

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

**KOMISIJI ZA POSLEDIPLOMSKE STUDIJE
TO THE COMMISSION FOR POSTGRADUATE STUDIES**

Na osnovu člana 94. Statuta i predloga Komisije za poslediplomske studije, Nastavno-naučno veće Farmaceutskog fakulteta Univerziteta u Beogradu, na sednici od 29.12.2011. god., donelo je odluku o imenovanju Komisije za ocenu ispunjenosti uslova za kandidata dipl. farmaceuta Ivanu Pantelić i naučne zasnovanosti teme doktorske disertacije pod naslovom: **"Dermalna raspoloživost lekova sa antiinflamatornim delovanjem iz podloga sa šećernim emulgatorom: komparativna primena metoda *in vitro*/*in vivo* karakterizacije"** Posle uvida u priloženi materijal i analize predmeta i cilja istraživanja, Komisija u sastavu: Doc. dr Snežana Savić, Prof. dr Jela Milić i Prof. dr Rolf Daniels podnosi sledeći:

According to the article 94 of the Statute and the recommendation of the Commission for Postgraduate Studies, on December 29th, 2011 the Academic Council of Faculty of Pharmacy, University of Belgrade, has appointed the Commission for the assessment of eligibility of the candidate and the scientific basis of the following subject of the doctoral dissertation:

"Dermal bioavailability of antiinflammatory drugs from sugar emulsifier stabilized bases: comparative application of *in vitro/in vivo* characterization methods"

After evaluation of the enclosed material and analysis of the subject and purpose of research, the Commission comprising: Assist. Prof. dr Snežana Savić, Prof. dr Jela Milić and Prof. dr Rolf Daniels would like to submit the following:

**IZVEŠTAJ
REPORT**

A. Biografija kandidata / Biography of the candidate

Ivana Pantelić rođena je 01.08.1982. godine u Beogradu, gde je završila osnovnu oglednu školu za francuski jezik "Vladislav Ribnikar" i III Beogradsku gimnaziju. Farmaceutski fakultet u Beogradu upisala je školske 2001/2002. godine, a diplomirala je 25.05.2007. sa prosečnom ocenom 8,81 i ocenom 10 na diplomskom ispitu.

Ivana Pantelić was born on August 1st 1982 in Belgrade where she has finished experimental elementary school for French language "Vladislav Ribnikar" and III Belgrade high school. She has commenced graduate studies in Pharmacy in the academic year of 2001/2002 and graduated from the Faculty of Pharmacy in Belgrade on May 25th 2007 with an average score of 8.81 and grade 10 on her diploma exam.

Doktorske akademske studije iz farmaceutske tehnologije na Farmaceutskom fakultetu u Beogradu upisala je školske 2008/2009. godine. U 2009. godini dobila je stipendiju Službe nemačke akademske razmene (DAAD) za jednomesečni istraživački boravak u Nemačkoj u

okviru specijalnog programa za Srbiju: Istraživačko iskustvo u Nemačkoj za studente i diplomce iz Srbije. Saradnju sa Institutom za farmaceutsku tehnologiju Eberhard-Karls Univerziteta u Tübingenu koju je tom prilikom ostvarila nastavila je kroz još jednu dvonedeljnu istraživačku posetu u septembru 2011. godine.

Mrs Pantelic has enrolled Doctoral academic studies in Pharmaceutical Technology at the Faculty of Pharmacy in the academic year 2008/2009. In 2009 she has received a scholarship from Deutsche Akademische Austausch Dienst (DAAD) for a one-month research stay in Germany as a part of a special programme for Serbia: Sonderprogramm Serbien – Forschungspraktika in Deutschland für Studierende und Graduierte aus Serbien. The attained collaboration with the Institute of Pharmaceutical Technology at the Eberhard-Karls Universität Tübingen was continued through another two-week research stay in September 2011.

Od oktobra 2007. godine radila je kao saradnik u nastavi do oktobra 2009. godine kada je izabrana u zvanje asistenta na Katedri za farmaceutsku tehnologiju i kozmetologiju Farmaceutskog fakulteta u Beogradu.

From October 2007 she was employed as an associate until October 2009 when she was promoted to the assistant position at the Department of Pharmaceutical Technology and Cosmetology, Faculty of Pharmacy in Belgrade.

Tokom 2010. godine učestvuje kao saradnik na projektu tehnološkog razvoja pod nazivom „Razvoj i karakterizacija koloidnih nosača za antiinflamatorne lekove“ (TR-19058), a od 2011. godine na projektu tehnološkog razvoja pod nazivom „Razvoj mikro- i nanosistema kao nosača za lekove sa antiinflamatornim delovanjem i metoda za njihovu karakterizaciju“ (TR-34031) i projektu osnovnih istraživanja pod nazivom „Razvoj molekula sa antiinflamatornim i kardioprotektivnim dejstvom: strukturne modifikacije, modelovanje, fizičko-hemijska karakterizacija i formulaciona ispitivanja“ (OI-172041) Ministarstva za prosvetu i nauku Republike Srbije.

Throughout 2010 she participated in the Project of technological development entitled „Development and characterization of colloidal carriers for anti-inflammatory drugs“ (TR-19058), and from 2011 in the Project „Development of micro- and nanosystems as carriers for anti-inflammatory drugs and the methods for their characterization“ (TR-34031) and the Project in fundamental research entitled „Development of molecules with anti-inflammatory and cardioprotective effects: structural modifications, modelling physicochemical characterization and formulation studies“ (OI-172041) funded by the Ministry of Education and Science of Republic of Serbia.

B. Spisak objavljenih radova / List of published papers:

I Rad u vrhunskom međunarodnom časopisu (M21)

- [1] Jaksic I, Lukic M, Malenovic A, Reichl S, Hoffmann C, Müller-Goymann C, Daniels R, Savic S. Compounding of a topical drug with prospective natural surfactant-stabilized pharmaceutical bases: physicochemical and in vitro/in vivo characterization. A ketoprofen case study. European Journal of Pharmaceutics and Biopharmaceutics 2012; 80 (1): 164-175.

II Rad u istaknutom međunarodnom časopisu (M22)

- [1] Savic S, Lukic M, Jaksic I, Reichl S, Tamburic S, Müller-Goymann C. An alkyl polyglucoside-mixed emulsifier as stabilizer of emulsion systems: The influence of colloidal structure on emulsions skin hydration potential. Journal of Colloid and Interface Science 2011; 358: 182–191.

III Rad u međunarodnom časopisu (M23)

- [1] Tasić-Kostov M, Reichl S, Lukic M, Jaksic I, Savic S. Two alkyl polyglucoside natural surfactants varying in the chain length in stabilization of lactobionic acid containing emulsions: physicochemical characterization and in vitro irritation potential assessment. *La Rivista Italiana Delle Sostanze Grasse* 2011; 88 (4): 256-264.
- [2] Tasic-Kostov M, Reichl S, Lukic M, Jaksic I, Savic S. Does lactobionic acid affect the colloidal structure and skin moisturizing potential of the alkyl polyglucoside-based emulsion systems? *Pharmazie* 2011; 66: 862-870.
- [3] Lukic M, Jaksic I, Krstonosic V, Cekic N, Savic S. A combined approach in characterization of an effective w/o hand cream: the influence of emollient on textural, sensorial and in vivo skin performance. *International Journal of Cosmetic Science* (*in press*); doi: 10.1111/j.1468-2494.2011.00693.

