljeve rada, Eksperimentalni deo, sa rezultatima i diskusijom za svaku od tri faze istraživanja, Zaključak i Literaturu. Na početku disertacije dat je sažetak rada na srpskom i engleskom jeziku, dok je na kraju rada data biografija autora i prilozi u okviru kojih su prikazani rezultati disertacije koji su do sada publikovani u okviru radova u naučnim časopisima. Disertacija je napisana jasnim i preglednim stilom i sadrži 48 slika, 47 tabela i 116literaturnih navoda.

*Doctoral dissertation candidate Jovana Kovačević, master of pharmacy, entitled **“Pharmaceutical development of enteric-coated pellets by application of quality by design concept and artificial intelligence techniques”**, is written on 164 pages and comprises five chapters: Introduction, The aims of research, Experimental part with results and discussion of three phases of the research, Conclusion and References. In the beginning of the dissertation a short abstract is given on both Serbian and English languages, while dissertation ends with the author’s biography and supplements containing results of the dissertation published in the scientific journals. Dissertation is written in a clear and concise style and contains 48 figures, 47 tables and 116 references.*

U **Uvodnom delu** su date teorijske osnove koje su od značaja za predmet proučavanja doktorske disertacije. Uvodni deo disertacije se sastoji iz dva odeljka. U prvom odeljku uvodnog dela izneti su osnovni principi na kojima se zasniva primena peleta kao višečestičnih sistema koji omogućavaju veću fleksibilnost u farmaceutskom razvoju i pružaju veću terapeutsku korist pacijentima u odnosu na pojedinačne farmaceutske oblike. Na koncizan i jasan način su prikazane glavne tehnike oblaganja koje se primenjuju u izradi peleta na laboratorijskom i industrijskom nivou. Za svaku od opisanih tehnika izrade ukratko su prikazani osnovni teorijski principi proizvodnog procesa kao i prednosti, osnovna ograničenja i potencijalni rizici koje nosi primena pojedinih tehnika i kojih treba biti svestan prilikom odabira određene proizvodne tehnike. Poseban osvrt dat je na potencijal svake od predstavljenih tehnika da izazove promenu kristalnog oblika lekovite supstance u toku procesa proizvodnje na šta je potrebno pažnju u fazi razvoja. U drugom odeljku uvodnog dela opisani su pristupi primene koncepta dizajna kvaliteta i soft computing tehnika veštačke inteligencije kao različiti pristupi koji mogu da se primene u cilju sticanja višeg stepena znanja o proizvodu u fazi razvoja. Predstavljeni su osnovni principi na kojima se zasniva analiza rizika i metodologija eksperimentalnog dizajna koji su alatke dizajna kvaliteta korišćene u pojedinim delovima istraživanja. Kako je u istraživanju za analizu rizika korišćena tehnika preliminarne analize opasnosti navedene su njene osnovne karakteristike. Predstavljeni su osnovni principi na kojima se zasniva metodologija eksperimentalnog dizajna, navedene su osnovne karakteristike dizajna prvog reda koji se koriste u skrining fazi istraživanja i metodologije površine odgovora koja se koristi u fazi optimizacije. Prikazani su osnovni principi soft computing tehnika veštačke inteligencije (multipla linearna regresija, stepwise regresija, laso regresija, grebenasta regresija, elastične mreže, regresiona stabla, ansambl regresionih stabala dobijen tehnikom "boosting", veštačke neuronske mreže) čija primenljivost u rešavanju regresionih problema u farmaceutskom razvoju je kasnije definisana kao jedan od ciljeva istraživanja. Detaljno je prikazan proces razvoja modela veštačkih neuronskih mreža i primeri primene veštačkih neuronskih mreža u farmaceutskoj tehnologiji. Opisan je i način na koji se ocenjuju razvijeni modeli.

The Introduction contains theoretical bases relevant for the subject of doctoral dissertation. The introduction is organized in two parts. The first part of the introduction gives basic principles of application of pellets as multiparticulate dosage forms which allow for greater flexibility in the pharmaceutical development and therapeutic benefits to the patients in

comparison with monolithic dosage forms. Some of the most widespread techniques of production of coated pellets in laboratory and industrial scale are given in a clear and concise manner. Basic theoretical principles of production process are briefly described for each presented production technique. Advantages, basic limitations and potential risks that application of certain technique carries are also given. In the development phase particular emphasis is put on the preservation of solid state form of the drug substance so evaluation of potential of each presented manufacturing technique to cause these changes is analyzed. The second part of the introduction comprises description of quality by design concept and soft computing techniques of artificial intelligence that can be used in the pharmaceutical development phase to gain enhanced knowledge about the product. Fundamentals of risk analysis and experimental design as quality by design tools used in certain parts of the research are presented. Owing to the fact that preliminary hazard analysis was the risk analysis technique used in the research, its main characteristics are presented. Basic principles of experimental design methodology are given, as well as main features of first order designs that are used in the screening phase and main features of response surface methodology that is used in the optimization phase. This chapter comprises the description of the principal characteristics of soft computing techniques of artificial intelligence (multiple linear regression, stepwise regression, lasso regression, ridge regression, elastic net, regression trees, ensemble of regression trees obtained by "boosting" technique, artificial neural networks) whose applicability in solving regression problems in the formulation development was later defined as one of the aims of the research. The development of artificial neural networks model is described in details with examples of their use in pharmaceutical technology. The method for evaluation of obtained models was described, too.

Ciljevi istraživanja su definisani kao ispitivanje različitih koncepata farmaceutskog razvoja gastrorezistentnih peleta u smislu ispitivanja različitih tehnika proizvodnje gastrorezistentnih peleta i različitih metodologija za postizanje višeg stepena znanja o proizvodu u fazi razvoja. Navedeni ciljevi su jasno definisani i na osnovu postavljenih ciljeva istraživanje je podeljeno u tri faze.

The aims of the research *are defined as evaluation of use of different concepts in the pharmaceutical development of enteric-coated pellets in terms of comparation of different techniques of manufacturing enteric-coated pellets and different methodologies for acquiring*

enhanced knowledge about the product during the development phase. These aims are clearly defined and based on the defined aims the whole research is divided into three phases.

Eksperimentalni deo. Eksperimenti koji čine doktorsku disertaciju realizovani su u okviru tri faze.

Experimental part. *Experiments from this doctoral dissertation have been conducted in three phases.*

U **prvoj fazi** istraživanja razmatrana je mogućnost primene različitih tehnika oblaganja peleta aktivnom supstancom i gastrorezistentnim filmom. Dat je pregled fizičko-hemijskih karakteristika duloksetin hidrohlorida koji je bio model supstanca u istraživanju, kao i pregled i obrazloženje za izbor ekscipijenasa korišćenih u tom delu istraživanja. Detaljnije je opisan gastrorezistentni polimer hipromeloza acetat sukcinat koji je korišćen. Ispitane tehnike oblaganja peleta aktivnom supstancom su sledeće: suvo oblaganje, oblaganje rastvorenom aktivnom supstancom i oblaganje suspendovanom aktivnom supstancom. Ispitane tehnike oblaganja gastrorezistentnim polimerom su: suvo oblaganje i oblaganje raspršivanjem suspenzije za oblaganje u kojoj je gastrorezistentni polimer rastvoren. Opisan je postupak izrade obloženih peleta koji podrazmeva oblaganje inaktivnih peleta sa tri sloja: slojem aktivne supstance, izolacionim slojem koji sprečava kontakt aktivne supstance i gastrorezistentnog polimera i gastrorezistentnim slojem koji sprečava razgradnju duloksetina u kiseloj sredini želuca. Dobijeni uzorci peleta su nakon svake faze oblaganja podvrgnuti detaljnoj karakterizaciji morfoloških osobina i fizičko-hemijskih karakteristika. U navedenom delu doktorske disertacije detaljno su opisane sve eksperimentalne procedure vezane za karakterizaciju uzoraka primenom skenirajuće elektronske mikroskopije (SEM) i optičke mikroskopije i softvera za analizu slike pomoću koga su određeni parametri: kružni prečnik peleta, stepen izduženosti, sferičnost projekcije i hrapavost površine. Sadržaj duloksetina je nakon svake faze proizvodnje u peletama ispitan metodom tečne hromatografije pod visokim pritiskom (HPLC) da bi se utvrdila efikasnost tehnika oblaganja, a takođe je ispitan i profil brzine rastvaranja peleta da bi se utvrdio uticaj nanesenog filma na oslobađanje duloksetina iz peleta. Nakon svake faze gravimetrijskom metodom u halogenom analizatoru je određen gubitak sušenjem peleta i određena im je i nasipna gustina. U okviru studije stabilnosti je HPLC metodom određivan sadržaj nečistoća duloksetina nakon 12, 24 i 36 meseci čuvanja gastrorezistentnih peleta u ambijentalnim uslovima.

