POLYMORFI

2007

FYSIKAALISEN FARMASIAN YHDISTYS

Fysikaalisen farmasian XVIII vuosittainen symposium:

LIPIDITUTKIMUS

ja farmaseuttiset sovellukset

Kuopio 25.1.2007

1988

SOCIETY OF PHYSICAL PHARMACY

ISSN: 1236-4002
1458-5820 (pdf)

Fysikaalisen farmasian XVIII vuosittainen symposium:

L I P I D I T U T K I M U S
j a f a r m a s e u t t i s e t
s o v e l l u k s e t

KUOPIO, MICROTEKNIA 3, 25.1.2007

XVIII SYMPOSIUMIN OHJELMA

9.00		ILMOITTAUTUMINEN JA AAMUKAHVI
9.30	Mika Pulkkinen (FFY, Kuopion yliopisto)	Symposiumin avaus
9.45	Anna-Maija Lampi (Helsingin yliopisto)	Lipids in foods and other biological materials
10.30	Reijo Käkelä (Helsingin yliopisto)	Current view of the structure and function of biological lipid membranes and analysis of the lipid profile by mass spectrometry-based lipidomics
11.15		Posteriesittelyt
11.45		LOUNAS JA POSTERINÄYTTELY
13.15	Susanne Wiedmer (Helsingin yliopisto)	Liposomes as coatings and carriers in electrophoresis
14.00		KAHVI
14.30	Marjo Yliperttula (Helsingin yliopisto)	Physicochemical characterization of liposome systems for pharmaceutical applications
15.15	Julia Lehtinen (Helsingin yliopisto)	Liposomal drug carriers for cancer treatment
15.35	Zanna Hyvönen (Kuopion yliopisto)	1,4-dihydropyridine derivatives as gene delivery system
15.55	Mika Pulkkinen (FFY, Kuopion yliopisto)	Symposiumin päätös
16.15		FYSIKAALISEN FARMASIAN YHDISTYKSEN VUOSIKOKOUS tutustuminen Farmaseuttisen lääketutkimuskeskuksen (FLK) toimintaan
18.00		ILALLISBUFFET JA PARHAAN POSTERIN PALKITSEMINEN

POLYMORFI 2007

FYSIKAALISEN FARMASIAN YHDISTYKSEN JÄSENLEHTI
SISÄLLYS

ESITYSABSTRAKTIT *1*

Lipids in foods and other biological materials *2*

Current view of the structure and function of biological lipid membranes and analysis of the lipid profile by mass spectrometry-based lipidomics *4*

Liposomes as coatings and carriers in electrophoresis *6*

Physicochemical characterization of liposome systems for pharmaceutical applications *7*

Liposomal drug carriers for cancer treatment *8*

1,4-dihydropyridine derivatives as gene delivery system *9*

POSTERIABSTRAKTIT *11*

Evaluation of mesoporous TCPSI, MCM-41, SBA-15 and TUD-1 materials as API carriers for oral drug delivery *12*

A novel dissolution microcalorimetry cell *13*

Detection of defensins from biological matrices with HPLC-ESI-MS *14*

Preferred orientation of crystallites affecs the intrinsic dissolution of aspirin and tolbutamide tablets *15*

The effect of texture on the intrinsic dissolution behaviour of pharmaceutical tablets *16*

Investigation of piroxicam monohydrate dehydration behaviour in compacts using multiple spectroscopic techniques *17*

Drug permeability in caco-2 cell monolayers is affected by use of 'physiological' donor solutions *18*

Bisphosphonate-induced ATP analog formation in different cancer cell lines *19*

Mesoporous silicon microparticles loading and release of peptide *20*

The effect of surface chemistry and porous properties of porous silicon on the dissolution of ibuprofen *21*

Effect of substrate ionization on the apparent affinity to P-glycoprotein Pgp-ATPase assay *22*

Different cellular distribution kinetics of testosterone, propranolol and quinidine *23*

VÄITÖSKIRJOJEN TIIVISTELMÄT *24*

The roughness and imaging characterisation of different pharmaceutical surfaces *25*

Caco-2 cell cultures in the assessment of intestinal absorption: Effects of some co-administered drugs and natural compounds in biological matrices *26*

Novel cationic amphiphilic 1,4-dihydropyridine derivatives for DNA delivery: structure-activity relationships and mechanisms *27*

Development of Analytical Methods for the Characterization of Absorption Cell Models *28*

Synthesis of Novel Cannabinoid CB1 Receptor Ligands *29*

Design, Synthesis and Characterization of Novel Water-Soluble Chitosan Derivatives 30
Molecular Modeling of the Endogenous Cannabinoid System - Usability of Modeling Results in Drug Design 31
Enzymatic Hydrolysis of the Endocannabinoid 2-Arachidonoylglycerol -Characterization and Inhibition in Rat Brain Membranes and Homogenates 32
GRADUJEN TIIVISTELMÄT 33
Scale-up of high-shear mixers- Understanding of wet granulation process 34
Lämpöherkillä polymeereillä päällystettyjen polystyreenipartikkelien ominaisuudet, sytotoksisuus sekä kiinnittyminen RAW 264 -makrofageihin ja Caco-2 soluihin 35
Adsorption of some drugs on microcrystalline cellulose in aqueous solution 36
Pintakuva-analyysi rakeiden prosessikäyttäytymisen tarkastelussa 37
Permeation mechanisms of salicylic acid and derivates across Caco-2 monolayers: involvment of transporter proteins 38
Kahden termoanalyyttisen menetelmän vertailu tutkittaessa amorfisen lääkeaineen kiteytymiskinetiikkaa 39
Partikkelien erottaminen dielektroforeesin avulla 40
Soluunottomekanismit ja niiden tutkiminen 41
Antibioottiset peptidit elimistön puolustusmekanismina 42
Proteiinien fysikaaliset olomuodot lääkevalmisteissa ja niiden stabiilius 43
Mikroemulsiot iholääkinnässä 44
Syklodekstriinikompleksoitumista tehostavat menetelmät 45
Biohajoavista polymeereistä valmistetut kirurgiset implantit erilaisten kudosvaurioiden hoitoon 46
Ihon epidermiksen solumallien rakennemorfologia ja lipidikoostumus 47
Solukalvoläpäisevyyden ennustammen in vitro menetelmin; PAMPA, RP-HPLC ja IAM-kromatografia 48
Dimetyloituneiden indolialkyyliamiinien käyttö alkuperäiskansojen keskuudessa ja nykyinen status 49
Prolyylioligopeptidaasi-inhibiittorien lipofiilisyyden määrittäminen 50
Kiinteiden geenilääkkeiden formulointi 51
KRYPTO 52
OSALLISTUJAT 53
KRYPTO RATKAISU 54

Päätoimittaja: Pekka Hoppu, Helsingin yliopisto
Pekka.Hoppu@helsinki.fi

Julkaisija: Fysikaalisen farmasian yhdistys ry
(www.physics.utu.fi/industrial/fyfa/)

PÄÄTOIMITTAJAN PALSTA

Tervehdys yhdistyksen jäsenet ja XVIII symposiumin osallistujat.

Kun viime vuonna pohdimme vuoden 2007 symposiumin aihetta ja sisältöä ilmaantui mielenkiintoa lipideihin ja rasvoihin. Aihe on monelle FFYn jäsenelle vieras, joten se täydentää hyvin aiheita mitä edellisissä symposiumeissa on käsitelty.

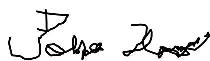
Rasvat ja lipidit ovat meille kaikille tuttuja joka päiväisessä arkielämässämme ja onhan rasvat sekä lipidit tärkeä rakenneosa elimistössämme. Myös päivittäisessä energian saannissa rasvat ovat tärkeä osa elämäämme. Kaikkien tuntemassa Kansanterveyslaitoksen (KTL) ruokaympyrässä tämän hetkinen rasvan päivittäinen saantisuositus kokonaisenergiasta on noin 30%. Joten myös ruoansulatuselimistömme on tullut hyvin tutuksi rasvojen kanssa. Valitettavasti osalla ihmisistä tämä päivittäinen kokonaisenergian saanti saattaa ylittyä aiheuttaen rasvaisen vararenkaan kertymistä vyötärölle. Vuoden 2002 KTLn arvion mukaan yli puolella suomalaisista on kertynyt liikakiloja, mikä saattaa aiheuttaa myös terveydellisiä ongelmia sekä ongelmia lääkeaineiden jakautumisessa elimistössä. KTL:n uusi terveys- ja ravintotutkimus alkaa helmikuussa 2007, josta uusia tuloksia saadaan loppuvuodesta.

Fysikaalisen farmasian näkökulmasta koetamme tämän vuoden symposiumissa tuoda kattavan näkökulman tämän hetkisestä lipiditutkimuksesta Suomessa ja lipidien perusominaisuuksista. Sehän kuuluu jo yhdistyksemme tavoitteisiin. Tavoitteemmehan on fysikaalisen farmasian tietouden edistäminen Suomessa. Esitelmien toivotaan avaavan uusia näkökulmia ja yhteistyömahdollisuuksia lipiditutkimuksessa ja yhteistyöverkon laajenemista. Toivottavasti jäsentemme ammattitaito kehittyy tässäkin symposiumissa tai Polymorfilehteä lukemalla, mistä seuraisi fysikaalisen farmasian tieteellisen ja teknisen tutkimuksen edistyminen niin lipiditutkimuksessa kuin myös muilla saroilla.

Polymorfilehti antaa tänäkin vuonna kattavan paketin farmasian alalla tehtävästä tutkimuksesta tiivistelmien muodossa. Sitten onnitteluni sanaristikkojen ystäville: tässä kuten edellisessäkin Polymorfissa on puuhaa kevättalven pimeisiin iltoihin.

Toivotamme kaikille lukijoillemme opettavaisia ja muistorikkaita hetkiä Polymorfin ja rasvojen ihmeellisessä maailmassa!