IV Rad u časopisu nacionalnog značaja (M52)

- [1] Jakšić I, Lukić M, Rajić M, Jončić-Savić K, Radulović V, Milić J, Savić S. Fizičko-hemijska karakterizacija i procena bezbednosti model podloga sa alkil poliglukozidnim emulgatorom za NSAIL, *Arhiv za farmaciju* 2010; 60: 26-47.
- [2] Ibrić S, Parojčić J, Jakšić I, Đurić Z. Farmaceutsko-tehnološki aspekti savremenih kardiovaskularnih lekova, *Arhiv za farmaciju* 2008; 58 (5-6): 432-442.
- [3] Vuleta G, Jakšić I, Lukić M, Savić S. Primena borne kiseline u farmaceutskim preparatima i kozmetičkim proizvodima – da ili ne? *Arhiv za farmaciju* 2008; 58 (4): 241 – 251.

V Saopštenje sa međunarodnog skupa štampano u celini (M33)

- [1] Jakšić I, Lukić M, Reichl S, Hoffmann C, Savić S. Komparativno in vitro/ex vivo/in vivo ispitivanje dermalne raspoloživosti ketoprofena iz emulzionih sistema na bazi alkil poliglukozida. II Kongres farmaceuta Bosne i Hercegovine sa međunarodnim učešćem, 17-20.11.2011. Banja Luka, Zbornik radova, str.192-194. (oralna prezentacija)
- [2] Lukić M, Jakšić I, Savić S. Razvoj optimalne formulacije dermokožmetičke emulzije za suhu kožu na bazi novog alkil poliglukozidnog emulgatora. II Kongres farmaceuta Bosne i Hercegovine sa međunarodnim učešćem, 17-20.11.2011. Banja Luka, Zbornik radova, str. 225-228.
- [3] Jaksic I, Lukic M, Milic J, Daniels R, Savic S. In vivo skin absorption of a model NSAID from alkyl polyglucoside emulsion vehicles: Influence of selected co-solvent and colloidal structure of the system, 5th Word Congress on Emulsions, Lyon, France, 12-14 October 2010, CD-ROM;
- [4] Lukic M, Jaksic I, Krajisnik D, Vuleta G, Savic S. Correlation of rheological and sensory properties and their comparison with in vivo effects of w/o creams for dry skin, 5th Word Congress on Emulsions, Lyon, France, 12-14 October 2010, CD-ROM;
- [5] Jakšić I, Lukić M, Milić J, Daniels R, Savić S. In vivo skin absorption of a model NSAID from alkyl polyglucoside emulsion vehicles: Influence of colloidal structure of the system and selected penetration enhancer, 7th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, Valletta, Malta, 8-11 March 2010, CD-ROM;

VI Saopštenje sa međunarodnog skupa štampano u izvodu (M34)

- [1] M. Lukic, I. Jaksic, R. Daniels, S. Savic; A C20/22 Alkyl Polyglucoside-Based Emulsion Vehicles: Adjusting of colloidal structure for better skin performance; *Skin Forum 12th Annual Meeting*; 2011, Frankfurt, Nemačka; CD-ROM

- [2] Jaksic I, Lukic M, Daniels R, Reichel S, Milic J, Savic S. Case by case physicochemical and in vitro/in vivo biopharmaceutical characterization of alkyl polyglucoside-based vehicles: ketoprofen as a model drug, 8th Central European Symposium on Pharmaceutical Technology, Graz, Austria, 16-18 September 2010; doi: 10.3797/scipharm.cespt.8.PDD32
- [3] Lukic M, Jaksic I, Daniels R, Vuleta G, Savic S. Natural surfactant of alkyl polyglucoside type: A physicochemical characterization of a new mixed emulsifier, 8th Central European Symposium on Pharmaceutical Technology, Graz, Austria, 16-18 September 2010; doi: 10.3797/scipharm.cespt.8.POT04
- [4] Lukić M, Jakšić I, Perović T, Tasić-Kostov M, Milić J, Savić S. Natural surfactants as potential pharmaceutical excipients in magistral drug preparation: physicochemical characterization and safety evaluation of model vehicles for NSAID, 69th International Congress of FIP, Istanbul, Turkey, 3-8 September 2009. p. 199.
- [5] Jakšić I, Lukić M, Kostić N, Milić J, Savić S. The influence of natural surfactant-based emulsion vehicles on in vitro liberation profiles of model NSAID, 69th International Congress of FIP, Istanbul, Turkey, 3-8 September 2009. p. 199.
- [6] Jakšić I, Lukić M, Jončić-Savić K, Milić J, Savić S. The influence of natural surfactant-based emulsion systems with and without isopropyl alcohol on skin absorption in vivo of model NSAID, 69th International Congress of FIP, Istanbul, Turkey, 3-8 September 2009. p. 199.
- [7] Lukić M, Jakšić I, Savić S. Skin performance of lactobionic and glycolic acid from emulsion and gel vehicles: short- and long-term application studies, Skin and Formulation, 3rd Symposium & Skin Forum, 10th Annual Meeting, Versailles, France, 9-10 March, 2009. p. 115.
- [8] Lukić M, Jakšić I, Savić S. The comparative effects of lactobionic and glycolic acid: an objective assessment of skin performance under occlusion, Skin and Formulation, 3rd Symposium & Skin Forum, 10th Annual Meeting, Versailles, France, 9-10 March, 2009. p. 115.

VII Saopštenje sa skupa nacionalnog značaja štampano u izvodu (M64)

- [1] Pantelić I, Lukić M, Čalija B, Jela M, Vuleta G, Savić S. Mogućnost primene alkil poliglukozidnih emulgatora različite dužine lanca u stabilizaciji nanočestičnih nosača aktivnih supstanci: preformulaciona ispitivanja. Simpozijum Biofarm 2011, Beograd, 27. oktobar 2011, P13.
- [2] Jakšić I, Lukić M, Milić J, Daniels R, Savić S. Komparativna in vitro/in vivo karakterizacija emulzionih podloga sa alkil poliglukozidnim emulgatorom kao nosača za diklofenak i ketoprofen, Arhiv za farmaciju 2010; 60 (5): 772-773. (V Kongres farmaceuta Srbije sa međunarodnim učešćem, Beograd, 13-17. oktobar 2010) – Treća nagrada za najbolju poster prezentaciju u okviru Sekcije za farmaceutsku tehnologiju i kozmetologiju;
- [3] Lukić M, Jakšić I, Vuleta G, Savić S. Formulisanje v/u emulzije za negu suve kože: optimalne reološke i senzorijske karakteristike, Arhiv za farmaciju 2010; 60 (5): 778-779. (V Kongres farmaceuta Srbije sa međunarodnim učešćem, Beograd, 13-17. oktobar 2010)
- [4] Lukić M, Jakšić I, Vuleta G, Savić S. Efikasnost kozmetičkih proizvoda – Koje efekte je moguće ispitati i kako? Arhiv za farmaciju 2010; 60 (5): 730-731. (Usmeno izlaganje - V Kongres farmaceuta Srbije sa međunarodnim učešćem, Beograd, 13-17. oktobar 2010)
- [5] Jakšić I, Lukić M, Milić J, Vuleta G, Savić S, Daniels R. Bioraspoloživost diklofenak-dietilamina iz emulzionih sistema na bazi alkil poliglukozidnog emulgatora: in vivo

tape stripping metod vs. in vitro oslobađanje, Simpozijum Biofarm 2009, Beograd, 22. oktobar 2009, P6.