*In the **first phase** of research, the possibility of preparation of pellets layered with drug and coated with enteric-coating by using different coating techniques is evaluated. An overview of physico-chemical characteristics of duloxetine hydrochloride that was the model drug in the research, as well as overview of used excipients and rationale for their inclusion in the formulation. An overview of physicho-chemical characteristics of hypromellose acetate succinate that was used for enteric coating of pellets is given. Drug layering techniques that were examined and compared were: powder layering, solution layering and suspension layering. Enteric coating techniques that were examined and compared were: dry coating and spray coating of suspension containing dissolved enteric polymer. The method of preparation of coated pellets is described and it is used to produce pellets having three different layers: drug layer, isolating layer that prevents contact of duloxetine hydrochloride and enteric coating polymer and enteric coating layer that prevents degradation of duloxetine in the stomach. Obtained samples of pellets were subjected to morphological and physicochemical characterization after each coating step. This part of the doctoral dissertation comprises description of all experimental procedures for characterization of morphological features of the samples by using scanning electron microscopy (SEM) and optical microscopy and image analysis software which were used for determining following parameters: circular diameter of pellets, aspect ratio, roundness and roughness . Efficacy of each evaluated coating technique is evaluated by determining the assay of duloxetine in pellets after each coating step. Dissolution profile of pellets was also examined after each coating step to asses the effect of the applied film on release of duloxetine from pellets. Bulk density and loss on drying of pellets were determined after application of each layer. In the course of stability study, levels of impurities of duloxetine were determined by using HPLC method. Impurities were detected in enteric-coated pellets stored in ambient conditions after 12, 24 and 36 months.*

U **drugoj fazi** istraživanja izvršena je optimizacija formulacije i procesa proizvodnje peleta obloženih aktivnom supstancom. Korišćene su tehnike oblaganja rastvorenom i suspendovanom aktivnom supstancom. U fazi skrininga je primenom frakcionog faktorijalnog dizajna tipa 2^{8-4} ispitan uticaj 8 ulaznih formulacijskih (viskozitet i udeo hidroksipropil metilceluloze 6 cp u tečnosti za oblaganje, rastvarač za pripremu tečnosti za oblaganje, prisustvo talka u formulaciji, koncentracija suvih supstanci u tečnosti za oblaganje) i procesnih promenljivih (protok ulaznog vazduha, temperatura proizvoda u fazi prskanja tečnosti za oblaganje i protok tečnosti za oblaganje) na prinos i koristan prinos proizvodnog

procesa. U cilju objašnjena rezultata faze skriniga pripremljeni su slobodni filmovi koji su sadržali duloksetin hidrohlorid i hidroksipropil metilcelulozu 6 cp u odnosima od 2:1 do 2.5 i slobodni filmovi koji su sadržali duloksetin hidrohlorid i povidon K-30 u odnosima od 1:1 do 1:2.6. Slobodni filmovi su analizirani tehnikom diferencijalne skenirajuće kalorimetrije. U navedenom delu doktorske disertacije detaljno su opisane sve eksperimentalne procedure vezane za fizičkohemijsku karakterizaciju uzoraka primenom diferencijalne skenirajuće kalorimetrije (*DSC*). Opisana je i priprema amorfnog duloksetin hidrohlorida primenom tehnike sušenja raspršivanjem iz metanolnog rastvora. Primenom centralnog kompozitnog dizajna i metodologije površine odgovora u fazi optimizacije detaljnije je ispitan efekat udela hidroksipropil metilceluloze u suspenziji za oblaganje i koncentracije suspenzije za oblaganje na efikasnost i koristan prinos procesa proizvodnje, na viskozitet suspenzije za oblaganje i vreme trajanja procesa. Razvijeni su modeli drugog reda koji su iskorišćeni za definisanje opsega ulaznih parametara pri kojima su u najvećoj mogućoj meri ispunjeni kriterijumi za željene vrednosti izlaznih parametara.

*In the **second phase** of research, optimization of formulation and production process of drug layered pellets was performed. Drug layering was performed by using solution and suspension coating in a fluid-bed device. Fractional factorial design* 2^{8-4} *was used to examine the influence of 8 formulation (viscosity and level of hypromellose 6 cp in the coating liquid formulation, solvent for preparation of coating liquid, presence of talc in the formulation, concentration of solids in the coating liquid) and process (inlet air flow rate, product temperature during spraying, spray rate) factors on yield and useful yield of the production process. In order to explain the results of the screening experiments free films containing duloxetine hydrochloride and hypromellose 6 cp in ratios from 2:1 do 2.5 were prepared. Free films containing duloxetine hydrochloride and povidone K-30 in ratios from 1:1 do 1:2.6 were prepared as well. Free films were subjected to analysis by differential scanning calorimetry (DSC) according to procedure described in this part of doctoral thesis. Preparation of amorphous duloxetine hydrochloride is described as well. In the optimization phase, central composite design and response surface methodology were employed to get a closer look to effect of level of hypromellose and solids content in the coating suspension on yield, useful yield, viscosity of coating suspension and time of production process. Second order models were developed and used to define the range of input variables within which the set criteria for target values of response variables are met to the greatest possible extent.*

U **trećoj fazi** istraživanja ispitivana je mogućnost primene soft computing tehnika veštačke inteligencije kao metodologija za postizanje višeg stepena znanja o proizvodu u fazi razvoja. Ispitane su sledeće tehnike: multipla linearna regresija, stepwise regresija, grebenasta regresija, laso regresija, elastične mreže, regresiona stabla, ansambl regresionih stabala dobijen tehnikom "boosting" i veštačke neuronske mreže. U ovom delu doktorske disertacije opisani su i softveri koji su korišćeni za primenu ovih tehnika. Za razvijanje modela korišćeni su istorijski podaci iz 19 eksperimenata izvedenih po principu proba i greška. Ispitan je uticaj 6 kvalitativnih i 10 kvantitativnih ulaznih promenljivih. Izlazi koji su praćeni su gastro-rezistencija i procenat oslobođenog duloksetina nakon 5, 10, 15, 20, 30, 45, 60 i 90 minuta testa ispitivanja brzine rastvaranja. Za razvijanje modela veštačkim neuronskim mrežama skup podataka je podeljen na skup za trening (80% podataka), test skup (10% podataka) i skup za validaciju (10% podataka). Za razvijanje model pomoću svih ostalih soft computing tehnika skup podataka je podeljen na skup za trening (90% podataka) i test skup (10% podataka). Performanse razvijenih modela su procenjene na osnovu rezultata na test skupu podataka. Favorizani su modeli sa nižim vrednostima korena kvadrata srednje greške (engl. root mean squared error – RMSE) za pojedinačne tačke profila oslobađanja duloksetina i modeli koji su imali viši faktor sličnosti predviđenih i eksperimentalno dobijenih profila oslobađanja duloksetina.

Sve metode korišćene u radu su u skladu sa savremenim standardima naučnoistraživačkog rada u naučnoj oblasti u kojoj je vršeno istraživanje. Prikazana metodologija omogućava dobijanje jasnih i nedvosmislenih rezultata kojim se ostvaruju postavljeni ciljevi istraživanja.

*The possibility of using soft computing techniques of artificial intelligence as a methodology for obtaining enhanced knowledge of the product during the development phase was explored in the **third phase** of the research. Following soft computing techniques were evaluated: multiple linear regression, stepwise regression, ridge regression, lasso regression, elastic net, regression trees, ensemble of regression tress obtained by "boosting" technique and artificial neural networks. In this part of the doctoral dissertation software used to apply these techniques were described. Historical data comprising results of 19 laboratory trials performed according to trial and error concept were used to develop models. Effects of 6 qualitative and 10 quantitative inlet variables were evaluated. Responses that were followed were gastric resistance and percent of released duloxetine after 5, 10, 15, 20, 30, 45, 60 and 90 minutes of dissolution test. For developing models by using artificial networks, data was*

divided to training set (80% of data), test set (10% of data) and validation set (10% of data). For developing models by all other soft computing techniques the data set was divided to training set (90% of data) and test set (10%) of data. Performance of models was evaluated on account of results obtained for test data. Models having lower root mean squared error (RMSE) of predicted release of duloxetine at single points of release profile as well as models having higher similarity factor between release predicted and experimentally obtained release profiles were favored.

All methods used in the doctoral dissertation are in accordance with the up-to-date standard of scientific work in this field of research. The presented methodology enables obtaining of clear and unambiguous results that meet defined aims of the research.