Helsingissä 22.1.2007



Pekka Hoppu

ESITYSABSTRAKTIT

LIPIDS IN FOODS AND OTHER BIOLOGICAL MATERIALS

Anna-Maija Lampi

Department of Applied Chemistry and Microbiology, P.O.Box 27 (Latokartanonkaari 11), 00014
University of Helsinki

Lipids consist of a large group of biological molecules that have versatile chemical and biological properties. Unlike other biological compounds, most lipids are insoluble in water which gives them special functional and chemical properties in living systems. In the 1980s, lipids were defined as natural compounds soluble in organic solvents and insoluble in water, but later the definition was revised because e.g. some lipids were found to be at least as soluble in water as in the solvents, and greater interest in structured compounds that were chemically modified from natural lipids. More recent definitions are based on the similarities in the synthesis of lipids or their functions, and not on their solubility. The definition by W.W. Christie [1] is: *"Lipids are fatty acids and their derivatives, and substances related biosynthetically or functionally to these compounds"*. This approach is currently widely accepted by lipid chemists and analysts. For lipid biologists, a new classification system and vocabulary for lipids were presented in 2005 [2], which were needed for the evaluating and cataloging the properties of lipids to build up "lipidomics" databases and thus for the systematization of lipid biology. A new naming system was needed, because the current IUPAC-IUB nomenclature dates from 1976 [3].

Lipids can be divided into simple lipids and complex lipids. Simple lipids are compounds that after hydrolysis yield one or two types of molecules while complex lipids are hydrolysed into at least three types of molecules. Lipid analysts might also name these classes as neutral and polar lipids, which characterize their chemical properties in a general manner. Simple lipids consist of fatty acids and their glycerol esters (mono-, di- and triacylglycerols), sterols and sterol esters and waxes. Many of them are esters, and in the esters the acid is a fatty acid and the alcohol is glycerol, sterol or a long chain alcohol. Simple lipids are usually hydrofobic and readily soluble in nonpolar solvents, but e.g. monoacylglycerols may also partition between the water and the lipid phases. In biological systems, the main function of triacylglycerols is to store energy while smaller acylglycerols also participate actively in metabolic pathways. Sterols are important constituents of membranes.

Complex lipids contain an even larger range of compounds. Three major subclasses are glycerophospholipids, glyceroglycolipids and sphingolipids. In the first two subclasses, the core of the molecule is a glycerol while in the third subclass it is a long-chain base with a hydroxyl group. Fatty acids form an ester or an amide bond, and the phosphorous-containing moieties and carbohydrates are liked to the hydroxyl groups. Complex lipids are polar, and some of them may even have a negative or a positive charge. They are amphipatic molecules and thus more soluble in polar organic solvents than non-polar ones. Furthermore e.g. mono- and digalactocyldiacylglycerols readily form colloids in water. Glycerophospholipids and sphingolipids are essential elements of biological membranes forming barriers between cells and cell organells, and modulate the membrane matrix for the activity of enzymes and function of proteins. In addition to these general functions, complex lipids have specific functions such as signalling and acting in immune response and as markers for diseases and stress. The first biologically active lipid was platelet activating factor that is closely related to the most common phospholipid namely phosphatidyl choline.

Fatty acids are the most important elements of lipids. They occur esterified or amidated in most lipids, but are rarely found as free components. Most chemical and physical properties of acylglycerols, and to a lesser extent in other lipids, are controlled by their fatty acid moieties. Fatty acids are long-chain mono-carboxylic acids with usually an even number of carbon atoms in a straight carbon chain. Fatty acids with 16 and 18 carbon atoms are the most common ones in animals and plants, while fatty acids with from four carbons are found in cow's milk and those with up to 24 carbon in e.g. fish. The length of the carbon chain has a great effect on the hydrofobicity, mobility and melt-

ing of the fatty acid. The number of the double bonds in a fatty acid directs the reactivity of the molecule. Saturated fatty acids and acyls are relatively stable molecules, but as the number of double bonds increases, the molecules become more susceptible to oxidative reactions. Similarly, the melting point of a fatty acid decreases as the unsaturation increases. Moreover, the position and geometry of double bonds in a fatty acid has a major effect on its physiological value. Fatty acids with two methylene interrupted *cis*-double bonds at the carbon number six from the methyl group (n-6; linoleic acid) and three respective bonds at the carbon number three (n-3; α-linolenic acid) are essential, i.e. a human cannot produce them and must obtain them from the food. They are essential for the synthesis of longer-chain fatty acids e.g. eicosanoids, and also needed for many other purposes. In general, monounsaturated and polyunsaturated fatty acids are considered to have more positive health effects than saturated fatty acids.

Traditionally when a food is evaluated for it lipids, it is the fat content and the fatty acid compositions that are looked at. Fat content is a diffuse expression, which may be considered as neutral lipids or may also include phospholipids. In most cases, the amounts of fat and lipids are close as in the case of milk, meat, fat fish or soyabean, but may have a great difference as in many green plant materials and fruits. For example, glyceroglycolipids contribute up to 20% of total lipids in cereal grains and up to 80% in the leaves of clover. In general, animals store energy in adipose tissue as fat. In milk, fat is covered by fat globule membranes to be dispersed in an aqueous matrix. Thus although in milk 96% of the lipids are triacylglycerols, in its fat globule membrane the proportion of phospholipids increases to 20%. These examples show that lipids are unevenly distributed in tissues and cells, and that due to the diverse chemical and physical properties of lipids they may have a variety of different functions in biological materials. Better understanding of the versatile properties of lipids has stimulated basic and applied research in this area, and new application of lipids are being introduced. Plant sterols are being enriched in foods to lower serum cholesterol, medium chain fatty acids are used to deliver lipid-soluble compounds, diacylglycerol oils are considered as low-energy alternatives and glycerogalactolipids as multifunctional emulsifiers and stabilators.

References

[1] Christie, W.W. The Lipid Library / All About Lipids. http://www.lipidlibrary.co.uk/lipids.html. Updated 19/12/2006.

[2] E. Fahy, S. Subramaniam, H.A. Brown, C.K. Glass, A.H. Merrill Jr., R.C. Mrphy, C.R.H. Raetz, D.W. Russell, Y. Seyama, W. Shaw, T. Shimizu, F.Spener, G. van Meer, M.S. VanNieuwenhze, S.H. White, J.L. Witztum, E.A. Dennis, A Comprehensive classification system for lipids, J. Lipid Res. 46, 2005, 839-861.

[3] IUPAC-IUB Commission of Biochemical Nomenclaturue. The nomenclature of lipids (recommendations 1976). http://www.chem.qmul.ac.uk/iupac/lipid/

CURRENT VIEW OF THE STRUCTURE AND FUNCTION OF BIOLOGICAL LIPID MEMBRANES AND ANALYSIS OF THE LIPID PROFILE BY MASS SPECTROMETRY-BASED LIPIDOMICS

Reijo Käkelä and Pentti Somerharju

Institute of Biomedicine, Department of Biochemistry and Developmental Biology, P.O. Box 63, 00014 University of Helsinki

Lipids form the basic structure of all biological membranes, and have also been found to be key players in a variety of physiological phenomena including signal transduction, intracellular trafficking and sorting of membrane bound molecules. Mammalian cells are estimated to contain 2000+ different lipid species. Apparently such a great variety of lipid molecules is needed for specific but still poorly known lipid-lipid and protein-lipid interactions essential for all living cells. Recently, two factors revolutionized lipid research and will likely lead to significant scientific breakthroughs in near future. They are *i)* novel theories of spatial organization of lipids in biological membranes, and *ii)* the advent of novel mass-spectrometric methods. "Lipidomics" i.e. mass spectrometry-based systems-level analysis of lipids and their interacting partners have become a new promising field of biomedical research with applications in drug and biomarker development.

i) At present it is firmly established that membrane lipids are not randomly distributed. Membranes contain caveolae and probably also lipid "rafts" which are cholesterol- and sphingolipid-enriched microdomains of membrane. Although many features like the dimensions and stability of s.c. lipid rafts are still disputed the rafts are assumed to sequester proteins in close proximity for efficient functional interactions [1]. This novel view of the dynamic lateral structure of the biological membranes has opened new vistas for experimental studies of protein-lipid interaction. In addition, the formation of membrane microenvironments must follow basic physicochemical laws (electric repulsion, fit of geometrical shape etc.) and thus their lipids may adopt regular superlattice-like distributions (Fig. 1) [2].

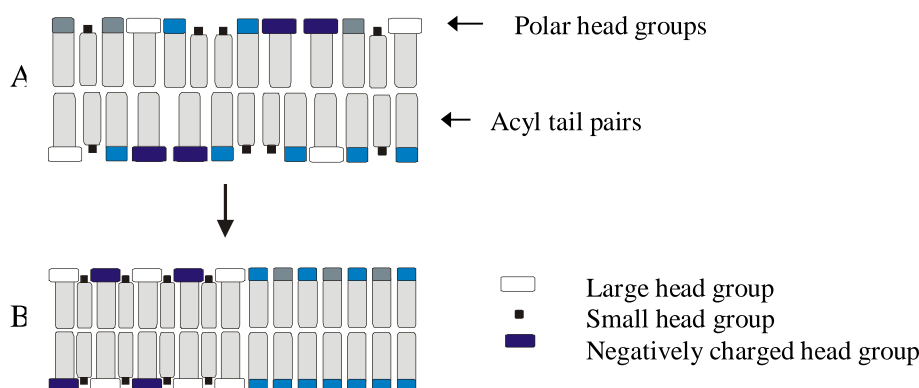


Figure 1. Instead of (A) random organization membrane lipids may form (B) phase-separated microdomains s.c. rafts (driven by geometric or hydrophobicity mismatch) the lipids of which may adopt regular distribution (driven by different polar head group geometry or charge).

ii) Detailed analyses of complex lipidomes have become feasible only recently due to the advent of novel mass spectrometric methods, particularly electrospray-ionization mass-spectrometry (ESI-MS). This "soft" ionization method allows quantitative analysis of hundreds of phospholipid molecular species with a sensitivity and speed which exceeds that of the traditional methods by several orders of magnitude [3]. In near future even trace components essential for signalling and regulatory functions can be determined quantitatively. ESI-MS combined with liquid chromatography (LC-ESI-MS) provides three dimensional data/maps that can be used for fast screening of a large number of biological or clinical samples (Fig. 2). Because of the great body of data produced even

in a single ESI-MS analysis, it is necessary to develop computerized data analysis tools and additional bioinformatics methods to correlate lipidomes (and compositional changes of them) with the physiological function under study [4].