- [6] Lukić M, Jončić K, Jakšić I, Stojanović B, Rajić M, Savić S. Profili oslobađanja model NSAIL iz različitih tipova nosača za topikalnu primenu: komparativna studija oslobađanja ketoprofena, Simpozijum Biofarm 2009, Beograd, 22. oktobar 2009, P9.
- [7] Lukić M, Jakšić I, Savić S, Tasić-Kostov M, Vesić S, Vuleta G. Laktobionska vs. glikolna kiselina: primena tehnika bioinženjeringa za objektivnu procenu stanja kože, XVIII Kongres udruženja dermatovenerologa Srbije, Beograd, 4.-6. jun 2009. (usmeno izlaganje)
- [8] Jakšić I, Savić S, Vuleta G. Biljni ekstrakti u kozmetičkim proizvodima za tretman celulita, XXVIII Savetovanje o lekovitim i aromatičnim biljkama, Vršac, 8.-11. oktobar 2008.

VIII Tehničko rešenje (M83)

- [1] Snežana Savić, Jela Milić, Milica Lukić, Ivana Jakšić, Mirjana Rajić, Katarina Jončić-Savić, Gordana Vuleta; Razvoj formulacije podloge na bazi prirodnog emulgatora alkil poliglukozidnog tipa za ex tempore izradu magistralnih lekova; 2009 – 2010; U okviru projekta TR 19058 finansiranog od strane Ministarstva nauke i tehnološkog razvoja R. Srbije.

C. Obrazloženje teme doktorske disertacije / Elaboration of the subject of the doctoral dissertation

1. Naučna oblast / Scientific field

Farmaceutska tehnologija / Pharmaceutical Technology

2. Predmet naučnog istraživanja / Subject of the scientific research

Predmet istraživanja doktorske disertacije je evaluacija komparativne primene različitih *in vitro* i *in vivo* tehnika biofarmaceutske karakterizacije lekova za primenu na koži u cilju sagledavanja potencijala i standardizacije *tape stripping* metode (metode sa trakama), kao obećavajuće *in vivo* metode za ispitivanje biološke raspoloživosti dermatoloških lekova. Apsorpcija u kožu model antiinflamatornih lekovitih supstanci različite rastvorljivosti (ketoprofen, diklofenak dietilamin i hidrokortizon) će se procenjivati iz emulzionih nosača na bazi šećernih emulgatora novije generacije (alternativne farmaceutske podloge), sa posebnim osvrtom na rasvetljavanje uticaja koloidne strukture primenjenih sistema i dodatka izabranih korastvarača - ubrzivača penetracije/penetracionih inhensera (izopropil alkohol, propilenglikol, glicerol) na dobijene permeacione/penetracione profile ovih lekova.

The subject of this doctoral dissertation is comparative application of different *in vitro* and *in vivo* techniques for biopharmaceutical characterization of topical drugs, with the aim of evaluating the potential of the tape stripping method as a prospective *in vivo* method for bioavailability assessment of dermatological drugs. Skin absorption of several model anti-inflammatory drugs of different solubility (ketoprofen, diclofenac diethylamine and hydrocortisone) will be assessed from emulsion carriers based on a novel sugar emulsifier (alternative pharmaceutical bases), with a special interest in evaluating the influence of the applied systems' colloidal structure and the addition of selected co-solvents/potential penetration enhancers (isopropyl alcohol, propylene glycol, glycerol) on the obtained permeation/penetration drug profiles.

3. Naučna zasnovanost predložene teme doktorske disertacije / Scientific basis of the proposed subject of the doctoral dissertation

Poslednjih godina, paralelno sa interesovanjem stručne javnosti za razvojem savremenih farmaceutskih oblika (biotehnoški lekovi, nosači mikro- i nano- veličina i dr) ističe se i potreba za tzv. individualizacijom terapije kojom bi se postigao ispravan izbor lekovite supstance, načina doziranja i optimalno oslobađanje iz primenjenog nosača (Florence i Lee, 2011; Nahata et al., 2008). Takođe, usled trenutne farmakoekonomske situacije, farmaceutska industrija racionalizuje svoju proizvodnju čime će tržište biti uskraćeno za određen broj farmaceutskih preparata (Minghetti et al., 2010). Iako se personalizacija terapije može postići magistralnom izradom lekova, konvencionalni nosači (podloge i vehikulumi), pogotovo za lekove koji se primenjuju na koži, često ne zadovoljavaju očekivanja pacijenata što se odražava na komplijansu (Krochmal, 2009). U tom smislu, javlja se potreba za uvođenjem novih farmaceutskih ekscipijenasa i odgovarajućih nosača/podloga radi unapređenja postojećih formulacija.

In the last decade, along with the growing interest in the research and development of contemporary dosage forms (biotechnological drugs, micro- and nano-carriers, etc) emerges a need for the so-called personalised medicines which would provide judicious selection of a drug substance, mode of its application and optimal drug release from the applied carrier (Florence and Lee, 2011; Nahata et al., 2008). Moreover, due to the current pharmacoeconomic situation, pharmaceutical industry is rationalizing its production which will deprive the market of a certain number of preparations (Minghetti et al., 2010). Although individualization of therapy may be accomplished by extemporaneous drug preparation, conventional carriers (bases and vehicles), especially those for topical application, often fail to satisfy patients' expectations and hence reflect compliance (Krochmal, 2009). Consequently, there is a growing demand for the introduction of novel pharmaceutical excipients and suitable carriers/bases, should the existing formulations be improved.

Pregledom novije literature može se uočiti da su se na polju stabilizatora emulzionih sistema posebno izdvojili nejonski mešani emulgatori prirodnog porekla iz grupe alkil poliglukozida koje odlikuje biodegradabilnost i povoljan dermatološki profil (Hoffmann i Platz, 2001; Holmberg, 2001; Metzger i Eissen, 2004; Savić et al., 2010). Dosad sprovedena preformulaciona i formulaciona ispitivanja alkil poliglukozidnih emulgatora ukazala su da emulzione sisteme stabilizuju građenjem kompleksnih struktura tipa lamelarnih tečnih kristala što otvara mogućnost poboljšanja aplikativnih karakteristika, vlažećeg efekta i/ili modifikovanog oslobađanja aktivnih supstanci iz takvih nosača (Savić et al., 2009).

Screening of the recent literature reveals a group of natural-origin non-ionic mixed emulsifiers of alkyl polyglucoside type with many favourable characteristics, especially their biodegradability and excellent dermatological profile (Hoffmann and Platz, 2001; Holmberg, 2001; Metzger and Eissen, 2004; Savic et al., 2010). Preformulation and formulation studies conducted so far have indicated that alkyl polyglucoside emulsifiers stabilize emulsion systems through formation of complex structures of lamellar liquid crystalline type which may improve a system's applicative properties, moisturising effect and/or induce modified release of active ingredients (Savic et al., 2009).