U okviru svake faze eksperimentalnog dela prikazani su dobijeni **rezultati**, zajedno sa **diskusijom** i poređenjem dobijenih rezultata sa trenutno dostupnim literaturnim podacima.

*Each section of the experimental part contains obtained **results** with the following **discussion** and comparison of the obtained results with the currently available literature data.*

Na kraju disertacije prikazani su **zaključci** u odnosu na prethodno postavljene ciljeve za svaku od sprovedenih faza istraživanja.

***Conclusions** are given at the end of dissertation in relation to predefined aims for each of the research phases.*

B. OPIS POSTIGNUTIH REZULTATA/*DESCRIPTION OF THE OBTAINED RESULTS*

U okviru **prve faze** istraživanja, uspešno su primenjene sledeće tehnike oblaganja peleta aktivnom supstancom: suvo oblaganje, oblaganje rastvorenom aktivnom supstancom i oblaganje suspendovanom aktivnom supstancom. Izolacioni film je na sve vrste peleta obloženih aktivnom supstancom nanošen tehnikom oblaganja suspenzijom. Takođe su uspešno proizvedene i gastrorezistentne pelete tehnikom suvog oblaganja i raspršivanjem suspenzije za oblaganje u kojoj je gastrorezistentni polimer rastvoren. Rezultati analize slika peleta sa optičkog mikroskopa i skenirajućeg elektronskog mikroskopa pokazale su da postoje

značajne razlike u morfološkim karakteristikama peleta obloženih tehnikom suvog oblaganja u odnosu na tehnike u koji je tečnost korišćena kao vehikulum za transport aktivne supstance do peleta. Pelete proizvedene tehnikom suvog oblaganja imaju lošije parametre oblika od peleta proizvedenim ostalim tehnikama t.j. širu raspodelu veličine sa prečnikom od 1084.4 μm ±141.1 μm, neravniju površinu sa hrapavošću od 0.84 ± 0.04 i lošiju sferičnost sa stepenom izduženosti od 1.17 ± 0.09 i sferičnošću projekcije od 0.77 ± 0.06. Pelete proizvedene oblaganjem rastvorene aktivne supstance bile su okarakterisane prečnikom 1270.0 μm ± 52.3 μm, hrapavošću od 0.97 ± 0.01, stepenom izduženosti od 1.09 ± 0.06 i sferičnošću projekcije od 0.90 ± 0.04. Pelete proizvedene oblaganjem suspendovanom aktivnom supstancom su okarakterisane prečnikom od 1086.2 μm ± 54.5 μm, hrapavošću od 0.97 ± 0.01, stepenom izduženosti od 1.11 ± 0.07 i sferičnošću projekcije od 0.89 ± 0.06. Ovakav trend rezultata parametara koji karakterišu oblik i osobine površine peleta se nastavljaju i u preostale dve faze proizvodnje gastrorezistentnih peleta. Gastrorezistentne suvo obložene pelete su se jedino po vrednosti parametra hrapavost (0.95 ± 0.01) približile vrednosti od 0.97 ± 0.01 koje su zabeležene za druge dve tehnike. Oslobađanje duloksetina iz peleta u svim fazama procesa proizvodnje je potpuno. Na osnovu izračunatog faktora sličnosti, f_2, profili oslobađanja duloksetina iz peleta obloženih suvim oblaganjem i oblaganjem suspendovanom aktivnom supstancom smatraju se sličnim (f_2=69.8). To nije slučaj sa peletama obloženim suspendovanom i rastvorenim duloksetin hidrohloridom (f_2=34.4) i suvo obloženih peleta i peleta obloženih duloksetin hidrohloridom iz rastvora (f_2=32.5). Izolacioni sloj usporava profil oslobađanja duloksetina iz peleta obloženih aktivnom supstancom iz rastvora i suspenzije u odnosu na pelete obložene samo slojem aktivne supstance toliko da se ne postoji sličnost profila oslobađanja – f_2=36.2 za pelete obložene duloksetinom iz rastvora i f_2=45.2 za pelete obložene aktivnom supstancom iz suspenzije. Uticaj izolacionog sloja nije tako veliki na oslobađanje iz suvo obloženih peleta tako da postoji sličnost profila oslobađanja peleta u prvoj i drugoj fazi procesa proizvodnje (f_2=59.6). Nakon nanošenja izolacionog filma postoji sličnost profila oslobađanja iz suvo obloženih peleta i peleta obloženih duloksetin hidrohloridom iz suspenzije (f_2=55.7) dok se profili preostalih parova formulacija ne mogu smatrati sličnim. Za postizanje slične gastrorezistencije bio je potreban oko 20% veći nanos gastrorezistentnog filma kada se ovaj film nanosi tehnikom suvog oblaganja, što je vrlo značajno. Film dobijen ovom tehnikom se pokazao permeabilnijim u kiseloj fazi testa ispitivanja brzine rastvaranja, ali manje permeabilan u fazi testa ispitivanja brzine rastvaranja koja se izvodi u fosfatnom puferu pH 6.8. Na osnovu izračunatih vrednosti faktora sličnosti f_2, ni jedan od parova ispitanih

formulacija nema slične profile oslobađanja iz gastrorezistentnih peleta, a ne postoji ni sličnost profila oslobađanja pojedinačnih formulacija nakon nanošenja izolacionog i gastrorezistentnog sloja. Rezultati određivanja sadržaja nečistoća duloksetina u gastrorezistentnim peletama u toku studije stabilnosti pokazali su da izolacioni sloj efikasno sprečava kontakt duloksetin hidrohlorida i hipromeloze acetat sukcinata jer je sadržaj duloksetin sukcinamida koji je proizvod njihove reakcije ispod gornje granice od 0.2%. U uzorku gastrorezistentnih peleta obloženih aktivnom supstancom iz rastvora nakon 36 meseci u ambijentalnim uslovima je određen sadržaj 1-naftola od 0.57% što je iznad gornje dozvoljene granice od 0.2%. Sadržaj ovog proizvoda hidrolize duloksetina je u druga dva ispitana uzorka u okviru specificiranih granica i iznosi 0.06.

*In the **first phase** of research, following techniques of drug layering of pellet were successfully applied: powder layering, solution layering and suspension layering. Isolating layer was applied to all drug layered pellets by using spray coating technique. Furthermore, enteric-coated pellets were successfully produced by using dry coating and spray coating of suspension containing dissolved polymer. Scanning electron micrographs and results of image analyses show that there is a significant difference in morphological characteristics of pellets produced by drug layering in comparison to techniques which use liquid as a vehicle for transportation of drug to pellets. Drug layered pellets have worse shape parameters than pellets produced by other two evaluated techniques, i.e. they have wider size distribution with circular diameter of 1084.4 μm ±141.1 μm, less smooth surface with roughness of 0.84 ± 0.04 and worse sphericity, as their aspect ratio was 1.17 ± 0.09 and roundness was 0.77 ± 0.06. Pellets produced by solution layering were characterized by circular diameter of 1270.0 μm ± 52.3 μm, roughness of 0.97 ± 0.01, aspect ratio of 1.09 ± 0.06 and roundness of 0.90 ± 0.04. Pellets produced by suspension layering were characterized by circular diameter of 1086.2 μm ± 54.5 μm, roughness of 0.97 ± 0.01, aspect ratio of 1.11 ± 0.07 and roundness of 0.89 ± 0.06. Such a trend of parameters of pellets' shape and surface characteristics is continued in the two remaining phases of production process – application of isolation layer and enteric-coating. The only shape parameter that had the similar value for for all three techniques in the end of production process was roughness as powdered layered pellets were found to have roughness of 0.95 ± 0.01, whereas other two techniques both had roughness of 0.97 ± 0.01. Release of duloxetine is complete in after all the phases of production process. Based on calculated similarity factor, f_2, after drug layering phase profiles of powder layered and suspension layered pellets could be considered similar (f_2=69.8). That was not the case*

suspension and solution layered pellets (f_2=34.4) and powder and solution layered pellets (f_2=32.5). Comparison of release profiles of drug layered and isolated pellets shows that isolating layer slowed down the release of duloxetine from solution and suspension layered pellets so much that there was no similarity in release profiles of pellets after these two phases of production. For solution layered pellets f2 factor was 36.2 and 45.2 for suspension layered pellets. Effect of isolating layer on the release of duloxetine was not so pronounced with powder layered pellets so that their release profiles could be considered similar after application of drug and isolating layer (f_2=59.6). After application of isolating layer, release profiles of suspension and powder layered pellets could be considered similar (f_2=55.7) whereas that was not the case for other pairs of evaluated manufacturing techniques. For obtaining similar gastric-resistance, 20% higher weight gain was required when enteric coating was applied to pellets by powder layering. Enteric-coating obtained by using this technique was more permeable in the acid stage of dissolution test, but less permeable in the buffer stage of dissolution test than the spray coated films. Based on calculated similarity factor f2, none of the pairs of investigated formulations shows similarities in the release profiles of duloxetine. Moreoveor, there was no similiarity between release profiles of single trials after application of isolating layer and enteric coating. Levels of impurities of duloxetine determined in enteric-coated pellets in the course of stability studies proved that isolating layer efficiently prevented contact of duloxetine hydrochloride and hypromellose acetate succinate because the content of duloxetine succinamide which is a product of their reaction was below the limit of 0.2%. Content of 0.57% of 1-naphthol was determined in the sample of solution layered pellets after 36 months of storage in ambient conditions which was above the limit of 0.2%. Content of 0.06% of this hydrolisis product of duloxetine found in other formulations was well below specified limit.