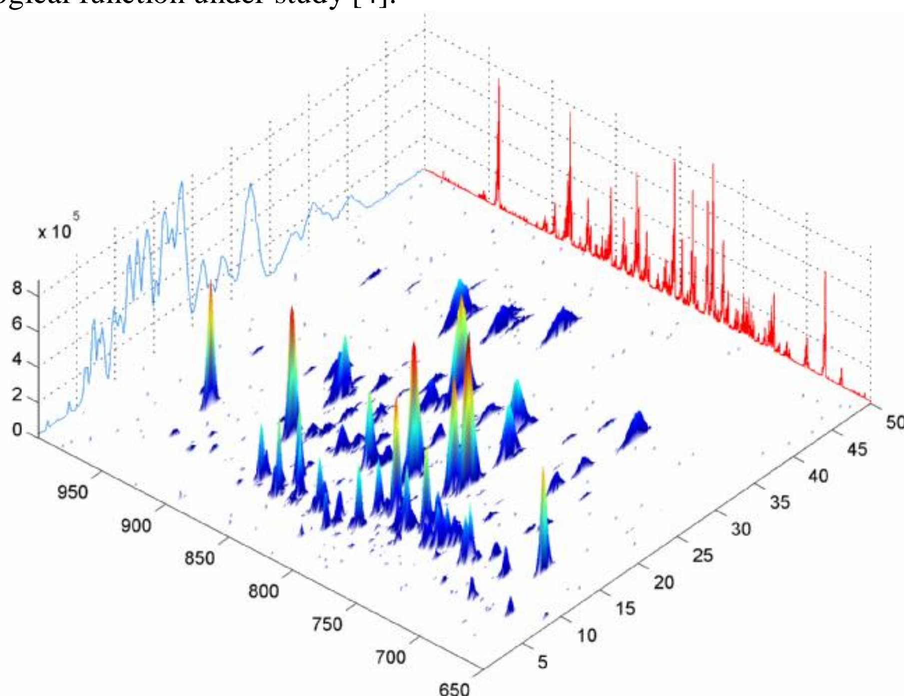


Figure 2. 3-D display (signal intensity 0-8x10^5; mass, m/z 650-1000; retention time, 0-50 min) of LC-ESI-MS analysis of the lipidome of mouse cerebrum [5].

References

[1] J.F. Hancock, Lipid Rafts: Contentious Only from Simplistic Standpoints, Nat. Rev. Mol. Cell Biol. 7 (2006) 456–62

[2] P. Somerharju, J.A. Virtanen, K.H. Cheng, Lateral Organisation of Membrane Lipids – The Superlattice View, Biochim. Biophys. Acta 1440 (1999) 32–48

[3] R. Käkelä, P. Somerharju, J. Tyynelä, Analysis of Phospholipid Molecular Species in Brains from Patients with Infantile and Juvenile Neuronal Ceroid Lipofuscinosis Using Liquid Chromatography Electrospray Ionization Mass Spectrometry, J. Neurochem. 84 (2003) 1051–1065

[4] P. Haimi, A. Upphoff, M. Hermansson, P. Somerharju, Software Tools for Analysis of Mass Spectrometric Lipidome Data, Anal. Chem. 78 (2006) 8324–8331

[5] M. Hermansson, A. Uphoff, R. Käkelä, P. Somerharju, Automated Quantitative Analysis of Complex Lipidomes by Liquid Chromatography/Mass Spectrometry, Anal. Chem. 77 (2005) 2166–2175

LIPOSOMES AS COATINGS AND CARRIERS IN ELECTROPHORESIS

Susanne Wiedmer

Laboratory of Analytical Chemistry, Department of Chemistry, University of Helsinki

We have demonstrated that phospholipid vesicles are suitable as carriers for analytes in electrokinetic capillary chromatography and as coating material in capillary electrochromatography. The present talk deals with physical and chemical factors affecting the use and effectiveness of phospholipid vesicles in capillary electrophoresis. The effect of temperature, pH, buffer component, together with the effect of composition and size of vesicles on the coating efficiency and on analyte interactions will be discussed. Low molar mass drugs have been used as model analytes in studies on analyte-phospholipid membrane interactions. Shielding of the fused-silica capillary surface with phospholipid membranes in the separation of proteins will be demonstrated. In addition, the use of proteins immobilized into phospholipid membranes as chiral selectors for the separation of amino acids will be discussed. Characterization of the phospholipid layer on silica has been made by quartz crystal microbalance technique and the results show that the formed layer is highly dependent on the phospholipid composition. Finally, the use of capillary electrophoresis, together with other spectroscopic techniques, in the characterization of PEGylated lipid membranes will be demonstrated.

PHYSICOCHEMICAL CHARACTERIZATION OF LIPOSOME SYSTEMS FOR PHARMACEUTICAL APPLICATIONS

Marjo Yliperttula

Division of Biopharmaceutics and Pharmacokinetics, P.O. Box 56 (Viikinkaari 5 E)
FI-00014 University of Helsinki, Finland, Marjo.yliperttula@helsinki.fi

Liposomes are hollow phospholipid vesicles that are formed due to self-assembly of amphiphilic lipids in water. They are increasingly used for pharmaceutical and medical applications. Liposomes can encapsulate hydrophobic molecules in the bilayer membrane and hydrophilic compounds in the aqueous internal cavity as well as amphiphilic substances. Drugs can interact with liposomes in several different ways depending on their solubility and polarity. Liposomes can be used as biocompatible carriers for small molecular weights drugs, peptides, proteins, plasmid DNA, antisense oligonucleotides and ribozymes.

A prerequisite for the use of loading liposomes as in vivo carriers is to develop methods allowing the controlled liposome preparation, particle size, shape, stability, surface changes, encapsulation rates, and also to determine the leakage kinetics of the entrapped substances. In order to determine and characterize the physicochemical properties of the liposomes, the techniques including Surface Tension Meter (TEM), viscometer, Dynamic Light Scattering (DLS), Electron Tomography Microscopy (ETM), Fluorescence spectroscopy, Phosphorous/Nitrogen content measurements are used. Each of the techniques has its own advantages and disadvantages and can be applied depending of the application the liposomes will be used. These issues will be discussed.

LIPOSOMAL DRUG CARRIERS FOR CANCER TREATMENT

Julia Lehtinen

Division of Biopharmacy, University of Helsinki, Finland
Drug Discovery and Development Technology Center, University of Helsinki, Finland

Liposomes have been used as carriers for many types of agents, for example chemotherapeutic agents, imaging agents, antigens, immunomodulators and genetic material. Liposomes can act as controlled release reservoirs and also protect drugs against degradation. With liposomes the drug can be targeted to the desired site of the body, and on the other hand, sensitive organs can be protected from toxic side effects (1).

Conventionally liposomes consist of phospholipids and cholesterol, but these conventional liposomes are easily recognized by reticuloendothelial system (RES) and they are taken up by liver and spleen. By adding polyethylene glycol (PEG) to the surface of liposomes, the circulation time of the liposomes can be increased remarkably (Figure 1).

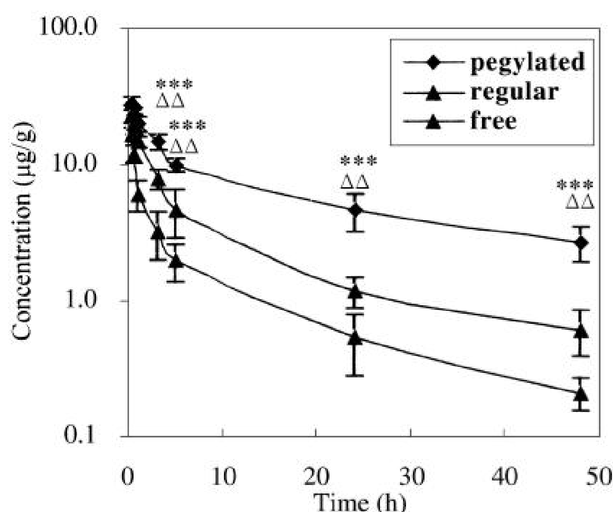


Figure 1. The profile of doxorubicin in the mouse blood after tail intravenous injection (2).

Doxorubicin-loaded Doxil® or Caelyx® is the only commercially available PEGylated liposome product. The efficacy of that passively targeted liposome is based on a long circulation time in the blood, leaky vasculature and poor lymphatic drainage of tumor site. The selectivity and efficacy can be further improved by adding targeting ligands (e.g. antibodies, peptides, carbohydrates) on the surface of the liposomes. These ligands can recognize antigens expressed on the tumor cells or neovascular endothelial cells.

The aim of our study is to target PEGylated liposomes to the angiogenic tumor vasculature. Targeting tumor vasculature rather than cancer cells has many advantages: [1]the treatment is selective and toxicity is minimal because angiogenesis in adults is limited to wound healing, ovulation, pregnancy and atherosclerosis. [2]Endothelial cells are easily accessible and [3]by destroying a few capillary endothelial cells a number of tumor cells dependent on them will also be destroyed. [4]Endothelial cells are genetically stable and do not become resistant to the therapy (3).

References:

1. G Storm and D J A Crommelin, Liposomes: quo vadis? PSTT 1 (1998) 19-31
2. W-L Lu et al. J Pharmacol Sci 95 (2004) 381-389
3. O Feron, TRENDS in Pharmacological Sciences 25 (2004) 536-542

1,4-DIHYDROPYRIDINE DERIVATIVES AS GENE DELIVERY SYSTEM

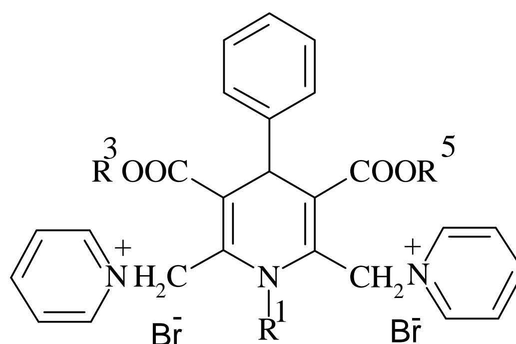
Zanna Hyvönen*, Marika Ruponen and Arto Urtti

*Department of Pharmaceutics, University of Kuopio, P.O. Box 1627, 70211 Kuopio, Finland

The biological functions of the body are mainly regulated by proteins, therefore, the absence, malproduction or too low production of a protein may be associated with many genetic and required diseases. Gene therapy is a promising new treatment modality in medicine. The principle of gene therapy is introduction of specific exogenous sequences of cDNA for the gene of interest into the target cell in order to produce therapeutic protein. The prerequisite for successful gene therapy is efficient and safe delivery of DNA into the target cells.