U oblasti istraživanja biofarmaceutskih karakteristika lekova koji se primenjuju na koži, uprkos postojanju brojnih *in vitro* metoda za ispitivanje permeacije i/ili penetracije, vlada veliko interesovanje za razvojem i standardizacijom pogodne *in vivo* metode koja bi dovoljno verno odražavala realne uslove primene (Au et al., 2010; Darlenski et al., 2009; Narkar, 2010). Osim u slučaju lokalne primene kortikosteroida (mogućnost primene vazokonstriktornog testa), pokazivanje biološke raspoloživosti i biološke ekvivalentnosti

lekova koji se primenjuju na koži zahteva sprovođenje skupih i dugotrajnih kliničkih studija (FDA CDER 1995, 1997). Kako bi se pojednostavilo ispitivanje raspoloživosti lekovitih supstanci iz drugih terapijskih grupa, poslednjih godina ispituje se mogućnost primene nekoliko *in vivo* metoda: mikrodijaliza kože, primena spektroskopije bliskog infracrvenog spektra (NIR), biopsija kože i *tape stripping* (TS) tehnika. S obzirom da su navedene metode ili izrazito invazivne ili zahtevaju primenu skupih uređaja, TS metod izdvojio se kao neinvazivna i ekonomična opcija (Narkar, 2010). TS (*skin stripping* ili metoda sa adhezivnim trakama) predstavlja tzv. dermatofarmakokinetički pristup procene penetracionih profila lekovite supstance kroz *stratum corneum* (SC) kao osnovnu barijeru kože. Zasniva se na sukcesivnom skidanju slojeva SC-a i sledstvenoj ekstrakciji i određivanju koncentracije lekovite supstance (Russell i Guy, 2009). FDA je 1998. godine izdao nacrt vodiča sa predloženim protokolom ispitivanja (FDA CDER, 1998) koji je povučen 2002. godine, nakon što su dobijeni varijabilni rezultati ispitivanja istih preparata od strane nezavisnih laboratorija, a zainteresovani istraživači pozvani su na dalji rad na optimizaciji date metode (N'Dri-Stempfer et al., 2009).

In spite of the numerous *in vitro* methods for studying permeation and/or penetration of topical drugs, in the field of their biopharmaceutical characterization there is an increasing demand for development and standardization of an appropriate *in vivo* method that could reflect real in-use conditions (Au et al., 2010; Darlenski et al., 2009; Narkar, 2010). With the exception of topical corticosteroids (possible application of the vasoconstriction assay), bioavailability and bioequivalence evaluation of topical drugs relies on clinical studies which are both time- and money-consuming (FDA CDER 1995, 1997). In order to facilitate characterization of other topical drugs, possible application of several *in vivo* methods is under evaluation: dermal microdialysis, near infrared spectroscopy (NIR), skin biopsy and tape stripping (TS) technique. Since the aforementioned methods are either highly invasive or require the use of expensive state-of-the art equipment, TS method has been distinguished as a non-invasive and economical option (Narkar, 2010). TS (or skin stripping) represents a so-called dermatopharmacokinetic approach for predicting drug penetration profiles across stratum corneum (SC) as the basic skin barrier. It is based on successive removal of SC layers, followed by their extraction and drug quantification (Russell and Guy, 2009). In 1998 FDA has issued a draft guidance proposing a protocol of the investigation (FDA CDER, 1998) which was withdrawn in 2002 after independent laboratories obtained variable results, and interested researchers were urged to continue their research on the method's optimization (N'Dri-Stempfer et al., 2009).

Dosadašnja ispitivanja bila su fokusirana na brojne faktore koji potencijalno mogu uticati na dobijene rezultate (inter- i intraindividualne razlike, uticaj debljine uklonjenog sloja SC-a, vrsta primenjene trake, i dr), kao i mehanicističko sagledavanje predloženih protokola primenom jednostavnih binarnih i ternarnih sistema (lekovita supstanca u jednom ili više rastvarača) (Boix-Montanes, 2011; Lademann et al., 2009). S druge strane, poznato je da se savremeni lekoviti preparati koji se primenjuju na koži obično sastoje od kompleksnih nosača, i u tom kontekstu treba napomenuti da primena TS tehnike u ispitivanju penetracionih profila lekovitih supstanci iz takvih nosača još uvek nije šire ispitana.

Research conducted so far have focused their attention on numerous factors which could potentially influence the obtained results (inter- and intra-individual variations, thickness of the removed SC, type of tapes used, etc), as well as mechanistic evaluation of the proposed protocols through simple binary and ternary systems (drug in one or more solvents) (Boix-Montanes 2011; Lademann et al., 2009). On the other hand, contemporary topical drug preparations usually comprise complex carriers, and it should be noted that the application of TS technique for the penetration assessment of drugs from such carriers has not been extensively addressed.

Trenutno dostupne *in vitro* metodologije biofarmaceutske karakterizacije lekova koji se primenjuju na koži koriste odgovarajuće difuzione ćelije u kojima su donorska i akceptorska faza odvojene pogodnom membranom. Iako istraživači na raspolaganju imaju membrane sintetskog, animalnog i humanog porekla, očigledno ograničenje ovog pristupa je nedostatak vijabilnog i potpornog tkiva, kao i metaboličke aktivnosti (Godin i Touitou, 2007). Uprkos činjenici da je uspostavljanje *in vitro/in vivo* korelacije u slučaju lekova koji se primenjuju na koži veliki izazov, očekuje se više uspeha sa parametrima dobijenim TS metodom i njihovom relacijom sa *in vitro* ispitivanjem oslobađanja/permeacije (Shah, 2005).

Currently available *in vitro* methods for biopharmaceutical characterization of topical drugs use diverse diffusion cells in which donor and receptor phases are separated by a membrane. Although membranes of synthetic, animal and human origin may be used, an obvious shortcoming of this approach is the lack of viable and backing tissue, as well as the metabolic activity (Godin and Touitou, 2007). In spite of the fact that establishing *in vitro/in vivo* correlation in case of topical drugs is still considered to be a challenging task, more success is anticipated with parameters obtained through the TS method and their relation with release/permeation data obtained *in vitro* (Shah, 2005).

4. Cilj istraživanja / The aim of the research

Cilj predložene doktorske disertacije je razvoj, fizičko-hemijska i biofarmaceutska karakterizacija emulzionih podloga stabilisanih prirodnim mešanim emulgatorom tipa alkil poliglukozida (od skora FDA sertifikiran farmaceutski ekscipijens cetostearil glukozid i cetostearil alkohol), koje bi našle primenu u magistralnoj praksi kao savremeni nosači lekova za primenu na kožu, kao i razvoj i optimizacija *tape stripping* metode kao preporučene *in vivo* tehnike za ispitivanje penetracije lekova kroz kožu, uz sprovođenje korelacije datih rezultata sa onim dobijenim ustanovljenim *in vitro* i *in vivo* metodama kroz nekoliko studija slučaja (ketoprofen, diklofenak dietilamin i hidrokortizon kao model lekovite supstance različite rastvorljivosti).

The aim of the proposed doctoral dissertation is development, physicochemical and biopharmaceutical characterization of emulsion bases stabilized with natural-origin mixed emulsifier of alkyl polyglucoside type (recently FDA certified pharmaceutical excipient cetearyl glucoside and cetearyl alcohol) which could serve as contemporary topical drug delivery systems in pharmaceutical compounding, as well as development and optimization of the tape stripping method as a recommended *in vivo* technique for skin penetration assessment of topical drugs, along with evaluation of its potential correlation with results obtained using already established *in vitro* and *in vivo* methods through several case studies (ketoprofen, diclofenac diethylamine and hydrocortisone as model drugs of different solubility).