U **drugoj fazi** istraživanja, tehnike eksperimentalnog dizajna su primenjene u optimizaciji formulacije i procesa proizvodnje peleta obloženih aktivnom supstancom. U fazi skrininga su pomoću frakcionog faktorijalnog dizajna tipa 2^{8-4} od 8 ulaznih formulacijskih i procesnih parametara izdvojena dva sa najvećim pozitivnim efektom na izlaze koji su praćeni: efikasnost procesa i koristan prinos. Neočekivano je primećen i veliki negativan uticaj koji je prisustvo rastvorenog duloksetina u tečnosti za oblaganje imalo na koristan prinos proizvodnog procesa usled izražene aglomeracije peleta u toku oblaganja. U cilju objašenjenja ovog negativnog efekta pripremljeni su slobodni filmovi sa rastvorenim i suspendovanim duloksetin hidrohloridom i hidroksipropil metilcelulozom 6 cp i analizirani su tehnikom DSC.

Rezultati su pokazali da u toku oblaganja peleta rastvorenim duloksetinom nastaje amorfni duloksetin čiji je sa Tg od oko 51 °C koja je bliska temperaturama proizvoda u toku oblaganja peleta. Sušenjem raspršivanjem iz metanolnog rastvora pripremljen je amorfni duloksetin hidrohlorid čija je DSC analiza pokazala Tg na oko 24°C. Prisustvo amorfnog duloksetina u peletama obloženim duloksetin hidrohloridom iz rastvora je dodatno potvrđeno analizom peleta tehnikom Raman spektroskopije. Na osnovu rezultata skrining eksperimenata razvijeni su modeli prvog reda koji su pokazali da je proces proizvodnje robustan u ispitanom opsegu procesnih parametara te je u fazi optimizacije pomoću centralnog kompozitnog dizajna i metodologije površine odgovora ispitan uticaj udela hidroksipropil metilceluloze 6 cp i koncentracije suvih supstancu na efikasnost procesa, koristan prinos, viskozitet suspenzije za oblaganje i trajanje proizvodnog procesa. Razvijeni su statistički značajni modeli (p-vrednost<0.05) drugog reda sa sledećim koeficijentima korelacije R^2: za efikasnost procesa R^2 = 0.9571, za koristan prinos R^2 = 0.9851, za viskozitet suspenzije za oblaganje R^2 = 0.9928 i za vreme trajanja procesa R^2 = 0.9584. Korišćenjem razvijenih modela i funkcije poželjnosti u softveru za obradu eksperimentalnih podataka definisani su optimalan udeo hidroksipropil metilceluloze i koncentracije suvih supstanci koji su potrebni za postizanje željene efikasnosti proizvodnog procesa veće od i korisnog prinosa proizvodnog procesa za što kraće vreme.

*In the **second phase** of the research, optimization of formulation and production process of drug layered pellets was carried out by using experimental design. In the screening phase,* 2^{8-4} *fractional factorial design was used to evaluate the impact of 8 formulation and process factors on process efficiency and useful yield, and 2 factors with the greatest positive impact were identified. Unexpectedly, it was observed that the presence of dissolved duloxetine in the coating liquid had the greatest negative impact on the useful yield due to extensive agglomeration of pellets during the layering process. To find the root cause of this agglomeration problem, free films containing dissolved and suspended duloxetine hydrochloride and hypromellose 6 cp were prepared and subjected to DSC analyses. They showed that during solution layering amorphous duloxetine is formed and that it's Tg of around 51 °C was close to product temperature during the coating process. The Tg of amorphous duloxetine produced by spray drying from methanol solution was around 24 °C . The presence of different solid state forms of duloxetine in solution and suspension layered pellets was also confirmed by Raman spectroscopy analyses. Results of screening experiments were used to develop the first order models that confirmed that the production process was robust within the examined range of process parameters and in the optimization phase central*

composite design and response surface methodology were used to get a closer look on the impact of level of hypromellose 6 cp and level of solids in the coating suspension on process efficiency, useful yield, viscosity of coating suspension and time of production process. Statistically significant (p-value <0.05) second order models were obtained with following correlation coefficients R2: process efficiency R^2 = 0.9571, useful yield R^2 = 0.9851, viscosity of coating suspension R^2 = 0.9928 and process time R^2 = 0.9584. Developed models and desirability function of experimental design software were used to define levels of hypromellose and solids in the coating suspension that enable obtaining target eficency and useful yield for minumum process time.

U **trećoj fazi** istraživanja soft computing tehnike veštačke inteligencije su uspešno primenjene za razvijanje modela koji povezuju kvalitativne i kvantitativne karakteristike formulacije gastrorezistentnih peleta sa gastrorezistencijom i količinom oslobođenog duloksetina nakon 5, 10, 15, 20, 30, 45, 60 i 90 minuta testa ispitivanja brzine rastvaranja u fosfatnom puferu pH 6.8. Za modelovanje oslobađanja su korišćeni istorijski podaci iz 19 laboratorijskih proba koje su izvedene po principu probe i greške. Elastične mreže su tehnika koja je imala najbolje faktore sličnosti između eksperimentalno dobijenih i predviđenih profila oslobađanja duloksetina za test formulacije (68.3 za formulaciju test 1 i 70.3 za formulaciju test 2). Međutim, modeli razvijeni ovom tehnikom nisu imali najmanje vrednosti RMSE za sve ispitane tačke profila oslobađanja tako da je zaključeno da bi se najbolja sličnost između eksperimentalno dobijenih i predviđenih profila oslobađanja duloksetina dobila kada bi se iskombinovale tehnike koje su imale najmanje greške predviđanja. Kada se ispitane tehnike kombinuju tako da se za predviđanja gastrorezistencije koristi ansambl regresionih stabala dobijen tehnikom "boosting", za oslobađanje duloksetina posle 5 i 10 minuta laso, odnosno grebenasta regresija, za oslobađanje duloksetina nakon 15 i 20 minuta elastične mreže, odnosno laso regresija, za oslobađanje duloksetina nakon 30 i 45 minuta ansambl regresionih stabala dobijen tehnikom "boosting" i veštačke neuronske mreže i za oslobađanje duloksetina nakon 60 i 90 minuta regresiona stabla, odnosno grebenasta regresija sličnost eksperimentalno dobijenih i predviđenih profila se poveća na 82.3 za formulaciju test 1 i 74.6 za formulaciju test 2.

*In the **third phase** of the research soft computing artificial intelligence techniques were successfully applied to develop models linking qualitative and quantitative enteric-coated pellets formulation variables with the gastric-resistance of pellets and amount of duloxetine*

released after 5, 10, 15, 20, 30, 45, 60 and minutes of buffer stage dissolution test. Data obtained in 19 laboratory trials conducted on trial and error basis were used for modeling. Elastic net was the technique whose models were the ones with the greatest similarity factor between experimentally obtained and predicted release profiles of duloxetine (f_2=68.3 for formulation test 1, f_2= 70.3 for formulation test 2). However, models developed by this technique did not have the lowest calculated RMSE for all points of release profile and it was therefore concluded that the highest similarity between experimentally obtained and predicted release profiles of enteric-coated pellets would be achieved when the single techniques having the lowest RMSE for single points of dissolution test would be combined together to predict the release profile. When evaluated techniques were combined so that the ensemble of regression trees obtained by "boosting" technique was used for predicting gastric-resistance, lasso and ridge regression for the release of duloxetine after 5 and 10 minutes respectively, elastic net and lasso regression for the release of duloxetine after 15 and 20 minutes respectively, ensemble of regression trees obtained by "boosting" technique and artificial neural network for the release of duloxetine after 30 and 45 minutes respectively and regression trees and ridge regression for the release of duloxetine after 60 and 90 minutes, similarity factor of predicted and experimentally obtained release profiles increased to 82.3 for the formulation test 1 and 74.6 for the formulation test 2.