Presently available gene delivery vehicles for somatic gene transfer can be divided to two categories: viral and non-viral. Viral vectors are the most efficient vehicles in gene delivery, but they have problems related to their safety and production. Therefore, synthetic DNA delivery agents (positively charged lipids and polymers) have been extensively studied. These systems are rather safe and easy to produce, but they have low transfection efficacy, especially in vivo. Although the physicochemical properties (size, zeta-potential etc.) of the carrier/DNA complexes may affect transfection efficacy, the structural features for optimal gene delivery are not yet clear. Therefore, design of more efficient gene delivery vehicles is very important.

In this study we introduce novel non-viral gene delivery system based on 1,4-dihydropyridine (1,4-DHP) structure, including the most promising group – double-charged amphiphiles with two quaternized nitrogens and different alkyl chain length at the positions 3 and 5 (Figure 1).



R^1	$R^3 = R^5$	Compound
H	$C_{10}H_{21}$	I
H	$C_{12}H_{25}$	II
H	$C_{14}H_{29}$	III
H	$C_{16}H_{33}$	IV
CH_3	$C_{12}H_{25}$	V

Figure 1. Structures of 1,4-dihydropyridine amphiphiles

In general, DNA delivery comes true on three levels: DNA condensation and complexation, endocytosis and nuclear entry. Negatively charged DNA molecules are usually condensed and/or complexed with positively charged carrier before delivery. These complexes (lipoplexes) are taken up by cells usually via endocytosis. The road of uptake determinates subsequent DNA release, trafficking and lifetime in the cell. Endocytosis is a multistep process involving binding to the cell surface, internalization, formation of endosomes, fusion with lysosomes and lysis. The low pH and enzymes

in the endosomes and lysosomes may destroy the lipoplexes and degrade DNA. Survived DNA must move then in the cytosol and dissociate from the lipoplex either before or after entering the nucleus in order to be transcribed and translated. Successful gene delivery agent should not fall apart or become inactivated by the extracellular components (e.g. albumin, glycosaminoglycans) in this multistep process of transfection.

We have investigated novel cationic amphiphilic 1,4-DHP derivatives for their ability to condense DNA and transfect the target cells in vitro, as well as their biophysical properties. The effect of extracellular and intracellular factors on the 1,4-DHP-mediated transfection was also examined. We have found that structural modifications of the amphiphiles, particularly the position and the amount of the charges per molecule, length of alkyl chains and change of substituents may affect the physicochemical and biological properties of the lipoplexes. The studies showed that besides having self-assembling properties, the amphiphiles are able to condense efficiently DNA and destabilize endosomal membranes. There was no correlation between size and zeta-potential of the complexes and their transfection efficacy, although positive zeta-potential was essential for transfection. The double-charged 1,4-DHP amphiphiles showed buffer capacity at endosomal pH that can contribute to their high transfection activity. Among 1,4-DHP derivatives, double-charged amphiphile with C12 long alkyl chains was the most efficient in transfecting the cells. After intravenous administration of this amphiphile/DNA complex into mice, the transgene expression was observed mainly in the liver. Moreover, intramuscular administration of the lipoplex resulted in some transfection in vivo.

Combination of amphiphiles with neutral lipid DOPE resulted in a serum-resistant transfection system. Whereas, inclusion of polyethylene glycol-lipid conjugates into the lipoplexes reduced transfection.

In conclusion, 1,4-DHP amphiphiles display high transfection efficacies in vitro and reveal some important structure-activity relationships and can be the basis for the further development of efficient gene delivery system for in vitro and in vivo.

POSTERIABSTRAKTIT

EVALUATION OF MESOPOROUS TCPSI, MCM-41, SBA-15 AND TUD-1 MATERIALS AS API CARRIERS FOR ORAL DRUG DELIVERY

T. Heikkilä [a, *], J. Salonen [a], J. Tuura [a], N. Kumar [b], T. Salmi [b], D. Yu. Murzin [b], M. S. Hamdy [c], G. Mul [c], L. Laitinen [d], A. M. Kaukonen [d], J. Hirvonen [e] and V-P. Lehto [a]

[a] Laboratory of Industrial Physics, Department of Physics, University of Turku, FI-20014 Turku, Finland
[b] Laboratory of Industrial Chemistry, Process Chemistry Centre, Åbo Akademi University, FI-20500 Turku, Finland
[c] Reactor and Catalysis Engineering (R&CE), Delft ChemTech, Delft University of Technology, Julianalaan 136, 2628 BL, Delft, The Netherlands
[d] Drug Discovery and Development Technology Center, University of Helsinki, Finland
[e] Division of Pharmaceutical Technology, Faculty of Pharmacy, University of Helsinki, FI-00014 Helsinki, Finland
[*] Graduate School of Materials Research, Turku, Finland

The feasibility of four mesoporous materials composed of biocompatible Si (TCPSi) or SiO_2 (MCM-41, SBA-15 and TUD-1) were evaluated for oral drug delivery applications. The main focus was to study the effect of the materials different pore systems (unidirectional/2D/3D) and their pore diameters (D_{BJH}), size distributions (PSD) and volumes (V_p) on the maximal drug load capacity and release profiles of an loaded API. Ibuprofen was used as the model drug and was loaded into the carriers via high concentration ethanol solutions. The materials were characterized with XRD and N2 sorption and the drug loads were determined with DSC, TG and HPLC. The unidirectional pore structure and high total pore volume were found to contribute positively to a high drug load capacity, with SBA-15 reaching a drug load of 1:1 in weight. Dissolution experiments were performed in HBSS buffers of pH 5.5, 6.8 and 7.4 to mimic the conditions in the small intestine. Remarkably, at pH 5.5 the dissolution rate of ibuprofen released from the mesoporous carriers is significantly faster compared to the standard bulk ibuprofen (86-63% vs. 25% released at 45 minutes), with the fastest release observed from the 3D pore network of TUD-1 carrier. The application of mesoporous carriers diminishes the pH dependency of ibuprofen dissolution (pK_a=4.4), providing an interesting prospect for the formulation of poorly soluble drug compounds.

Table I. Pore characteristics and ibuprofen loads of the mesoporous carriers.

Material	S_{BET} (m^2/g)	D_{BJH} (nm)	PSD (nm)	V_p (cm^3/g)	Porosity (%)	Drug load (wt%/carrier)
TCPSi	282	11.0	2-30	0.860	68	41.5
MCM-41	1063	2.6	2-3	0.717	65	59.2
SBA-15	625	6.9	5-12	1.067	73	102.0
TUD-1	453	4.9	2-20	0.556	57	49.5

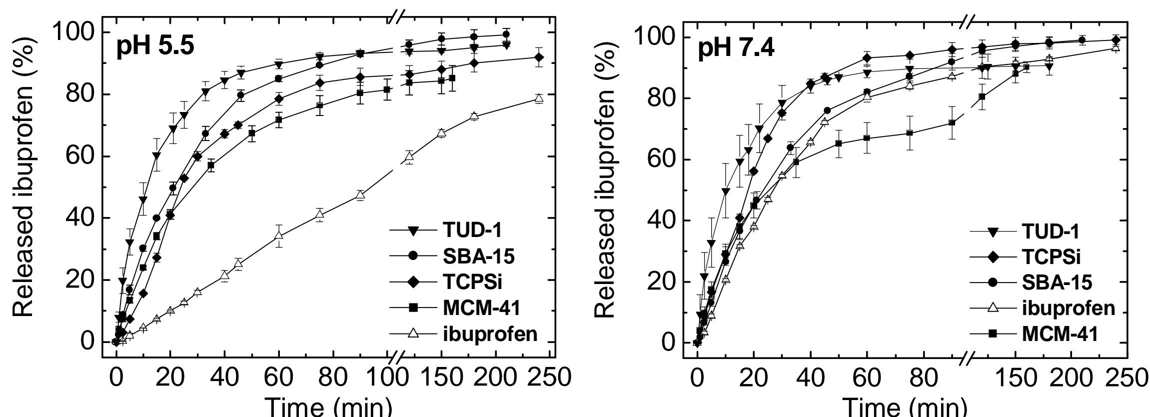


Fig. 1. Ibuprofen dissolution profiles at HBSS buffer of pH 5.5 and 7.4.

A NOVEL DISSOLUTION MICROCALORIMETRY CELL

O-P. Hämäläinen, M. Aarnio and V-P. Lehto

Laboratory of Industrial Physics, Department of Physics, University of Turku, FI-20014, Turku, Finland

The aim of the present study was to develop and test a new dissolution microcalorimetry vessel to determine the heats of solution for pharmaceutical powders with sample sizes of few milligrams or even below.

Heat of solution ($\Delta_{sol}H$) is an important characteristic of solvent-solute interaction. Knowledge of $\Delta_{sol}H$ can be used for example to detect different polymorphs of a pharmaceutical or to compare the stabilities of different polymorphs. Traditionally $\Delta_{sol}H$
has been determined with "macrocalorimeters", which is accompanied by need of large amounts of sample material and excessive measurement times with poorly dissolved materials.

Microcalorimetric dissolution equipment have been developed before (for example [1]), but the present apparatus is based on a different principle (Figure 1). The cell is designed as an accessory for Thermal Activity Monitor (TAM) 2277 (ThermoMetric Ab). The sample is placed on top of a membrane filter selected compatible with the solvent used. Prior to entering the sample ampoule, the solvent is flown through a heat exchanger to assume the measurement temperature of TAM and then through the sample dissolving it. Any portion of the sample material flown through the filter is presumed to be completely dissolved. The solution is withdrawn from the cell via heat exchanger similar to previously mentioned. The heat flow associated with the reaction is monitored with a computer.

The cell was tested by dissolving KCl (recommended by IUPAC as a calibration reagent [2]) and NaCl in purified water and poorly dissolving ibuprofen in water-ethanol mixture at 298 K. $\Delta_{sol}H$ values for KCl and NaCl were determined and found similar to those presented in literature.

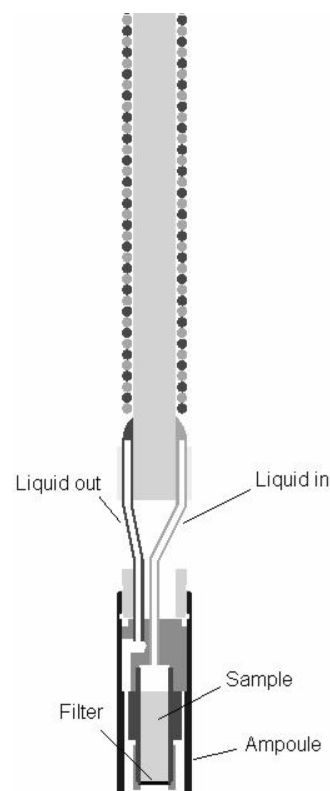


Figure 1: Construction of dissolution cell.