5. Metodologija naučnog istraživanja / Methodology of the scientific research

Eksperimentalni rad biće podeljen u tri faze:

Experiments will be organized in three phases:

U okviru prve faze biće izrađene različite model podloge stabilisane šećernim mešanim emulgatorom iz grupe alkil poliglukozida (cetostearil glukozid i cetostearil alkohol). U skladu sa opštim principima farmaceutske prakse, biće formulisane podloge jednostavnog sastava kako bi potencijalno odgovarale uslovima magistralne izrade, uz variranje dodatka različitih korastvarača i potencijalnih ubrzivača penetracije (izopropil alkohol u koncentraciji od 10 % (m/m), glicerol 20 % (m/m) i propilenglikol 20 % (m/m)). Izabrane model lekovite supstance (ketoprofen, diklofenak dietilamin i hidrokortizon) različitih fizičko-hemijskih osobina (rastvorljivost, logP, amfifilna priroda) biće inkorporirane suspendovanjem u izrađene

podloge kako bi se procenio potencijal same podloge (specifične koloidne strukture) i/ili dodatog korastvarača da rastvori suspendovani lek i time utiče na njegovu termodinamičku aktivnost (što će biti procenjeno deskriptivno – polarizacionom svetlosnom mikroskopijom, i precizno - izračunavanjem saturacione koncentracije model lekovitih supstanci u formulisanim podlogama). Nakon izrade, pripremljeni uzorci (same podloge i aktivni uzorci) biće podvrgnuti fizičko-hemijskoj karakterizaciji (mikroskopska analiza: svetlosna, polarizaciona i transmisiona elektronska mikroskopija; merenje pH i električne provodljivosti; reološka analiza: kontinualna i oscilatorna reologija; termalne metode: diferencijalna skenirajuća kalorimetrija i termogravimetrijska analiza), skriningu biofarmaceutskih karakteristika (*in vitro* ispitivanje brzine oslobađanja model lekovitih supstanci kroz sintetske membrane uz karakterizaciju dobijenih profila oslobađanja zasnovanu na različitim matematičkim modelima) i *in vitro/in vivo* ispitivanju bezbednosnih profila (*in vitro* ispitivanje citotoksičnosti upotrebom modela rekonstruisanog humanog epiderma i *in vivo* merenje eritema indeksa, transepidermalnog gubitka vode (TEGV), pH kože i vlažnosti *stratum corneum*-a primenom savremenih tehnika bioinženjeringa kože). U cilju procene stabilnosti izrađenih podloga relevantna ispitivanja biće ponovljena nakon 1, 3 i 6 meseci čuvanja uzoraka na sobnoj temperaturi. Paralelno sa podlogama stabilisanim šećernim emulgatorom, biće pripremljene i ispitane odgovarajuće referentne podloge na bazi Nejonskog hidrofilnog krema (Deutsches Arzneibuch 2006), kao podloge potvrđenog, farmakopejskog kvaliteta.

In the first phase of the research different model bases stabilized with a sugar mixed emulsifier of alkyl polyglucoside type (cetearyl glucoside and cetearyl alcohol) shall be prepared. In accordance with the usual principles in pharmaceutical practice, bases of relatively simple composition will be formulated, which could potentially be suitable for extemporaneous preparation settings. Furthermore, the addition of several co-solvents and potential penetration enhancers (isopropyl alcohol in concentration of 10 % (m/m), glycerol 20 % (m/m) and propylene glycol 20 % (m/m)) will be varied. The selected model drugs (ketoprofen, diclofenac diethylamine and hydrocortisone) differing in physicochemical characteristics (solubility, logP, amphiphilic nature) shall be suspended in the prepared bases in order to evaluate the potential of the very base (specific colloidal structure) and/or the added co-solvent to dissolve the suspended drug and hence affect its thermodynamic activity. This will be estimated both descriptively (light microscopy) and precisely by assessing the concentration of saturation of model drugs in the prepared bases. After their preparation, the samples (bases and corresponding active samples) will be submitted through physicochemical characterization (microscopy: light, polarization and transmission electron microscopy; pH and conductivity measurements; rheological analysis: continual and oscillatory rheology; thermal analysis: differential scanning calorimetry and thermogravimetric analysis), screening of biopharmaceutical characteristics (*in vitro* release testing through synthetic membranes along with the characterization of the obtained release profiles using different mathematical models) and *in vitro/in vivo* safety assessment (*in vitro* cytotoxicity assay with a reconstructed human epidermis-based model, and *in vivo* measurements of erythema index, transepidermal water loss (TEWL), pH of the skin and stratum corneum hydration using skin bioengineering techniques). In order to evaluate stability of the prepared samples relevant measurements shall be reassessed after 1, 3 and 6 months of storage at room temperature. Along with the bases stabilized with the sugar emulsifier, reference samples based on Non-ionic hydrophilic cream (Deutsches Arzneibuch 2006), as a base of the established, pharmacopoeial quality, will be prepared and characterized in parallel.

Druga faza rada biće posvećena razvoju *tape stripping* metode kao neinvazivne i ekonomične tehnike *in vivo* procene dermalne bioraspoloživosti lekova uz variranje vrste primenjenih adhezivnih traka (vrsta adheziva, nosača adheziva i dimenzije traka) i procesa

ekstrakcije lekovite supstance sa primenjenih traka (vreme sonifikacije i centrifugiranja traka u etanolu 70 % (V/V)) u cilju optimizacije protokola ispitivanja. Kako bi se dobili validni rezultati, protokol ispitivanja će obuhvatiti i gravimetriju traka (merenje mase traka pre i nakon primene na osetljivoj analitičkoj vagi) i merenje TEGV-a, kao parametra integriteta kožne barijere. Dobijeni parametri će primenom nakoliko matematičkih relacija omogućiti izračunavanje relativne debljine uklonjenog SC (tzv. normalizacija debljine uklonjenog SC-a) u cilju umanjenja uticaja intra- i interindividualnih razlika unutar seta ispitanika. Zatim će biti procenjen potencijal razvijene TS metode za ispitivanje penetracionih profila model lekovitih supstanci iz uzoraka formulisanih i ispitanih u okviru prve faze eksperimentalnog rada, kao farmaceutskih oblika realne kompleksnosti. Od interesa je ispitati potencijal TS metode da ukaže na značaj same mikrostrukture primenjenog emulzionog nosača i/ili dodatog ubrzivača penetracije u realnim uslovima primene lekova koji se nanose na kožu. Paralelno sa primenom TS metode, biće sprovedena biofarmaceutska ispitivanja koja su trenutno preporučena od strane regulatornih agencija za lekove koji se primenjuju na koži: ispitivanja permeacije primenom Franz-ovih difuzionih ćelija (i) *in vitro* - kroz veštačke konstrukte kože, i (ii) tzv. *ex vivo* - kroz izolovani humani *stratum corneum*. Dobijeni rezultati biće razmatrani sa aspekta moguće korelacije sprovedenih *in vitro* testova sa podacima koje pruža TS metoda predloženog protokola.

The second phase of the research will be directed towards development of the tape stripping method as a non-invasive and inexpensive technique for *in vivo* dermal bioavailability assessment, with emphasis on the variation of the type of the tapes used (composition of the adhesive, the backing layer and tape dimensions) and the extraction process of a drug from the applied tape (duration of sonification and centrifugation of the tapes in ethanol 70 % (V/V)) for the purpose of optimizing the protocol of investigation. In order to obtain valid results, the protocol of the study will also embody the gravimetric analysis of the tapes (tape measurements prior to and after its application by means of a sensitive analytical balance) and TEWL assessment, as a parameter of skin barrier integrity. Through several mathematical equations, the obtained parameters will provide relative depth of the removed SC (so-called normalization of the SC thickness) with the purpose of diminishing the inevitable influence of the intra- and inter-individual differences within the set of the volunteers. Further, the potential of the developed TS method will be estimated for the penetration assessment of the active samples formulated and characterized in phase one of the research, as drug delivery systems of real complexity. Moreover, it is of interest to assess the potential of the TS method to demonstrate the influence of the very microstructure of the applied emulsion system and/or the added penetration enhancer in real in-use conditions of topical drug application. Several methods of biopharmaceutical characterization accepted by the regulatory agencies for topical drugs will be conducted in parallel with the TS method: permeation studies with Franz diffusion cells (i) *in vitro* – through artificial skin constructs, and (ii) so-called *ex vivo* – through isolated human stratum corneum. The obtained results will be evaluated for the potential correlation of the conducted *in vitro* methods with data offered by the TS technique of the suggested protocol.