C. UPOREDNA ANALIZA REZULTATA SA PODACIMA IZ LITERATURE/ COMPARISON OF THE OBTAINED RESULTS WITH THE PUBLISHED DATA

Primena peleta u farmaceutskoj industriji je u porastu. Postoji više različitih tehnika oblaganja kojima se pelete mogu oblagati aktivnim supstancama i funkcionalnim i nefunkcionalnim filmovima koje se primenjuju na laboratorijskom i industrijskom nivou, a tehnika koja će biti izabrana za proizvodnju obloženih peleta zavisi od fizičko-hemijskih karakteristika aktivne supstance, odnosno stepena funkcionalnosti filma koji se nanosi kao i od karakteristika gotovog proizvoda. Kao model lekovita supstanca u istraživanju je korišćen duloksetin hidrohlorid koji spada u BCS klasu 2 lekovitih supstanci (Pandya i autori, 2015), pokazuje polimorfizam (Marjo i autori, 2011) i u velikoj meri se degradira na pH vrednostima nižim od 2.5 (Chhalotiya i autori, 2010) što sve predstavlja izazov u farmaceutskom razvoju. Jedan od ciljeva farmaceutskog razvoja proizvoda je i postizanje višeg stepena znanja o proizvodu, a to znanje se kasnije koristi i u svim drugim fazama životnog ciklusa proizvoda. To znanje može

biti predstavljeno matematičkim modelima koji povezuju određene ulazne parametre (karakteristike i količine materijala u formulaciji, vrednosti procesnih parametara) sa kritičnim atributima kvaliteta proizvoda. Modeli mogu da se razviju na osnovu rezultata dobijenih na sistematičan način primenom eksperimentalnog dizajna kao jedne od glavnih alatki koncepta dizajna kvaliteta i korišćenjem soft computing tehnika koje analizom istorijskih podataka prikupljenih na nesistematičan način mogu da utvrde veze između ulaznih parametara i kritičnih atributa kvaliteta proizvoda.

The use of pellets in the pharmaceutical industry is in constant increase. In the laboratory and industrial scale there are more different ways of producing pellets layered with drug substances or coated with functional and nonfunctional films. The manufacturing technique of choice depends on physicho-chemical characteristics of the drug substance, the degree of functionality of coating and characteristics of the finished product. The model drug substance used in this research was duloxetine hydrochloride which belongs to BCS class 2 of drug substances (Pandya i autori, 2015), shows polymorphism (Marjo et al., 2011) and to the great extent is degraded at pH values below 2.5 (Chhalotiya et al., 2010) which altogether presents a challenge in the pharmaceutical development. One of the aims of pharmaceutical development is to gain enhanced knowledge of the product that can later be used in all the other phases of product lifecycle. That knowledge can be represented by mathematical models linking input variables (characteristics and quantities of materials in the formulation, values of process parameter) with critical quality attributes of the product. Models can be developed on account of results obtained in a systematical way by application of experimental design as one of the main tools of quality by design concept and historical data gathered in a non-systematical way. This kind of data can be analyzed by soft computing techniques to find the links between input variables and critical quality attributes of the product.

U literaturi postoje podaci o oblaganju peleta aktivnom supstancom suvim oblaganjem (Varhosaz i autori, 2009; Nastruzzi i autori, 2000), oblaganjem iz rastvora (McConnel i autori, 2009; Nikowitz i autori (2013) i oblaganjem iz suspenzije (Suhrenbrock i autori, 2011; Sinchaipanid i autori 2004). U literaturi postoje podaci o proizvodnji peleta obloženih funkcionalnim filmom primenom tehnika suvog oblaganja i raspršivanjem rastvorenog ili dispergovanog polimera (Terebesi i Bodmeier, 2010; Pearnchob i Bodmeier, 2003; Obara i autori, 1999). U literaturi za sada nisu dostupni podaci uporedni podaci o proizvodnji lekom obloženih peleta metodama suvog oblaganja, oblaganja iz rastvora i oblaganja iz suspenzije.

Takođe, u literaturi nisu dostupni podaci koji upoređuju tehnike oblaganja peleta duloksetina gastrorezistentnim filmom tehnikama suvog oblaganja i oblaganja suspenzijom za oblaganje u kojoj je gastrorezistentni polimer rastvoren. Rezultati prikazani u disertaciji pokazuju da se sve tri tehnike oblaganja peleta aktivnom supstancom mogu uspešno primeniti u proizvodnji obloženih peleta tako da se postigne ciljani sadržaj duloksetina i njegovo potpuno oslobađanje iz peleta. Lošije morfološke osobine u smislu sferičnosti projekcije, stepena izduženosti i hrapavosti suvo obloženih peleta u odnosu na pelete obložene aktivnom supstancom iz rastvora i suspenzije su bile očekivane. Nastruzzi i autori (2000) su prikazali SEM mikrografije peleta ibuprofena proizvedenih tehnikom suvog oblaganja u konvencionalnom uređaju za oblaganje sa imerzionim mačevima na kojima su jasne nepravilnosti površine ovako proizvedenih peleta, ali parametri oblika nisu kvantifikovani. Sinchaipanid i autori (2004) su zaključili da glatkiju površinu imaju pelete koje su proizvedene tehnikom koja obezbeđuje bolje kretanje peleta u uređaju za oblaganje što je u skladu sa rezultatima ovog istraživanja. Jedini parametar oblika koji je kvantifikovan je srednji prečnik peleta, vrednost standardne devijacije ovog parametra koji ukazuje na širinu raspodele veličine peleta je oko 55 μm što je u nivou vrednosti (52 – 54 μm) dobijene za pelete obložene u uređaju sa fluidizirajućim kretanjem vazduha u ovom istraživanju. U literaturi nisu pronađeni podaci o parametrima oblika suvo obloženih peleta i peleta obloženih iz rastvora i suspenzije, ali poređenjem SEM mikrografija i rezultata za parametre: stepen izduženosti, sferičnost projekcije i hrapavost dobijenih u ovom istraživanju i SEM mikrografija i parametara oblika koje su objavili Yeung i Rein (2015) za pelete proizvedene tehnikom hot melt ekstruzije zaključuje se da se softver za analizu slike može koristiti za pouzdanu kvantifikaciju parametara oblika peleta proizvedenih suvim oblaganjem i oblaganjem iz rastvora i suspenzije. Za postizanje gastrorezistencije od manje od 2% oslobođenog duloksetina u kiseloj fazi testa brzine rastvaranja gastrorezistentnih peleta bio je potreban oko 20% veći nanos gastrorezistentnog filma ukoliko se on nanosio na pelete tehnikom suvog oblaganja, a ne raspršivanjem suspenzije za oblaganje. Slično se može zaključiti i na osnovu dostupnih literaturnih podataka (Pearnchob i Bodmeier, 2003; Obara i autori, 1999; Kablitz i autori 2008). Veći nanos gastrorezistentnog filma uslovio je sporiji profil oslobađanja iz suvo obloženih gastrorezistentnih peleta što je takođe nađeno i od strane Pearnchob i Bodmeier-a (2003) koji su oblagali pelete Eudragit®-om RS, šelakom i etil celulozom. Nema literaturnih podataka o hemijskoj stabilnosti gastrorezistentnih peleta duloksetina.

There are literature data about powder layering (Varhosaz et al., 2009; Nastruzzi et al., 2000), solution layering (McConnel et al., 2009; Nikowitz et al., 2013) and suspension layering of pellets (Suhrenbrock et al., 2011; Sinchaipanid et al., 2004). There are also literature data on production of pellets coated with functional films by using spray coating of functional polymer solutions and dispersions and by using powder coating techniques (Terebesi and Bodmeier, 2010; Pearnchob and Bodmeier, 2003; Obara et al., 1999). There were no data found in the literature that compared three drug layering techniques (powder layering, solution layering and suspension layering) with the same model drug. Moreover, there were no comparative data about enteric-coating of duloxetine pellets by using dry coating and spray coating. Results presented in doctoral dissertation show that all three evaluated drug layering techniques could be successfully applied to produce duloxetine layered pellets with aimed content of duloxetine and its complete release in the dissolution test. Worse morphological characteristics of powder layered pellets in terms of roundness, aspect ratio and roughness in comparison to spray coated pellets were expected. Nastruzzi et al. (2000) presented SEM micrographs of pellets produced by powder layering in conventional coating pan with immersion swords. They clearly show irregularities of pellets' surface, but shape parameters were not quantified. Sinchaipanid et al. (2004) concluded that pellets produced in the equipment that enabled better movement of product had smoother surface which is accordance with the results of this research. The only shape parameter that was quantified by Sinchaipanid et al. (2004) was mean diameter of pellets and standard deviations of this parameter that point to the width of the pellet size distribution were around 55 μm which is comparable to standard deviations of 52 – 54 μm that were obtained for drug layered pellets in the fluid-bed device in this research. There were no literature data found about aspect ratio, roundness and roughness of pellets produced by powder, solution and suspension layering. However, comparation of SEM micrographs and image analyses results for the shape parameters: aspect ratio, roundness and roughness presented in this research and SEM micrographs and shape parameters of hot melt extruded pellets published by Yeung and Rein (2015) it was concluded that image analysis software could be used to reliably quantify shape parameters of powder, solution and suspension layered pellets. To achieve gastric-resistance of less than 2% of duloxetine released in the acid stage of the dissolution test around 20% higher weight gain of enteric coating was required when enteric-coating was applied to pellets by using dry coating instead of spray coating. The same can be concluded from the available literature data (Pearnchob and Bodmeier, 2003; Obara et al., 1999;

Kablitz et al., 2008). There were no literature data about chemical stability of enteric coated pellets of duloxetine.