References

[1] M.Bastos, G.Bai, E.Qvarnström, I.Wadsö, Thermochim.Acta 405 (2003) 21-30
[2] I.Wadsö, R.N.Goldberg, Pure Appl.Chem.vol.73,no.10, (2003) 1625-1639

DETECTION OF DEFENSINS FROM BIOLOGICAL MATRICES WITH HPLC-ESI-MS

Jussi Tervonen, Ilpo Jääskeläinen and Seppo Auriola

University of Kuopio, Department of pharmaceutical chemistry, Kuopio, Finland
jussi.tervonen@uku.fi

Defensins are cationic, 3-6 kDa molecular weight peptides that are found in most human tissues and fluids. They are part of the innate immunity system and have direct antibacterial, antifungal and antiviral properties and can indirectly enhance the immune system. There is a lot of research of the properties of defensins and several methods to measure the activity of the genes, but there is very little development of qualitative and quantitative methods for the peptides in different biological fluids. In this study a pre-treatment and detection method was developed for different biological samples: human saliva and pancreatic juice and rat ileum. The pre-treatment of a biological sample consists of gel permeation chromatography and reversed phase chromatography. Detection was done with mass spectrometry.

Samples were treated with 30% formic acid to precipitate large proteins. The first separation was done with gel permeation chromatography: TSKgel 4000 and 2000 in a series, isocratic 20% acetonitrile +0,1% trifluoroacetic acid. The fractions acquired were separated again with RP-HPLC, using a silica C4 column with 300Å pore size. LTQ linear ion trap mass spectrometer was used to detect the peptides.

Different columns were tested for RP-HPLC, and C4 with 300Å pore size was found to be the best. The reason for this is, that there's a significant carryover of peptides in columns with larger side chains and smaller pore sizes. This is probably due to the smaller active surface of the column, which can strongly bind the cationic peptides.

Human neutrophil defensins (HNP) nos. 1, 2, and 3 were detected in human saliva. HNPs 1, 2, 3 and 4 were detected in human pancreatic juice. Rat neutrophil defensins 2 and 4 were detected in a sample of rat intestine (ileum). Next target will be colostrum, which contains a lot of antibacterial components. Also a quantitation method is being developed. This enables the studying of the effect nutrition or illnesses to the level of defensins in humans and animals.

PREFERRED ORIENTATION OF CRYSTALLITES AFFECS THE INTRINSIC DISSOLUTION OF ASPIRIN AND TOLBUTAMIDE TABLETS

Mikko Tenho[a,c], Jaakko Aaltonen[b] and Vesa-Pekka Lehto[a]

[a] Laboratory of Industrial Physics, University of Turku, FI-20014 Turku, Finland
[b] Division of Pharmaceutical Technology, University of Helsinki, FI-00014 Helsinki, Finland
[c] Graduate School of Materials Research, Turku, Finland

Introduction

Preferred orientation of crystallites (or texture) often occurs when the particles of the sample powder are needle or plate-like. When this kind of powder is compacted into tablets the degree of preferred orientation normally increases. In a previous study we found out that reducing the preferred orientation by grinding the powder to be tabletted slightly increases the intrinsic dissolution rate. However, the effects of grinding remained unclear. In the present study the samples with different texturization behavior were prepared by using different recrystallization methods. That way the powders to be studied all had virgin surfaces and different kind of textures could be obtained. Assuming that different crystal planes possess different energetics and therefore also different dissolution properties the differences in the intrinsic dissolution rates should be bigger.

Materials and Methods

Three different batches of aspirin and tolbutamide were used. The preferred orientation of the compacted tablets was measured with several different methods using both ordinary and texture goniometry. All the crystallographic parameters of the samples except texture were similar. The channel flow technique was used to determine the intrinsic dissolution rate of the samples.

Results, Discussion and Conclusions

Aspirin tablet samples had different textures whereas the texture of the tolbutamide samples was of similar nature and only the amount of preferred orientation varied between the samples (Figure). Generally, the more texture there was in the sample the slower intrinsic the dissolution rate. However, in the case of aspirin the different texture determination methods did not give consistent texture values in all cases. This could be due to the more complicated crystal structure of aspirin. Nevertheless, based on the present study in the case of aspirin and tolbutamide the preferred orientation should be taken into account when interpreting the results of the intrinsic dissolution studies. Moreover, there could be a possibility to modify the dissolution behaviour of pharmaceuticals by modifying their texture.

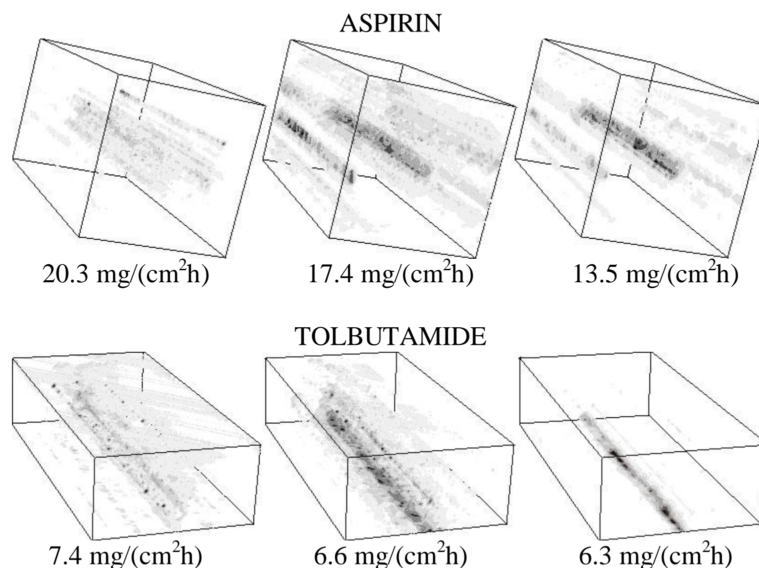


Figure. The orientation distribution functions and the intrinsic dissolution rates of the aspirin and tolbutamide samples.

THE EFFECT OF TEXTURE ON THE INTRINSIC DISSOLUTION BEHAVIOUR OF PHARMACEUTICAL TABLETS

Mikko Tenho[a,c], Paula Heinänen[b], Veli Pekka Tanninen[b] and Vesa-Pekka Lehto[a]

[a] Laboratory of Industrial Physics, University of Turku, FI-20014 Turku, Finland
[b] Orion Corporation, Orion Pharma, P.O. Box 65, FI-02101 Espoo, Finland
[c] Graduate School of Materials Research, Turku, Finland

Preferred orientation of crystals, i.e. texture, is often a problem when interpreting the x-ray diffractograms of powdered samples, especially if the sample has needle or plate-like particles. As the different crystal planes possess different surface energetics, also the behaviour of non-randomly distributed powder samples could differ from those that are randomly distributed even though samples are made of the same material. Since texturization often takes place due to the compaction of pharmaceutical tablets, the possible effects of texture must be considered when performing accurate tablet studies. The purpose of this study was to examine the effects of texture on the intrinsic dissolution rate (IDR) of tablets. The texture of the tablets compacted from several active pharmaceutical ingredients (API) with various solubility properties was investigated prior to the IDR experiment. Moreover, the texture of the samples was measured also after the dissolution tests. Two different tablets were prepared from each API, one tablet had big needle and/or platelike particles and the other tablet was compacted from the ground powder. This procedure allowed studying texturized and less texturized tablets, which were both made from the same API. The results indicate that the effect of texture can be seen in the IDR studies. However, the extent of this effect is rather low compared to the effect of polymorphism or amorphicity, for example. Moreover, the effect is compound specific. Nevertheless, in every case the samples with less texture dissolved slightly faster (Table).

Table. The preferred orientation and IDR values of the samples. The texture of the samples was characterized with three various methods. (The structure of entacapone was not available.)

sample	half width of pole figure [°]	texture parameter		intrinsic dissolution rate [mg/(cm^{2}h)]
		structure refinement	comparison method	
unground acetylsalicylic acid	11.2	0.48	0.13	17
ground acetylsalicylic acid	27.8	0.65	0.24	18
unground tolbutamide	8.4	0.47	0.21	7.1
ground tolbutamide	20.6	0.66	0.62	7.7
unground carbamazepine	27.4	2.34	0.15	0.96
ground carbamazepine	39.8	1.54	0.18	0.96
unground entacapone	13.7	-	0.12	1.1
ground entacapone	22.4	-	0.33	1.2

INVESTIGATION OF PIROXICAM MONOHYDRATE DEHYDRATION BEHAVIOUR IN COMPACTS USING MULTIPLE SPECTROSCOPIC TECHNIQUES

K. Kogermann[a,b], J.A. Zeitler[c,d], C.J. Strachan[a,c,e], T. Rades[c], P.F. Taday[d], M. Pepper[d], J. Heinämäki[a], J. Rantanen[f]

[a]Division of Pharmaceutical Technology, University of Helsinki, Finland
[b]Department of Pharmacy, University of Tartu, Estonia
[c]University of Otago, New Zealand
[d]TeraView Ltd, UK
[e]Viikki Drug Discovery Technology Centre, University of Helsinki, Finland
[f]Department of Pharmaceutics and Analytical Chemistry, The Danish University of Pharmaceutical Sciences, Denmark

The dehydration/ hydrate formation may occur during pharmaceutical manufacturing and storage, and there is an enormous need to control and understand these processes. Vibrational spectroscopic techniques including infrared, near-infrared (NIR) and Raman spectroscopy are especially useful for studying dehydration/ hydrate formation. Firstly, they probe the molecular level, and can therefore potentially be used to understand these transformations on this level. Secondly, they are non-destructive and can measure transitions in real time. Terahertz pulsed spectroscopy (TPS) is a very recent vibrational spectroscopy technique that operates in the far-infrared region of the electromagnetic spectrum. It directly probes crystal structure and intermolecular bonding. Recently, it has been shown that TPS can be used to monitor dehydration of pharmaceutical hydrates.

This study consists of two parts. In the first, the effect of compact preparation on the dehydration of piroxicam monohydrate (PRXMH) was investigated using *in situ* TPS. Different sample preparation methods were trialled (mixed sample, surface sample and middle layer sample) and the effect of sample preparation on dehydration was verified for surface layer compacts using powder X-ray diffractometry (XRPD), Raman spectroscopy and NIR spectroscopy. The reproducibility was checked by using triplicate heating experiments.