U trećoj fazi TS tehnika će biti poređena sa vazokonstriktornim testom za lekove iz grupe kortikosteroida kao, sa izuzetkom kliničkih studija, jedinim *in vivo* ispitivanjem dermalne bioraspoloživosti i bioekvivalentnosti prihvaćenim od strane regulatornih tela (FDA Guideline, 1998). Komparacija dve *in vivo* metode (TS kao tzv. dermatofarmakokinetički, i vazokonstriktorni test kao farmakodinamski pristup) biće sprovedena na primeru hidrokortizona iz uzoraka formulisanih i pripremljenih u okviru prve faze istraživanja.

In the third phase, TS technique will be compared to the vasoconstriction assay for the topical corticosteroids as, with the exception of clinical studies, the only *in vivo* method for dermal bioavailability and bioequivalence assessment accepted by the regulatory agencies

(FDA Guideline, 1998). Comparison of the two *in vivo* methods (TS as a dermatopharmacokinetic, and skin blanching assay as a pharmacodynamic approach) will be conducted with hydrocortisone-loaded samples prepared in the phase one of the research.

U cilju sveobuhvatne karakterizacije uzoraka biće primenjene sledeće metode ispitivanja:

In order to conduct a comprehensive characterization of the samples, the following methods shall be employed:

Uvid u specifičnu koloidnu mikrostrukturu podloga stabilisanih alkil poliglukozidnim emulgatorom biće izvršen primenom polarizacionog mikroskopa Carl Zeiss ApoTome Imager Z1 (Zeiss, Göttingen, Nemačka) integrisanog sa digitalnom AxioCam ICc1 kamerom i AxioVision 4.6 softverskim paketom. Dublji i pouzdaniji uvid u složenu anizotropnu strukturu emulzionih podloga (formiranje kompleksnih lamelarnih gel i tečno-kristalnih struktura) biće postignut primenom transmisije elektronske mikroskopije (tehnika lomljenja zamrznutog uzorka).

Insight into the specific colloidal structure of the bases stabilized with the alkyl polyglucoside emulsifier will be obtained by polarization microscope Carl Zeiss ApoTome Imager Z1 (Zeiss, Göttingen, Germany) integrated with a digital AxioCam ICc1 camera and AxioVision 4.6 software. A deeper and more precise understanding of the complex anisotropic structure of the emulsion systems (formation of the complex lamellar gel and liquid crystalline structures) is expected to be achieved through transmission electron microscopy (freeze-fracture technique).

Potencijal koloidne strukture podloge i/ili variranih korastvarača da rastvori suspendovani lek i na taj način utiče na termodinamičku aktivnost model lekovitih supstanci biće procenjen primenom svetlosne mikroskopije (Carl Zeiss ApoTome Imager Z1, Zeiss, Göttingen, Nemačka; bez primene λ -pločice), i to deskriptivno - uvidom u snimljene mikrografije uveličanja 200x i 400x, i precizno - izračunavanjem saturacione koncentracije model lekovitih supstanci u formulisanim podlogama.

The potential of the base's colloidal structure and/or the varied co-solvents to dissolve the suspended drug and hence influence the thermodynamic activity of the model drugs will be evaluated using light microscopy (Carl Zeiss ApoTome Imager Z1, Zeiss, Göttingen, Germany; without λ -plate), i.e. descriptively – from the captured micrographs at magnifications 200x and 400x, and precisely – evaluating the model drugs' concentration of saturation in the formulated bases.

Ispitivanje stabilnosti uzoraka biće sprovedeno kroz merenje pH vrednosti (Hanna instruments HI 9321, Michigan, SAD), električne provodljivosti (konduktometar CDM 230, Radiometer, Brønshøj, Danska) i reoloških parametara (kontinualna i oscilatorna reologija) primenom reometra Rheolab MC 120 (Paar Physica, Ostfildern, Nemačka) inicijalno (7 dana nakon izrade), posle 1, 3 i 6 meseci čuvanja uzoraka na sobnoj temperaturi.

Stability assessment of the samples will be evaluated through pH (Hanna Instruments HI 9321, Michigan, USA), conductivity (Conductometer CDM 230, Radiometer, Brønshøj, Denmark) and rheological measurements (continual and oscillatory rheology) using a Rheolab MC 120 rheometer (Paar Physica, Ostfildern, Germany) initially (7 days after preparation), after 1, 3 and 6 months of storage at room temperature.

Procena faznih prelaza i načina inkorporiranja vode u emulzionom sistemu/farmaceutskoj podlozi izvršiće se primenom diferencijalnog skenirajućeg kalorimetra Mettler DSC 820 (Mettler Toledo, Giessen, Nemačka) i uređaja za termogravimetrijsku analizu Netzsch STA 409PG (Netzsch, Selb, Nemačka).

Phase transitions and the mode of water incorporation within the emulsion system/pharmaceutical base will be evaluated using differential scanning calorimetry (Mettler DSC 820, Mettler Toledo, Giessen, Germany) and thermogravimetry (Netzsch STA 409PG, Netzsch, Selb, Germany).

Ispitivanje brzine oslobađanja model lekovitih supstanci iz uzoraka biće sprovedeno u uređaju za *dissolution test* sa mini-lopaticama (Erweka DT 600, Heusenstamm, Nemačka) primenom VanKel Enhancer[®] ćelija (VanKel Industries Inc., CA, SAD) uz membrane od celulozaacetata. Liberaciona kinetika model lekova biće procenjena primenom više matematičkih modela koji potencijalno odgovaraju polučvrstim emulzionim sistemima (kinetika nultog reda, prvog reda, Higuchi i Hixon-Crowell model) pomoću softverskog paketa OriginPro 8 (OriginLab Corporation, Northampton, UK).

Dissolution testing of the investigated active samples will be conducted in a dissolution apparatus equipped with a mini-paddle system (Erweka DT 600, Heusenstamm, Germany) using VanKel Enhancer[®] cells (VanKel Industries Inc., CA, USA) through cellulose acetate membranes. Liberation kinetics of the model drugs will be evaluated through several mathematical models potentially suitable for semisolid emulsion systems (zero-order, first order, Higuchi and Hixon-Crowell kinetics) in OriginPro 8 software (OriginLab Corporation, Northampton, UK).

Uvid u permeacione profile biće izvršen primenom modifikovanih Franz-ovih difuzionih ćelija u kojima će donorska (ispitivani uzorak) od akceptorske faze (fosfatni pufer pH 7,4 (USP31)) biti odvojene (i) veštačkim konstruktom kože (*in vitro*) i (ii) izolovanim humanim *stratum corneum-om* (tzv. *ex vivo* pristup). Permeacija će biti praćena tokom 30 h u odgovarajućim vremenskim intervalima, a koncentracija lekovitih supstanci u eluatima biće određena primenom HPLC metode (HPLC, Waters, Eshborn, Nemačka).

Permeation profiles will be investigated using modified Franz diffusion cells where the donor phase (the investigated drug-loaded sample) and receptor phase (phosphate buffer pH 7,4 (USP31)) will be separated by (i) artificial skin constructs (*in vitro*) and (ii) isolated human stratum corneum (so-called *ex vivo* approach). Permeation will be monitored over 30 h in predetermined time points, while the drug concentration in the withdrawn aliquots will be quantified using an appropriate HPLC method (HPLC, Waters, Eshborn, Germany).