Da bi se na sistematičan način ispitao uticaj formulacijskih karakteristika i parametara proizvodnog procesa na prinos i koristan prinos procesa oblaganja inaktivnih peleta duloksetinom korišćena je metodologija eksperimentalnog dizajna čija primena je široko opisana u literaturi (Mihajlović i autori, 2011; Suhrenbrock i autori, 2011; Đuriš i autori, 2013). U fazi skrininga korišćenjem farkacionog faktorijalno dizajna tipa 2^{8-4} ispitan uticaj 8 formulacijskih i procesnih parametara na prinos i koristan prinos proizvodnog procesa. Na osnovu rezultata skrining eksperimenata razvijeni su matematički modeli prvog reda iz kojih je zaključeno da je proces robustan u okviru ispitanog opsega procesnih parametara. Neočekivano je otkriveno da je aglomeracija peleta u toku proizvodnog procesa znatno izraženija ako je duloksetin hidrohlorid rastvoren u tečnosti za oblaganje. Postoje literaturni podaci da neke lekovite supstance mogu da budu plastifikatori za određene polimere pa je posumnjano da je to slučaj sa našom model supstancom i hidroksipropil metilcelulozom (Wu i McGinity, 1999). To je provereno tako što su pripremljeni slobodni filmovi iz rastvora ili suspenzija duloksetina i hidroksipropil metilceluloze 6 cp i analizirani diferencijalnom skenirajućom kalorimetrijom što je postupak opisan u literaturi (Sakata i Yamaguchi, 2011). DSC analize su pokazale da duloksetin hidrohlorid ne plastificira hidroksipropil metilcelulozu već da u toku oblaganja peleta iz rastvora duloksetina nastaje amorfni duloksetin sa temperaturom staklastog prelaza bliskoj temperaturama proizvoda u toku procesa oblaganja. Hidroksipropil metilceluloza je poznati inhibitor kristalizacije (Tajarobi i autori, 2011; Zhao i autori 2012) koja sprečava kristalizaciju duloksetina prilikom oblaganja peleta duloksetin hidrohloridom iz rastvora. U literaturi su nađeni podaci o izdvajanju amorfnog oblika lekovitih supstanci u toku oblaganja peleta rastvorenim aktivnim supstancama i polimerima (Nikowitz i autori, 2013; Zhang i autori 2008), ali ne postoje podaci o nastanku amorfnih formi čiji je Tg toliko nizak da onemogućava proces oblaganja usled izražene aglomeracije peleta. Prisustvo različitih oblika čvrstog stanja u peletama obloženim duloksetinom iz rastvora i suspenzije potvrđeno je i Raman spektroskopije za koju postoje literaturni podaci da može da se koristi u ovu svrhu (Hao i autori, 2010). U fazi optimizacije je korišćenjem centralnog kompozitnog dizajna i metodologije površine odgovora optimizovan udeo hidroksipropil metilceluloze i koncentracije suvih supstanci u suspenziji za oblaganje tako da se postigne što veća efikasnost i koristan prinos proizvodnog procesa za što kraće vreme. Dodatno je u ovoj fazi praćen i viskozitet suspenzija za oblaganje. Razvijeni su modeli drugog

reda sa R^2>0.90 koji su pokazali da je koncentracija suvih supstanci je faktor sa najvećim efektom na efikasnost proizvodnog procesa, koristan prinos i vreme trajanja procesa oblaganja. Udeo hidroksipropil metilceluloze u formulaciji i njen kvadratni efekat imali su najveći efekat na koristan prinos. Ovi rezultati su uporedivi sa rezultatima Suhrenbrock i autora (2011) koji su ispitivali uticaj udela PVA-PEG graft kopolimera, koncentraciju suspenzije za oblaganje i veličinu čestica hidrohlorotiazida na efikasnost proizvodnog procesa. Korišćena je funkcija poželjnosti softvera za eksperimentilni dizajn za definisanje opsega udela vezivnog sredstva i koncentracije čvrstih supstanci pri kojima se istovremeno i u najvećoj meri ispunjavaju ciljni kriterijumi postavljeni za više izlaza što je postupak koji je opisan u literaturi (Singh i autori, 2012).

Experimental design methodology was employed to evaluate the influence of formulation characteristics and parameters of production process of drug layering of pellets which is a methodology widely described in the literature (Mihajlović et al., 2011; Suhrenbrock et al., 2011; Đuriš et al., 2013). Results of the screening experiments were used to develop first order models that implied that production process was robust within the range of examined process parameters. It was unexpectedly found that the agglomeration of pellets was much more pronounced during the solution layering process. There are literature data about plasticizing properties of drugs (Wu i McGinity, 1999) and it was suspected that it was the case with duloxetine hydrochloride and hypromellose. To check this, free films prepared with solutions and suspensions of duloxetine and hypromellose 6 cp were prepared and analyzed by using DSC which is a common method of analyzing film coatings (Sakata and Yamaguchi, 2011). DSC analyses showed that during solution layering process formation of amorphous duloxetine occurs and that Tg of amorphous duloxetine has a Tg close to product temperatures during the coating process. Hypromellose is a known crystallization inhibitor (Tajarobi et al., 2011; Zhao et al., 2012) which prevents crystallization of duloxetine during the solution layering of pellets. There are literature data about formation of amorphous substances during solution layering (Nikowitz et al., 2013; Zhang et al., 2008), but there are no literature data about the formation of amorphous forms with a Tg so low that makes the solution layering impossible. The presence of different solid state forms of duloxetine in solution and suspension layered pellets was confirmed by Raman spectroscopy analysis which can be used for this purpose according to literature data (Hao et al., 2010). In the optimization phase central composite design and response surface methodology were employed to optimize the level of hypromellose and level of solids in the coating suspension to

obtain as good process efficiency and useful yield as possible for as little time possible. Additionally, viscosity of coating suspensions was examined. Second order models with R^2>0.90 were developed. They implied that concentration of solids was the factor with greatest positive effect on process efficacy, viscosity of coating suspension and process time. Level of hypromellose 6 cp and its quadratic effect had the greatest positive impact on useful yield. These results are comparable with the results of Suhrenbrock et al. (2011) who examined the influence of level of PVA-PEG graft copolymer, level of solids in the coating suspension and particle size of hydrochlorothizide on process efficency. Desirability function of experimental design software was used to define the range level of binder and solids that, to the greatest possible extent, fulfill set target criteria for more than one response variable which is method described in the literature (Roopa et al., 2012).