In the second part, non-isothermal dehydration of PRXMH from surface layer compacts was monitored *in situ* by the more commonly used spectroscopic methods, Raman and NIR spectroscopy (reflective mode), and the results compared with those obtained using TPS (transmission mode). Non-isothermal measurements were performed using three different heating rates (3, 5 and 7 $Kmin^{-1}$). Non-isothermal variable temperature XRPD (VT-XRPD) was used to verify the solid-state transformation from PRXMH to piroxicam form I (PRXAH).

The study showed that the polymorphic form of piroxicam was unchanged after compact preparation, but the dehydration behaviour of PRXMH was strongly dependant on the sample preparation method. All spectroscopic techniques could be used to monitor the phase transformation from PRXMH to PRXAH on the surface of the compacts during the non-isothermal dehydration. However, these methods suggested different dehydration temperature ranges, which can largely be attributed to the different sampling depths of the three methods. Raman spectroscopy appeared to have the largest surface bias while transmission TPS could monitor dehydration throughout samples several millimetres thick. TPS could be used to observe the water vapour leaving the sample.

DRUG PERMEABILITY IN CACO-2 CELL MONOLAYERS IS AFFECTED BY USE OF 'PHYSIOLOGICAL' DONOR SOLUTIONS

S. Eskola[a], N.Patel[b], M. Stewart[b], B. Forbes[b]

[a]Faculty of Pharmacy, Viikinkaari 9, 00014 University of Helsinki , Finland. email: sini.eskola@helsinki.fi
[b]King's College London, Pharmaceutical Science Research Division, Franklin-Wilkins Building, 150 Stamford Street, London, SE1 9NH, UK.

Introduction and aim of the study

Simulated intestinal fluid representing the fasted state in the intestinal lumen (FaSIF) has been used as a biorelevant medium in permeability experiments using the Caco-2 drug absorption model (Ingels et al 2004). The use of FaSIF resulted in a reduction in the permeability lipophilic drugs compared to permeability measured using standard transport medium. The recently reported development of a biocompatible simulated intestinal fluid to represent the fed state in the intestinal lumen (FeSIF; Patel et al, 2006) provides an opportunity to investigate the effect of using a wider range of biorelevant conditions on drug permeability in the Caco-2 system. The aim of this study was to measure the permeability of propranolol, metoprolol, imipramine and digoxin in Caco-2 cells using FaSIF and FeSIF as biorelevant media and Hanks' balanced salt solution (HBSS) as standard test medium.

Methods

Caco-2 cells (passage 60-73) were seeded in polyester Transwell® cell culture inserts (pore diameter 0.4 µm, surface area 1.13 cm2) at a seeding density of 6.6×104 cells/cm2 and grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% v/v foetal bovine serum, 1% v/v non-essential amino acids (100x), 1% v/v L-glutamine (200 mM) and gentamicin (50 mg/mL). Cells were cultured at 37oC in an atmosphere of 5% CO2 and used for experiments at 21-28 d. Test solutions containing 0.02-0.05 mM radiolabelled drug were prepared in FaSIF, FeSIF and HBSS and absorptive permeability was measured over 2 h as described previously (Patel et al, 2006). Transepithelial electrical resistance (TER) was measured before and after experiments and drug recovery was calculated.

Results

No change in TER (> 400 Ω cm2) was measured over the course of the experiments. The transport rate of each compound was linear under all experimental conditions used. Metoprolol and digoxin recovery was complete and was not affected by the donor matrix. The mass balance (% recovery) for propranolol and imipramine was low in HBSS (77 and 50 %, respectively), higher in FaSIF (86 and 76 %, respectively) and complete in FeSIF (101 and 93 %, respectively). The permeability of propranolol, metoprolol and imipramine was reduced by using FaSIF to 64-72% compared to that measured using HBSS. This effect was enhanced by using FESIF, with a reduction in permeability to 19-22% compared to that measured using HBSS. No effect of the donor matrix on the permeability of digoxin was observed.

Conclusions

The findings with FaSIF are consistent with those reported previously (Ingels et al, 2004). With FeSIF the effects on drug permeability observed when using FaSIF were enhanced, and can be attributed to increased micellar encapsulation of drug in bile salt:lecithin mixed micelles reducing free drug concentration. The FaSIF and FeSIF provide the opportunity to perform experiments under more physiological conditions than standard transport medium. However, the relevance in vivo of the effects on permeability measured in vitro requires further investigation.

References

Patel, N. et al (2006) Drug Dev. Ind. Pharm. **32**: 1-11.
Ingels, F. et al (2004) Int. J. Pharm. **274**: 221-232.

BISPHOSPHONATE-INDUCED ATP ANALOG FORMATION IN DIFFERENT CANCER CELL LINES

Johanna Kuokkanen[a], H. Mönkkönen[a,b], A. Evans[b], I. Holen[b], M. Jauhiainen[a], S. Auriola[c] and J. Mönkkönen[a]

[a]Department of Pharmaceutics, University of Kuopio, Finland
[b]The University of Sheffield, Sheffield, UK
[c]Department of Pharmaceutical Chemistry, University of Kuopio, Finland

Bisphosphonates (BPs) are effective inhibitors of tumour-induced bone destruction. Recent studies have demonstrated that BPs inhibit growth, attachment and invasion of cancer cells in culture and promote apoptosis. The mechanisms responsible for the observed anti-tumour effects of BPs are beginning to be elucidated.

Recently we reported that nitrogen-containing bisphosphonates (N-BPs) induce formation of a novel ATP analog (ApppI) as a consequence of the inhibition of FPP synthase in the mevalonate pathway, and the subsequent accumulation of isopentenyl diphosphate (IPP). Similarly to AppCp-type ATP analog of non-nitrogen containing BPs (non-N-BPs), ApppI is able to induce apoptosis in cells. It is possible that BP-induced ATP analogs may account for the anti-tumour effects of these drugs.

We evaluated here the IPP/ApppI accumulation induced by zoledronic acid (a N-BP), protein prenylation and clodronate (a non-N-BP) metabolism to $AppCCl_2p$ in various cancer cell lines. Zoledronic acid –induced IPP/ApppI accumulation and clodronate metabolism to $AppCCl_2p$ were determined in cell extracts by mass spectrometry. The effect of zoledronic acid on protein prenylation was determined by measurement of the accumulation of unprenylated Rap1A by Western Blot.

There were marked differences in zoledronic acid –induced IPP/ApppI formation and clodronate metabolism between different cancer cell lines. The production of cytotoxic ATP analogs in different tumour cells after bisphosphonate treatment is likely to depend on the activity of enzymes, such as FPP- or aminoacyl-tRNA synthetase, responsible for ATP analog formation. The ability of the cancer cells to metabolize the cytotoxic compounds varies providing a potential new mechanism contributing to the specificity of bisphosphonates against different tumour cell types.

MESOPOROUS SILICON MICROPARTICLES LOADING AND RELEASE OF PEPTIDE

Juha Mönkäre[a], Joakim Riikonen[b], Eero Mella-Aho[c], Mika Pulkkinen[a], Vesa-Pekka Lehto[b], Karl-Heinz Herzig[c], Kristiina Järvinen[a] and Jarno Salonen[b]

[a] Department of Pharmaceutics, University of Kuopio, P.O. Box 1627, FI-70211 Kuopio, Finland
[b] Laboratory of Industrial Physics, Department of Physics, University of Turku, FI-20014 Turku, Finland
[c] A. I. Virtanen Institute for Molecular Sciences, Department of Biotechnology and Molecular Medicine, P.O. Box 1627, FI-70211 Kuopio, Finland

Clinical use of peptides is currently limited especially because of their poor bioavailability and short duration of action in vivo. To overcome these limitations new peptide carriers must be developed. The aim of this work was to characterize the loading and controlled release of peptide from thermally oxidized (TOPSi) and thermally hydrocarbonized (THCPSi) porous silicon microparticles. Earlier mesoporous silicon microparticles have been used to enhance the dissolution of poorly soluble small drug molecules[1] but they could be also used to control drug release.

In the present study the model drug was a ghrelin antagonist (M_w 930 g/mol). Ghrelin is a very strong food intake stimulant. Therefore, its antagonists are of clinical interest. The peptide was loaded into porous silicon microparticles with different surface chemistries: TOPSi surface is hydrophilic, formed by bonds between silicon and oxygen. 2) THCPSi surface is hydrophobic, consisting of silicon, carbon and hydrogen. The peptide loading degree of microparticles was analysed by thermogravimetry as described earlier[2]. The peptide was loaded into TOPSi microparticles (size < 100µm) both from water and methanol and the loading degrees were 15 and 32 % (w/w), respectively. The loading solution was evaporated after the loading was completed. The peptide loading into THCPSi microparticles (size < 100µm) was performed from methanol and the loading degree was 10 % (w/w). In the case of THCPSi microparticles, particles were filtrated from methanol after the loading was completed.

The *in vitro* release tests were performed in 1.5 – 4 ml of phosphate buffered saline (PBS) at pH 7.4 in the test tubes that were placed in the water bath shaker (+37 ˚C and 120 rpm). Test tubes were centrifuged before collecting the sample. The released ghrelin antagonist was analysed with HPLC-UV. Most of the peptide was released in 30 minutes from TOPSi microparticles (amount approximately 1 mg) and complete release was reached after 60 minutes regardless of the loading degree. The peptide release from THCPSi microparticles (amount approximately 1 mg) lasted up to four hours.

In conclusion, mesoporous silicon particles can be loaded with ghrelin antagonist. Our results suggest that THCPSi microparticles can sustain and control peptide release *in vitro* and thus might increase peptide bioavailability *in vivo*.

ACKNOWLEDGEMENTS
The work was support in part by the Finnish Academy (PEPBI consortium # 117906).

REFERENCES
[1] Salonen J, Laitinen L, Kaukonen A.M, Tuura J, Björqvist M, Heikkilä T, Vähä-Heikkilä K, Hirvonen J and Lehto V-P. J. Control. Release 108 (2005) 362-374
[2] Lehto V-P, Vähä-Heikkilä K, Paski J and Salonen J. J. Therm. Anal. Calorim. 80 (2005) 393-397

THE EFFECT OF SURFACE CHEMISTRY AND POROUS PROPERTIES OF POROUS SILICON ON THE DISSOLUTION OF IBUPROFEN

J. Riikonen[a], T. Limnell[b, c], J. Salonen[a], A.M. Kaukonen[b], L. Laitinen[b], J. Hirvonen[c], V.-P. Lehto[a]

[a] Department of Physics, University of Turku, FI-20014 Turku, Finland
[b] Drug Discovery and Development Technology Center, FI-00014 University of Helsinki, Finland
[c] Division of Pharmaceutical Technology, Faculty of Pharmacy, FI-00014 University of Helsinki, Finland

Mesoporous silicon microparticles can be used to improve or delay the release of drugs. The improving effect is mostly due to lesser ordering of the drug molecules in the pores compared to crystalline bulk drug. This is because of the small pore size and the interaction between drug molecules and the pore wall. In the present work release of model drug ibuprofen was studied from five different porous silicon microparticles to clarify the effect of porous properties and surface chemistry of the particles on the dissolution.