Tokom **razvoja i optimizacije tape stripping metode** biće procenjena pogodnost primene dve vrste adhezivnih traka (D-squame[®], CuDerm, Dallas, SAD i 3M[™] Transpore[™], 3M HealthCare, UK). Takođe, u cilju standardizacije procesa ekstrakcije leka sa trake biće varirano vreme izlaganja traka sa medijumom za ekstrakciju ultrazvuku (Sonorex RK102H, Bandelin, Berlin, Nemačka) i vreme/brzina centrifugiranja (Tehtnica, Zelezniki, Slovenija). Paralelno sa sprovođenjem protokola TS-a, merenje TEGV-a biće vršeno u različitim fazama uklanjanja *stratum corneuma* osetljivom sondom uređaja Tewameter[®] TM210 (Courage+Khazaka, Keln, Nemačka). Sadržaj lekovite supstance u eluatima biće precizno određen primenom ultra visokoefikasne tečne hromatografije sa masenom spektroskopijom (UHPLC-MS).

In the scope of the **development and optimization of the tape stripping method**, the potential use of two types of adhesive tapes (D-squame[®], CuDerm, Dallas, USA and 3M[™] Transpore[™], 3M HealthCare, UK) will be compared for the purpose. Additionally, as a potential contribution to the standardization of the drug extraction process, the applied tapes will be submitted to various duration of sonification (Sonorex RK102H, Bandelin, Berlin, Germany) and duration/speed of centrifugation (Tehtnica, Zelezniki, Slovenia). During the TS protocol, TEWL will be measured in different phases of SC harvesting using a sensitive probe of Tewameter[®] TM210 (Courage+Khazaka, Köln, Germany). Drug content in the obtained

eluates will be precisely determined by means of ultra high performance liquid chromatography-mass spectrometry (UHPLC-MS).

Aspekt bezbednosti ispitivanih podloga sa i bez dodatka izabranih korastvarača/potencijalnih ubrzivača penetracije biće procenjen (i) *in vitro* ispitivanjem citotoksičnosti na ćelijskim kulturama (rekonstruisani humani epiderm) koje je bazirano na Mosmanovoj MTT redukcionoj metodi, i (ii) primenom savremenih *in vivo* tehnika bioinženjeringa kože kojima će se dobiti uvid u sledeće parametre: a) hidratacija SC i b) pH kože primenom uređaja Cutometer[®] MPA 580, c) TEGV uređajem Tewameter[®] TM210 i d) eritema indeks primenom aparata Mexameter[®] MX18 (svi proizvođača Courage+Khazaka, Keln, Nemačka).

Safety aspect of the investigated bases with and without the addition of the selected co-solvents/potential penetration enhancers will be assessed by (i) *in vitro* cytotoxicity assay on cell cultures (reconstructed human epidermis) based on the Mosman's MTT reduction method, and (ii) *in vivo* skin bioengineering techniques which will provide information on the following parameters: a) SC hydration and b) pH of the skin using Cutometer[®] MPA 580, c) TEGV using Tewameter[®] TM210 and d) erythema index by means of Mexameter[®] MX18 (all Courage+Khazaka, Köln, Germany).

Analiza vazokonstriktornog efekta nakon lokalne primene hidrokortizona iz izrađenih uzoraka biće procenjen neinvazivnom tehnikom bioinženjeringa kože primenom uređaja Mexameter[®] MX18 (Courage+Khazaka, Keln, Nemačka). Na ovaj način, merenjem efekta izbeljivanja kože (eng. *skin blanching assay*) procenjuje se brzina i obim difuzije leka u dermalnu vaskulaturu, što je u direktnoj korelaciji sa dermalnom bioraspoloživosti leka.

Vasoconstriction effect after topical application of hydrocortisone-loaded samples will be evaluated by a skin bioengineering technique using Mexameter[®] MX18 (Courage+Khazaka, Köln, Germany). Hence, through this skin blanching assay both rate and extent of drug diffusion into the dermal vasculature could be assessed, which is in direct correlation with its dermal bioavailability.

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7. Očekivani rezultati / Anticipated results

Kao ishod prve faze eksperimentalnog rada očekuje se da će primenjeni šećerni emulgator alkil poliglukoziidnog tipa pokazati dobar potencijal za stabilizaciju podloga jednostavnog sastava koje bi našle primenu kao tzv. *ready-to-use*, univerzalne podloge u magistralnoj izradi lekova koji se primenjuju na koži. Imajući u vidu da je za primenjeni emulgator pokazana sposobnost formiranja kompleksnih lamelarnih tečno-kristalnih faza, očekuje se da će se prisustvo specifičnih anizotropnih struktura uočiti primenom polarizacije i transmisije elektronske mikroskopije. Takođe, smatra se da će karakterizacija izrađenih podloga primenom reoloških merenja, ispitivanjem pH vrednosti i električne provodljivosti pružiti zadovoljavajuće podatke o stabilnosti formulisanih podloga tokom 6 meseci čuvanja na sobnoj temperaturi (uobičajeni rok trajanja magistralno izrađenih podloga tipa hidrofilnog krema). Dodatno, s obzirom da je u pitanju emulgator prirodnog porekla koji se već uspešno koristi na polju kozmetologije, formulisani uzorci bi trebalo da poseduju i dobre aplikativne i senzorijske osobine koje bi zadovoljile kriterijume savremenog pacijenta u pogledu kvaliteta i očekivanih karakteristika farmaceutskih podloga. Očekuje se da će specifična koloidna mikrostruktura formulisanih podloga biti sposobna da emulguje i zadrži izopropil alkohol, glicerol i propilenglikol u ispitivanim koncentracijama, kao korastvarače koji bi mogli da utiču na bolje oslobađanje, permeaciju i penetraciju izabranih aktivnih supstanci. Ispitivanje formulisanih podloga kroz tri studije slučaja (ketoprofen, diklofenak dietilamin i hidrokortizon) trebalo bi da ukaže na ekvivalentne ili unapređene liberacione profile model lekova u poređenju sa odgovarajućim referentnim podlogama konvencionalnog sastava. Konačno, ali ne i najmanje važno, od formulisanih podloga se očekuje dobar *in vitro/in vivo* bezbednosni i dermatološki profil čak i nakon dodatka izabranih korastvarača/ubrzivača penetracije, kao potencijalnih iritanasa.

The first phase of the research is expected to demonstrate satisfactory potential of the applied sugar emulsifier of alkyl polyglucoside type for stabilization of relatively simple bases that could find use as the so-called ready-to-use, universal bases in pharmaceutical compounding of topical drugs. Taking into consideration that the emulsifier is known to generate complex lamellar liquid crystalline phases, it is expected that the presence of such specific anisotropic structures will be confirmed with polarization and transmission electron microscopy. Additionally, the physicochemical characterization of the prepared bases through rheological measurements, pH and conductivity assessment is expected to prove the formulated bases stable during 6 months of storage at room temperature (usual expiry date of extemporaneously prepared hydrophilic creams). Moreover, since this natural-origin emulsifier is already successfully applied in cosmetics, the investigated samples should also possess good applicative and sensory properties that would meet high expectations of a modern patient concerning their quality. Further, it is expected that the specific colloidal microstructure of the investigated bases will be able to sustain isopropyl alcohol, glycerol and propylene glycol in the selected concentrations, as co-solvents that could induce enhanced release, permeation and penetration of the chosen drugs. Through three case studies (ketoprofen, diclofenac diethylamine and hydrocortisone), the investigated samples should demonstrate equivalent or enhanced liberation profiles when compared to the conventional, reference samples. Last but not least, the investigated bases are expected to possess satisfactory *in vitro/in vivo* safety and skin performance profile, even after the incorporation of the selected co-solvents/penetration enhancers, as potential irritants.