Relativno je lako analizirati rezultate iz dobro osmišljenog dizjna eksperimenata. Međutim, nekada dostupni podaci nisu prikupljeni prema tačno utvrđenom eksperimentalnom planu ili modeli prvog, odnosno drugog reda nisu pogodni za opisivanje sistema usled izražene nelinearnosti. Tada je modele moguće razviti primenom soft computing tehnika veštačke inteligencije. U literaturi je opisana primena nekih od ovih tehnika kao što su veštačke neuronske mreže i regresiona stabla, samih ili u kombinaciji sa metodologijom eksperimentalnog dizajna, za razvijanje modela koji su korišćeni u optimizaciji formulacije i procesa (Mihajlović i autori 2011; Mendyk i autori, 2010; Ronowicz i autori, 2015). U doktorskoj disertaciji prikazani su rezultati modelovanja oslobađanja duloksetina iz gastrorezistentnih peleta pri čemu su korišćeni istorijski podaci iz laboratorijskih proba izvedenih po principu probe greške. Poređenjem eksperimentalno dobijenih i predviđenih profila oslobađanja test formulacija utvrđeno je da modeli razvijeni tehnikama laso regresije, grebenaste regresije, elastične mreže, regresionih stabala i ansamblom regresionih stabala dobijenih tehnikom "boosting" daju faktore sličnosti f_2 veće od 50, a da su najviše vrednosti faktora sličnosti, f_2 izračunati za model razvijen tehnikom elastične mreže i iznose 68.3 za formulaciju test 1 i 70.3 za formulaciju test 2. Ibrić i autori (2002) su u svom istraživanju modelovali oslobađanje aspirina iz matriks tableta, rezultate su prikupili primenom eksperimentalnog dizajna i dobili faktore sličnosti, f_2, od 55.28 i 61.45. Petrović i autori (2012) su koristili različite tipove veštačkih neuronskih mreža da bi modelovali oslobađanje diklofenaka i kofeina iz matriks tableta i poređenjem sličnosti eksperimentalno dobijenih i predviđenih profila oslobađanja dobili rezultate u opsegu od 49.3 do 86.9. Chansanroj i autori (2011) su modelovali oslobađanje iz matriks tableta primenom tehnika veštačkih neuronskih

mreža i samorganizujućih mapa i poređenjem sličnosti eksperimentalno dobijenih i predviđenih profila oslobađanja model supstance dobili f_2 vrednosti u opsegu od 42.3 do 95.8. Može se zaključiti da su performanse modela razvijenih soft computing tehnikama prikazanih u ovom istraživanju uporedive sa performansama modela opisanih u literaturi. Vrednosti RMSE modela razvijenog pomoću elastične mreže za test skup podataka kreću se u opsegu od 0.43 za oslobađanje duloksetina posle 5 minuta do 6.07 za procenat oslobođene supstance nakon 20 minuta i slične su opsegu vrednosti RMSE od 1.85 do 5.19 koji su dobili Ibrić i autori (2002) za modele dobijene veštačkim neuronskim mrežama. Iako je model razvijenim pomoću elastične mreže predvideo profile oslobađanja najsličnije eksperimentalno dobijenim profilima, vrednosti RMSE za pojedine tačke profila predviđenih ovom tehnikom nisu najniže u odnosu na sve ispitane tehnike. Kombinovanjem pojedinačnih tehnika koje su za datu tačku profila oslobađanja imale najnižu vrednost RMSE, opseg RMSE vrednosti se smanjio na 0.08 za gastrorezistenciju predviđenu ansamblom regresionih stabala do 3.56% za oslobađanje duloksetina nakon 30 minuta testa ispitivanje brzine rastvaranja, predviđeno istom tehnikom. Faktor sličnost eksperimentalno dobijenih i predviđenih profila oslobađanja se povećala na 82.3 za formulaciju test 1 i 74.6 za formulaciju test. Oslobađanje duloksetina iz gastrorezistentnih peleta je kompleksan dinamički proces. Ponašanje sistema se menja u toku vremena pa je zato teško jednom tehnikom izvršiti predviđanja za sve vremenske tačke profila oslobađanja. Za svaku ispitanu vremensku tačku drugačiji uslovi su bili optimalni za modelovanje što je uslovljeno složenošću formulacije, odnosno prisustvom više različitih slojeva i njihove interakcije u konačnom proizvodu i u medijumu za ispitivanje brzine rastvaranja.

Primena laso regresije, grebenaste regresije, elastičnih mreža i ansambla regresionih stabala dobijenih tehnikom "boosting" u modelovanju oslobađanja lekovite supstance iz gastrorezistentnih peleta nije opisana u literaturi i može se smatrati jedinstvenim naučnim doprinosom ove disertacije.

It is relatively easy to analyze the results obtained by using well planned design of experiments. However, sometimes available data are not collected according to systematical experimental plan, or first and second order models are not suitable for describing the system due to its nonlinearity. In these cases models can be developed by using artificial intelligence soft computing techniques. Some of these techniques, including artificial neural networks and regression trees, were reported in the literature to have been used in optimization of

formulation and process (Mihajlović et al., 2011; Mendyk et al., 2010; Ronowicz et al., 2015), alone or combined with experimental design methodology. The doctoral thesis comprises results of modeling release of duloxetine from enteric-coated pellets while using the historical data from laboratory trials performed according to trial and error concept. Comparation of experimentally obtained and predicted release profiles of test formulations it was found that similarity factors greater than 50 were obtained by using following techniques: lasso regression, ridge regression, elastic net, regression trees and ensemble of regression trees obtained by "boosting" method. The single technique with the highes f2 values for test formulation was the elastic net whose models enabled obtaining f2 value of 68.3 for formulation test 1 and 70.3 for formulation test 2. Ibrić et al. (2002) modelled release of aspirine form matrix tablets, the results were obtained by using design of experiments and obtained similarity factors of 55.28 and 61.45 for test formulations. Petrović et al. (2012) employed different types of artificial neural netwroks to model the relase of diclofenac and coffeine from matrix tablets and obtained similarity factors of 49.3 to 86.9 for test formulations. Chansanroj et al. employed artificial neural networks and self-organizing maps to model drug release from matrix tablets and obtained f2 values from 42.3 to 95.8 for test formulations. It can be concluded that performances of models developed by soft computing techniqes described in this research are comparable to performance of models described in the literature. RMSE values of model developed by using elastic net were within the range of 0.43, for release of duloxetine after 5 minutes of dissolution test, to 6.07 for the release of duloxetine after 20 minutes. The range of obtained RMSE values of the model obtained by the elastic net is similar to the RMSE range of 1.85 to 5.19 obtained by Ibrić et al. for the models obtained by using artificial neural networks. Although the model developed by the elastic predicted release profiles that were most similar to the ones experimentally obtained, RMSE values for single points of the release profile were not the lowest among all tested techniques. Combining techniques that had the lowest RMSE for the particular point of the dissolution profile led to tightening of the range of RMSE from 0.08 for gastric-resistance predicted by the ensemble of regression trees obtained by "boosting" technique to 3.56 for the release of duloxetine after 30 minutes of the dissolution test, predicted by the same technique. The similarity factor of experimentaly obtained and predicted release profiles increased to 82.3 for formulation test 1 and 74.6 for formulation test 2. Release of duloxetine from enteric coated pellets is a complex, dinamic process. The system constantly changes during the dissolution test and its therefore difficult to predict release of duloxetine in the all time points of the release profile by using a single technique. Different conditions were optimal for

modelling the release for each time point which was caused by the complexity of the formulation, i.e. the presence of three different layers in the product as well as their interactions in the product and in the dissolution medium.

Application of lasso regression, ridge regression, elastic net and ensemble of regression tress obtained by the "boosting" in modelling the release of drug substance from enteric-coated pellets has not been described in the literature and and can be considered as a unique scientific contribution of this doctoral dissertation

Literatura/*References*

1. Chansanroj, K., Petrovic , J., Ibric , S., Betz, G., 2011. Drug release control and system understanding of sucrose esters matrix tablets by artificial neural networks. Eur. J. Pharm. Sci. 44, 321–331.
2. Djuris, J., Medarevic, D., Krstic, M., Djuric, Z., Ibric, S., 2013. Application of quality by design concepts in the development of fluidized bed granulation and tableting process. J Pharm Sci 102:1869– 1882.
3. Hao, H., Su, W., Barrett, M., Caron, V., Healy, A., and Glennon, B., 2010. Calibration-Free Application of Raman Spectroscopy to the Monitoring of Mannitol Crystallization and Its Polymorphic Transformation. Org. Process Res. Dev.**,** 14, 1209–1214.
4. Kablitz, C.D., Kappl, M., Urbanetz, N.A., 2008. Parameters influencing polymer particle layering of the dry coating process. Eur. J. Pharm. Biopharm., 69, 760–768.
5. McConnell, E.L., Macfarlane, C.B., Basit, A.W., 2009. An observational study on the influence of solvent composition on the architecture of drug-layered pellets. Int.J. Pharm., 380, 67 – 71.
6. Mendyk, A., Kleinebudde, P., Thommes, M.,Yoo, A., Szleka, J., Jachowicz, R., 2010. Analysis of pellets with use of artificial neural networks. Eur. J. Pharm. Sci. 41, 421–429.
7. Mihajlović, T., Ibrić, S., Mladenović, A., 2011. Application of Design of Experiments and Multilayer Perceptron Neural Network in Optimization of the Spray-Drying Process, Dry. Technol., 29:14, 1638-1647.
8. Nastruzzi, C., Cortesi, R., Esposito, E., Genovesi, A., Vecchio, C., Menegatti, E., 2000. Influence of formualtion and process parameters on pellet production by powder layering technique. AAPS Pharm. Sci. Tech. 1(2), 14 – 25.
9. Nikowitz, K., Pintye-Hódi, K., Regdon, G. Jr., 2013. Study of recrystallization in coated pellets – Effect of coating on API crystallinity. Eur. J. Pharm. Sci., 48, 563 – 571.
10. Obara, S., Maruyama, N., Nishiyama, Y., Kokubo, H., 1999. Dry coating: an innovative enteric coating method using a cellulose derivative. Eur. J. Pharm. Biopharm., 47, 51–59.
11. Pearnchob, N., Bodmeier, R., 2003. Dry polymer powder coating and comparison with conventional liquid-based coatings for Eudragit RS, ethylcellulose and shellac. Eur. J. Pharm. Biopharm., 56, 363–369.
12. Petrović, J., Ibrić, S., Betz, G., Đurić, Z., 2012. Optimization of matrix tablets controlled drug release using Elman dynamic neural networks and decision trees. Int. J. Pharm., 428, 57– 67
13. Ronowicz, J., Thommes, M., Kleinebudde, P., Krysinski, J., 2015. A data mining approach to optimize pellets manufacturing process based on a decision tree algorithm. Eur. J. Pharm. Sci., 73, 44–48.
14. Sakata, Y., Yamaguchi, H., 2011. Effects of calcium salts on thermal characteristics of hydroxypropyl methylcellulose films. J. Non-Cryst. Solids., 357, 1279-1284.