Ibuprofen was loaded from ethanol solution into five different porous silicon microparticles with three different surface treatments: as anodized (as-anod), thermally oxidized (TOPSi), annealed thermally oxidized (ann-TOPSi), thermally carbonized (TCPSi) and annealed thermally carbonized (ann-TCPSi). Annealing affects only the porous properties by increasing the pore size of materials. The surface chemistry is the same for similar samples with or without annealing.

Loading degrees were measured independently with TG, pycnometry and HPLC. The achieved loading degrees are presented in Table I. Dissolution experiments were performed in buffered Hank's balanced salt solution (HBSS) at pH 5.5 at +37 °C. The results are shown in Figure 1. Two properties of the samples seemed to dominate the release of ibuprofen: crystallinity of ibuprofen and the pore size. The first is dominating the beginning of the release profile and the latter is dominating the end of the release profile. Samples ann-TOPSi and TCPSi were found to show the fastest overall release.

Table I. Loading degrees and calculated standard deviations

Sample	Av. loading degree (%)	SD (%)
as anod	36,8	4,8
TOPSi	36,5	2,4
ann-TOPSi	30,9	2,9
TCPSi	30,9	3,6
ann-TCPSi	39,6	1,5

Acknowledgements
The financial support from the Academy of Finland is acknowledged (grant no. 211048).

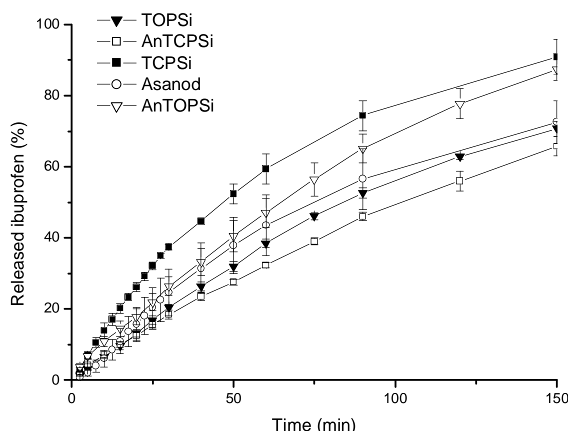


Figure 1. Release profiles of loaded ibuprofen at HBSS, pH 5,5.

EFFECT OF SUBSTRATE IONIZATION ON THE APPARENT AFFINITY TO P-GLYCOPROTEIN PGP-ATPASE ASSAY

Heikkinen Aki T, Mönkkönen Jukka

Department of Pharmaceutics, University of Kuopio, Finland

The objective of this study was to determine the effect of pH environment on the apparent affinity of basic, neutral and acidic substrates to P-glycoprotein using Pgp-ATPase assay.

The Pgp-ATPase assay was performed using human P-glycoprotein expressing insect (High Five, BTI-TN5B1-4) cell membranes purchased from BD Biosciences. The apparent affinity of two basic (verapamil and quinidine), two neutral (testosterone and digoxin) and one acidic (monensin) substrates to P-glycoprotein was determined at pH 6.8 and at pH 7.4. The apparent affinity of basic substrates was higher at pH 7.4 than at pH 6.8 (Km for verapamil was 13µM and 22µM and for quinidine 7µM and 24µM at pH 7.4 and pH 6.8, respectively). There was no significant difference in apparent affinity of neutral and acidic substrates at different pH climates (Km for testosterone was 348µM and 347µM, for digoxin 101µM and 134µM and for monensin 3.6µM and 3.0 µM at pH 7.4 and at pH 6.8, respectively).

The effect of pH on the apparent affinity of basic substrates was expected because there is a strong body of evidence that P-glycoprotein binds its substrates inside the lipid bilayer [1, 2] and the distribution of basic and acidic drugs between aqueous and lipid phases is substantially affected by pH (clogD for verapamil is 2.33 and 1.77, for quinidine 1.81 and 1.25, and for monensin 0.67 and 1.2 at pH 7.4 and at pH 6.8, respectively). However, the expected effect of pH on the apparent affinity of acidic monensin to P-glycoprotein was not seen. Further studies are thus needed to explain this different behavior of basic and acidic substrates in Pgp-ATPase assay. The negligible effect of pH on the apparent affinity of the neutral substrates to P-glycoprotein suggests that the change in pH probably does not affect on P-glycoproteins substrate binding *per se.*

References

1. Shapiro, A.B. *et al.* P-glycoprotein-mediated Hoechst 33342 transport out of the lipid bilayer. Eur. J. Biochem. 1997;250:115-121.
2. Loo, T.W. and Clarke, D.M. Do drug substrates enter the common drug-binding pocket of P-glycoprotein through "gates"? Biochem. Biophys. Res. Commun. 2005;329:419-422

DIFFERENT CELLULAR DISTRIBUTION KINETICS OF TESTOSTERONE, PROPRANOLOL AND QUINIDINE

Timo Korjamo, Paavo Honkakoski, Jukka Mönkkönen

Department of Pharmaceutics, University of Kuopio, Finland

Purpose:
To compare the kinetics of the cellular distribution of testosterone, propranolol and quinidine (with P-glycoprotein inhibitor) in Caco-2 cells during a permeability experiment.

Methods:
Permeability experiments were conducted in the apical-to-basolateral direction with testosterone (50 µM), propranolol (50 µM) and quinidine (10 µM, with P-glycoprotein inhibitor GF120918) up to 90 minutes. At each time point, samples from donor and receiver compartments and from the cell monolayer were taken. The distribution of the compound and the recovery was calculated at each time point. The data was fitted into a kinetic simulation model that contained the donor and receiver compartments and a cellular phase containing either one or two compartments. Fitting was done using MatLab-software.

Results:
The total recoveries were between 92 % and 97 %. The peak amount of testosterone, propranolol and quinidine in the cellular compartments were about 9 %, 21 % and 23 %, respectively. This peak was achieved by 5, 15 and 30 minutes accordingly. Fitting procedure revealed that the behaviour of testosterone could be described with a one compartment model indicating classical rapid equilibration in the cells. Propranolol and quinidine, however, required a two-compartment model indicating biphasic cellular retention. The ratio of apparent cellular concentration to apical (donor) concentration remained fairly constant with testosterone for the whole experiment while it increased considerably (2 to 3 fold) with propranolol and quinidine.

Conlusions:
Cellular distribution of lipophilic molecules is not necessarily a rapid process but may proceed for a considerable time. Thus in addition to rapid decrease in mass balance, accumulation to the cells can reduce the concentration gradient long during the permeability experiment, and the end point mass balance correction may be inadequate. The biphasic nature of distribution may be due to rapid entrance to the cell membrane and slower equilibration to intracellular compartments.

VÄITÖSKIRJOJEN TIIVISTELMÄT

THE ROUGHNESS AND IMAGING CHARACTERISATION OF DIFFERENT PHARMACEUTICAL SURFACES

Paulus Seitavuopio

Helsinki University Printing House, University of Helsinki, Faculty of Pharmacy, 2006, 91 p.

The surface properties of solid state pharmaceutics are of critical importance. Processing modifies the surfaces and effects surface roughness, which influences the performance of the final dosage form in many different levels. Surface roughness has an effect on, e.g., the properties of powders, tablet compression and tablet coating. The overall goal of this research was to understand the surface structures of pharmaceutical surfaces. In this context the specific purpose was to compare four different analysing techniques (optical microscopy, scanning electron microscopy, laser profilometry and atomic force microscopy) in various pharmaceutical applications where the surfaces have quite different roughness scale. This was done by comparing the image and roughness analysing techniques using powder compacts, coated tablets and crystal surfaces as model surfaces.
It was found that optical microscopy was still a very efficient technique, as it yielded information that SEM and AFM imaging are not able to provide. Roughness measurements complemented the image data and gave quantitative information about height differences. AFM roughness data represents the roughness of only a small part of the surface and therefore needs other methods like laser profilometer are needed to provide a larger scale description of the surface. The new developed roughness analysing method visualised surface roughness by giving detailed roughness maps, which showed local variations in surface roughness values. The method was able to provide a picture of the surface heterogeneity and the scale of the roughness. In the coating study, the laser profilometer results showed that the increase in surface roughness was largest during the first 30 minutes of coating when the surface was not yet fully covered with coating. The SEM images and the dispersive X-ray analysis results showed that the surface was fully covered with coating within 15 to 30 minutes. The combination of the different measurement techniques made it possible to follow the change of surface roughness and development of polymer coating. The optical imaging techniques gave a good overview of processes affecting the whole crystal surface, but they lacked the resolution to see small nanometer scale processes. AFM was used to visualize the nanoscale effects of cleaving and reveal the full surface heterogeneity, which underlies the optical imaging. Ethanol washing changed small (nanoscale) structure to some extent, but the effect of ethanol washing on the larger scale was small. Water washing caused total reformation of the surface structure at all levels.

CACO-2 CELL CULTURES IN THE ASSESSMENT OF INTESTINAL ABSORPTION: EFFECTS OF SOME CO-ADMINISTERED DRUGS AND NATURAL COMPOUNDS IN BIOLOGICAL MATRICES

Leena Laitinen

Helsinki University Printing House, University of Helsinki, Faculty of Pharmacy, 2006, 73 p.

Several different in vitro absorption models are used in the screening of new drug candidates. One of them is the Caco-2 model, a widely used in vitro model for small intestinal absorption. Caco-2 cells, which originate from a colon carcinoma, differentiate spontaneously to cells that resemble mature small intestinal enterocytes and express carrier proteins similar to the small intestine, and can therefore be used for the assessment of active transport processes during intestinal absorption. Due to variation in cell line differentiation and selection of sub populations, permeability data obtained from different laboratories is seldom directly comparable. Hence, the use of several drugs or compounds with known permeability characteristics are recommended for model standardisation and the use of internal standards in every experiment could offer a solution to the problem.