S obzirom na broj ključnih parametara TS metode koji će biti varirani u drugoj fazi istraživanja, očekuje se da će se definisati optimalan protokol ispitivanja koji će pružati pouzdane rezultate uprkos brojnim izvorima varijabilnosti karakterističnim za većinu *in vivo*

metodologija. Kao potencijalna tehnika ispitivanja dermalne raspoloživosti lekova kompleksnog sastava, očekuje se da će pokazati zadovoljavajuću osetljivost nakon isključivanja mogućih interferencija (uticaj primenjenog adheziva, uklonjenih korneocita i sastojaka formulacije). Kroz ispitivanja uzoraka iz prve faze rada, od optimizovane TS metode očekuje se da, u poređenju sa prihvaćenim *in vitro* i *ex vivo* ispitivanjima oslobađanja i permeacije, bude dovoljno diskriminatorna da ukaže na značajan uticaj koloidne mikrostrukture i/ili pojedinih primenjenih ubrzivača penetracije. S obzirom da je u pitanju metoda koja se bazira na realnim uslovima primene lekova na koži, očekuje se da će pored parametara brzine i stepena penetracije leka kroz *stratum corneum*, kao suštinsku kožnu barijeru, pružiti informacije o specifičnim lek-koža interakcijama kroz uticaj primenjenog nosača na kohezivnost korneocita.

Having in mind the number of TS parameters about to be varied in the second phase of the research, it is anticipated that an optimal protocol of the TS method could be defined, which would offer valid results in spite of the numerous sources of variability common for the most *in vivo* studies. As a prospective technique for dermal bioavailability assessment of topical drugs of complex composition, it is expected to demonstrate satisfactory sensitivity after diminishing possible interferences (influence of the adhesive, stripped corneocytes and formulation constituents). Throughout the investigation of the samples prepared in the first phase of the research, the optimized TS method, when compared to the established *in vitro* and *ex vivo* release/permeation studies, is expected to be discriminative enough to indicate significant influence of the colloidal structure and/or some of the incorporated penetration enhancers. Since it is a method based on real in-use conditions of topical drug application, apart from the parameters of rate and extent of drug penetration through SC as a fundamental skin barrier, the method is expected to provide information on specific drug-skin interactions via the influence of the applied carrier on the corneocytes cohesiveness.

Konačno, komparativnim ispitivanjem TS tehnike i vazokonstriktornog testa (eng. *skin blanching assay*) nakon *in vivo* dermalne primene kortikosteroida očekuje se da će kao ishod treće faze eksperimentalnog rada na primeru hidrokortizona iz formulisanih uzoraka biti pokazana dobra korelacija između TS metode optimizovanog protokola i prihvaćene analize farmakodinamskog odgovora kortikosteroida na koži. Očekivani rezultati druge i treće faze istraživanja ukazali bi na značajan potencijal TS tehnike kao *in vivo* metode procene dermalne bioraspoloživosti i bioekvivalentnosti koja bi se mogla uvrstiti i u rutinske metode biofarmaceutske karakterizacije lekova koji se primenjuju na koži, posebno u situacijama procene generičkih lekova, kako bi se kao krajnji ishod moglo očekivati izostavljanje dela ili ukupnih kliničkih ispitivanja novih, a pre svega generičkih dermatoloških lekova.

Finally, comparative assessment of the TS technique and the vasoconstriction test (skin blanching assay) after *in vivo* topical application of corticosteroids (i.e. hydrocortisone from the investigated samples), a good correlation between the TS method of the optimized protocol and the acknowledged analysis of the topical corticosteroids' pharmacodynamic effect is expected as the outcome of the third phase of the research. Anticipated results of the phase two and three of the research would imply a considerable potential of the TS technique as an *in vivo* method for dermal bioavailability and bioequivalence assessment that may find its place as one of the routine methods for biopharmaceutical characterization of topical drugs. This would be of considerable importance, especially in case of generic drugs' evaluation, and could eventually allow omitting a part of or even completely excluding clinical trials of (primarily generic) dermatological drugs.

D. Zaključak

Na osnovu uvida u podatke koje je kandidat Ivana Pantelić navela u prijavi doktorske disertacije, a posle analize istih, Komisija smatra da je predložena tema doktorske disertacije naučno zasnovana (na osnovu pregleda dostupne literature iz oblasti formulacije, fizičkohemijskih ispitivanja i *in vitro/in vivo* metoda karakterizacije emulzionih sistema za primenu na kožu). Podaci vezani za formulaciju i složenost strukture koloidnih sistema stabilisanih alkil poliglukozidnim emulgatorom prirodnog porekla, kao i izbor variranih korastvarača/potencijalnih penetracionih inhensera i model lekovitih supstanci različitih fizičkohemijskih osobina, ukazuju na potrebu za sveobuhvatnom karakterizacijom formulisanih sistema koja bi, nakon razmatranja aspekata stabilnosti, bezbednosti, efikasnosti i aplikativnih osobina, mogla dovesti do razvoja savremenih emulzionih podloga tipa ulje-u-vodi namenjenih *ex tempore* izradi magistralnih dermatoloških lekova, što je od značaja u individualizovanoj terapiji niza dermatoloških oboljenja.

Based on the information that the candidate Ivana Pantelić has stated in the application of the doctoral dissertation, and after their thorough analysis, the Commission believes that the suggested subject of the doctoral dissertation is scientifically founded (based on the review of the available literature in the field of formulation, physicochemical characterization and *in vitro/in vivo* methods for the assessment of emulsion systems intended for topical application). Data concerning the formulation and the overall complexity of the structure of the colloidal systems stabilized with a natural-origin alkyl polyglucoside emulsifier, as well as the varied co-solvents/potential penetration enhancers and model drugs of different physicochemical characteristics, imply that there is a need for a comprehensive characterization of the formulated systems that could, after proper stability, safety and efficacy considerations, lead to development of contemporary emulsion bases of oil-in-water type intended for *ex tempore* preparation of dermatological drugs, which is of interest in personalized therapy of diverse dermatological conditions.

Variranje više parametara protokola *in vivo tape stripping* metode (ključni faktori koji mogu uticati na dobijene rezultate, poput vrste adhezivnih traka i postupka ekstrakcije leka) uz sledstvenu komparativnu evaluaciju predložene metode sa prihvaćenim *in vitro* biofarmaceutskim i *in vivo* farmakodinamskim ispitivanjima, pruža mogućnost optimizacije ove *in vivo* tehnike ispitivanja dermalne raspoloživosti lekova kompleksnog sastava. Rezultati ovih ispitivanja bi mogli doprineti uspostavljanju konačne verzije Vodiča ovog ispitivanja.

Varying several key parameters of the *in vivo tape stripping* protocol (factors that could influence the obtained results, such as the type of the adhesive tapes used and the procedure of drug extraction) along with the comparative evaluation of the suggested method with already established *in vitro* biopharmaceutical and *in vivo* pharmacodynamic investigation, offers the possibility of optimization of this *in vivo* technique for dermal bioavailability assessment of complex drug dosage forms, which may contribute to the definition of the method's final Guidance.

Prema mišljenju članova Komisije tema doktorske disertacije je aktuelna i opravdana. Članovi Komisije predlažu Nastavno-naučnom veću da se Ivani Pantelić odobri izrada doktorske disertacije pod navedenim naslovom.

According to all the members of this Commission, the subject of the doctoral dissertation is both contemporary and relevant. The members of the Commission suggest that the Academic Council of Faculty of Pharmacy should allow Ivana Pantelic to resume work on the doctoral dissertation of the aforementioned subject.

Beograd, 13.02.2012. god.
Belgrade, 13.02.2012.

Članovi Komisije:
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