15. Sinchaipanid, N., Chitropas, P., Mitrevej, A., 2004. Influences of Layering on Theophylline Pellet Characteristics. Drug. Dev. Tech., 9(2), 163-170.
16. Singh, G., Pai, R.S., Devi, V.K., 2012. Optimization of Pellets Containing Solid Dispersion Prepared by Extrusion/Spheronization Using Central Composite Design and Desirability Function. J. Young. Pharm., 4(3), 146-156.
17. Suhrenbrock, L., Radtke, G., Knop, K., Kleinebudde, P., 2011. Suspension pellet layering using PVA-PEG graft copolymer as a new binder. Int. J. Pharm., 412, 28 – 36.
18. Tajarobi, F., Larsson, A., Matic, H., Abrahmsén-Alami, S., 2011. The influence of crystallization inhibition of HPMC and HPMCAS on model substance dissolution and release in swellable matrix tablets. Eur. J. Pharm. Biopharm., 78, 125–133.
19. Terebesi, I., Bodmeier, R., 2010. Optimised process and formulation conditions for extended release dry polymer powder-coated pellets. Eur. J. Pharm. Biopharm. 75, 63 – 70.
20. Varshosaz, J.,Tavakolia, N., Serria, A., 2009. Preparation and in vitro characterization of piroxicam enteric coated pellets using powder layering technique. Pharm. Dev.Tech. 14(3), 305-311.
21. Wu C, McGinity JW. 1999. Non-traditional plasticization of polymeric films. Int J Pharm 177:15–27.
22. Yeung, C., Rein, H., 2015. Hot-melt extrusion of sugar-starch-pellets. Int. J. Pharm., 493: 390-403.
23. Zhang, X., Sun, N., Wu, B., Lu, Y., Guan, T., Wu, W., 2008. Physical characterization of lansoprazole/PVP solid dispersion prepared by fluid-bed coating technique. Powder Technol., 182 (3), 480-485.
24. Zhao, M., Barker, S.A., Belton, P.S., McGregor, C., Craig, D.Q.M., 2012. Development of fully amorphous dispersion of a low Tg drug via co-spray drying with hydrophilic polymers. Eur. J. Pharm. Biopharm., 82, 572 – 579.

D. OBJAVLJENI REZULTATI KOJI ČINE DEO DISERTACIJE/PUBLISHED RESULTS WHICH ARE PART OF THE DOCTORAL DISSERTATION

Radovi objavljeni u naučnim časopisima međunarodnog značaja

1. Kovačević, J., Ibrić, S., Đuriš, J., Kleinebudde, P., 2016. Application of the design of experiments in optimization of drug layering of pellets with an insight into drug polymer interactions. Int. J. Pharm., 506, 312-319. (M23) *IF 3,650/2014*

2. Kovačević, J., Mladenović, A., Đuriš, J., Ibrić, S., 2016. Evaluation of powder, solution and suspension layering for the preparation of enteric coated pellets. Eur. J. Pharm. Sci., 85, 84-93. (M23) *IF 3,350/2014*

E. ZAKLJUČAK - OBRAZLOŽENJE NAUČNOG DOPRINOSA DISERTACIJE/ CONCLUSION-JUSTIFICATION OF SCIENTIFIC CONTRIBUTION OF THE DOCTORAL DISSERTATION

Doktorska disertacija kandidata Jovane Kovačević, magistra farmacije, fokusirana je na farmaceutski razvoj gastrorezistentnih peleta uz korišćenje koncepta dizajna kvaliteta i soft computing tehnika veštačke inteligencije, što predstavlja tematiku koja je veoma aktuelna i značajna, kako sa aspekta naučnoistraživačkog rada, tako i sa aspekta farmaceutske industrije. Detaljnom analizom priložene doktorske disertacije Komisija je konstatovala da je disertacija prikazana na jasan i pregledan način i da su svi postavljeni ciljevi doktorske disertacije u potpunosti realizovani. Eksperimentni su organizovani i sprovedeni u skladu sa savremenim standardima u proučavanoj naučnoj oblasti što je omogućilo dobijanje rezultata kojima se ostvaruju prethodno postavljeni ciljevi doktorske disertacije. Kandidat je u doktorskoj disertaciji na sveobuhvatan način razmotrio tri različita pristupa za proizvodnju peleta obloženih aktivnom supstancom i dva pristupa proizvodnji gastro-rezistentnih peleta, kao i primenu koncepta dizajna kvaliteta i soft computing tehnika veštačke inteligencije u cilju postizanja višeg stepena znanja o proizvodu u fazi farmaceutskog razvoja. Na kraju doktorske disertacije prikazani su zaključci koji su izvedeni na osnovu dobijenih rezultata i podataka dostupnih u literaturi. I pored toga što se oblast koju je kandidat istraživao veoma intezivno proučava poslednjih nekoliko godina, sa velikim brojem pratećih publikacija, kandidat je uspeo da dâ originalan doprinos u rešavanju ispitivanih problema. Sve ovo je potkrepljeno činjenicom da su rezultati ove doktorske disertacije do sada publikovani u dva rada u međunarodnim naučnim časopisima.

Doctoral dissertation of candidate Jovana Kovačević, master of pharmacy, is focused on the pharmaceutical development of enteric-coated techniques while using quality by design concept and soft computing techniques of artificial intelligence, which presents a topic that is relevant and important for both research work and pharmaceutical industry. After detailed review of submitted doctoral dissertation, Committee ascertains that dissertation is presented in clear and concise manner and that all defined aims of doctoral dissertation are fully accomplished. All experiments are organized and conducted in accordance with up-to-date standards in the studied research field, which enabled obtaining results that meet the predefined aims of dissertation. In this doctoral dissertation, candidate has considered in a comprehensive manner three different approaches for producing drug layered pellets, two different approaches of producing enteric-coated pellets, as well as quality by design concept

and soft computing artificial intelligence techniques as means of acquiring enhanced knowledge of the product in the pharmaceutical development phase. At the end of doctoral dissertation, conclusions are given based on the obtained results and available literature data. Although investigated research field is intensively studied in the last few years, resulting in large number of publications, candidate succeeded to give an original contribution in solving the studied problems. This is supported with the fact that up to now the results of this doctoral dissertation are published in two papers in international scientific journals.

Na osnovu svega izloženog, može se zaključiti da je kandidat ispunio postavljene ciljeve u doktorskoj disertaciji pod nazivom „**Farmaceutski razvoj gastrorezistentnih peleta uz primenu koncepta dizajna kvaliteta i tehnika veštačke inteligencije“**, te predlažemo Nastavno-naučnom veću Farmaceutskog fakulteta da prihvati Izveštaj i omogući kandidatu mag. farm. Jovani Kovačević odbranu doktorske disertacije.

Based on the above consideration, it can be concluded that candidate fulfill defined aims of doctoral dissertation entitled ***„Pharmaceutical development of enteric-coated pellets by application of quality by design concept and artificial intelligence techniques“****, and therefore we advise the Academic Council of the Faculty of Pharmacy-University of Belgrade to accept this Report and permits candidate Jovana Kovačević defense of this doctoral dissertation.*

Beograd/Belgrade,
31/05/2016

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