In this study, the simultaneous use of five to ten drugs as internal standards was evaluated. Drugs with different permeability characteristics (high and low permeability, passive and active transport and active efflux) were used to detect their possible effects on cell viability, monolayer integrity and possible interactions during permeability across Caco-2 cell monolayers. After validation of the use of several drugs simultaneously as internal standards, the usefulness of the method was further probed by testing the possible interactions during absorption between drugs and plant extracts that are used as food supplements, functional foods, or natural laxatives. These extracts contain several active compounds, such as flavonoids, alkyl gallates, or anthraquinones, which are able to partition into the cell membranes and thus affect e.g. the fluidity of the membranes, or diffuse across the cell monolayers, either by using active transport mechanisms or passively.

Five to ten drugs in one experiment at individual 50 µM concentrations did not cause problems in cell viability (MTT test) or monolayer integrity (transepithelial electrical resistance (TEER) measurements, and 14C-mannitol diffusion test). The individual permeability values in the cocktails correlated closely with those obtained from single-drug experiments, except when active transport of drugs was involved. Drugs with passive permeability can be included in cocktails; no interactions between them are expected. Drugs, which occupy same binding sites of a transport protein, cannot be included as internal standards. According to the results, standard testing of Caco-2 functionality does not require the use of more than 3-4 compounds with very low, low, moderate and high permeability (including substrates for transporters), depending on the characteristics of the studied drug candidates. To probe the effects of plant extracts on the permeability of co-administered drugs, standard drugs with known absorption behaviour can be used. Flax seed extract, for example, decreased the permeability of all co-administered drugs. When flax seeds are used as a laxative, fluids with dissolved drugs are adsorbed on the fibers of flax seed, leading to decreased drug absorption. The effects of other plant extracts, which contain high concentrations of different flavonoids and alkyl gallates, are difficult to predict, because their interactions may be mediated via several active transport proteins, such as OCT, MDR1 and different MRP´s, or additionally via the effects on the cell membrane fluidity, as the permeability experiments with different flavonoids and alkyl gallates proved.

Whereas several food-drug interactions have often been attributed to the inhibition of drug metabolizing enzymes, information regarding the effects of food components on transporters during absorption, distribution and excretion of drugs is still limited. Caco-2 cell monolayers, when expressing active transport and efflux proteins, are therefore very well suitable as in vitro model for this type of studies.

NOVEL CATIONIC AMPHIPHILIC 1,4-DIHYDROPYRIDINE DERIVATIVES FOR DNA DELIVERY: STRUCTURE-ACTIVITY RELATIONSHIPS AND MECHANISMS

Zanna, Hyvönen

Kuopio University Publications A. Pharmaceutical Sciences 92. 2006. 95 p.

Transcription and subsequent translation of an aberrant gene result in the absence, rnalproduction or too low production of a protein. This may result in disease state, since proteins regulate many of the biological functions of the body. The principle of gene therapy is introduction of specific exogenous sequences of cDNA for the gene of interest into the target cell in order to repair abnormal production of protein. Delivery of DNA into the cells must be efficient and safe. Viral vectors are the most efficient vehicles in gene delivery, but they have problems related to their safety and production. Consequently, design of more efficient and safe non-viral DNA delivery agents is very important. Non-viral synthetic agents, such as positively charged polymers, lipids and peptides have been extensively studied as systems for gene transfer. They have been found to be safe and easy to produce, but they show low transfection efficacy, especially in vivo.

The objective of the present study was to develop a new efficient delivery agent with the essential structural features for successful gene delivery in vitro and in vivo. More than 50 1,4-dihydropyridine (1,4-DHP) derivatives were synthesized and screened for their ability to condense DNA and transfect the target cells in vitro. Furthermore, the most promising group of 1,4-DHPs was selected and the biophysical properties, cellular uptake, intracellular kinetics and transfection efficacy of their complexes with DNA (lipoplexes) were investigated. These studies showed that some 1,4-DHP amphiphiles are effective gene transfer agents in cell culture. Besides having self-assembling properties, the amphiphiles are able to condense efficiently DNA and destabilize endosomal membranes. The size and zeta-potential of the lipoplexes were dependent on the compound-DNA charge ratio. There was no correlation between size and zeta-potential of the complexes and their transfection efficacy, although positive zeta-potential was essential for transfection. Structural modifications of the amphiphiles, particularly the position and the amount of the charges per molecule, length of alkyl chains and change of substituents may affect the physicochemical and biological properties of the h'poplexes. The double-charged 1,4-DHP amphiphiles are more efficient and less toxic towards all examined cell lines than single-charged derivatives. Double-charged amphiphiles show also buffer capacity at endosomal pH that can contribute to their high transfection activity. Among 1,4-DHP derivatives, double-charged amphiphile with Ci2 long alkyl chains was the most efficient in transfecting the cells. After intravenous administration of this amphiphile lipoplex into mice, the transgene expression was observed mainly in the liver. Moreover, intramuscular administration of the lipoplex resulted in some transfection in vivo.

The effect of extracellular and intracellular factors on the 1,4-DHP-mediated transfection was also examined. The negatively charged exogenous glycosaminoglycans (GAGs) and serum components interfere with the transfection efficacy of 1,4-DHP amphiphiles. The strength of interaction of GAGs with the lipoplexes correlates with overall anionic charge density of the GAG chain. The main serum protein albumin does not prevent the cellular uptake of the lipoplexes, but alters their intracellular kinetic in such a way that transfection is impeded. Combination of amphiphiles with neutral lipid DOPE resulted in a serum-resistant transfection system Whereas inclusion of polyethylene glycol-lipid conjugates into the lipoplexes reduced transfection efficacy.

In conclusion, the present study shows that several 1,4-DHP amphiphiles display high transfection efficiencies in vitro and reveal some important structure-activity relationships and can be the basis for the further development of gene delivery system in vitro and in vivo. The investigation of the mechanisms of the cell uptake of 1,4-DHP lipoplexes, their intracellular trafficking and nuclear entry is extremely important for the optimization of the amphiphile-mediated transfectioa

DEVELOPMENT OF ANALYTICAL METHODS FOR THE CHARACTERIZATION OF ABSORPTION CELL MODELS

Palmgren, Joni J.

Kuopio University Publications A. Pharmaceutical Sciences 95. 2006. 115 p.

In pharmaceutical research, several techniques are used to screen drug candidates, varying from in silica calculations to in vivo studies in humans. In vitro approaches have become the preferred method for the screening of drug candidates. Cell-based models are especially common for the determination of in vitro ADME (absorption, distribution, metabolism, and excretion) properties of drug candidates.

Traditionally, a single compound has been administered to a cell model and from which samples are collected and analyzed with an analytical method. On the other hand, cassette dosing (having several compounds in a mixture) is a fast and powerful approach to study ADME properties and is commonly used to decrease time, labor, and expenditures. Cassette dosing provides the opportunity for higher throughput both in ADME studies and in sample analyses. New analytical technologies have enabled the use of cassette dosing in pharmaceutical research. For example, the combination of mass spectrometry and HPLC is a suitable analytical method for ADME studies, because of its sensitivity and selectivity.

The objectives of this study were to develop cassette dosing and various analytical methods (1) to study the permeability properties of absorption cell models, and (2) to evaluate drug loss during in vitro cell studies. The specific aims were (a) to develop analytical method for the analysis of neutral and charged PEG molecules, and to use this method to evaluate the permeation of polyethylene glycols (PEG) through a corneal epithelial cell line, (b) to develop a cassette dosing mixture and analytical method for the characterization of absorption properties of the Caco-2 cell monolayer, (c) to develop extraction and analytical methods for the quantitation of cholesterol and plant sterols from cultured cells and to estimate cellular uptake of sterols, and (d) to develop a new cassette dosing mixture and analytical methods, and use these methods to determine drug loss during in vitro cell studies.

The results from this study show that the developed high performance liquid chromatography-electrospray ionization-mass spectrometric (HPLC-ESI-MS) method can be applied to study paracellular permeability of charged and neutral PEGs through biological membranes. Furthermore, conventional ultaviolet/fluorescence HPLC can be used for high throughput cassette analyses, and cassette dosing is suitable for permeability studies with Caco-2 cell monolayers. The results further indicate that the cellular uptake of sterols and the amount of endogenous cholesterol in Caco-2 cells can be quantified by a newly developed HPLC-atmospheric pressure chemical ionization (APCI)-MS method. Finally, HPLC-ESI-tandem mass spectrometry (MS/MS) is a sensitive and selective method for the study of drug loss.

In conclusion, the present study shows that cassette dosing and analytical methods (based on liquid chromatography and various detection methods) are suitable for the characterization of absorption cell models and can be used to study the loss of drug content during in vitro experiments. Moreover, this data could be useful in the development of in vitro cell procedures.

National Library of Medicine Classification: QV 744, QV 771, QV 38, QU 325, QU 120

Medical Subject Headings: chemistry, pharmaceutical; drug evaluation, preclinical; pharmaceutical preparations; pharmacokinetics; absorption; tissue distribution; metabolism; permeability; chemistry, analytical; cell line; cells, cultured; epithelium, corneal; membranes; polyethylene glycols; caco-2 cells; cholesterol; phytosterols; chromatography, high pressure liquid; spectrometry, mass, electrospray ionization

SYNTHESIS OF NOVEL CANNABINOID CB1 RECEPTOR LIGANDS

Parkkari, Teija H.

Kuopio University Publications A. Pharmaceutical Sciences 97. 2006. 148 p.

The past fifteen years have been an exciting time for the cannabinoid research. Our understanding of the endogenous cannabinoid system (ECS) has continued to reveal the significant physiological role of this system in both the central nervous system (CNS) and peripheral tissues. The ECS has proved to be an important neuronal modulator with a novel mechanism of action, and therefore, a novel target for the drug discovery. Although cannabinoids still suffer from a negative reputation mainly due to the recreational use of marijuana, the exponential growth in a number of cannabinoid related patent applications is evidence that even in the near future, synthetic cannabinoids will achieve general acceptance as therapeutically significant agents.

The present study was focused on the design of novel metabolically stable endocannabinoid type CB1 receptor ligands, with the basis of the design based on the previously reported structure activity relationships of endocannabinoids. The synthesized compounds were divided into four different groups according to their chemical structures; (i) derivatives of arachidonyl alcohol, (ii) reversed amides of N-arachidonoyl ethanolamide (AEA), (iii) dimethylheptyl (DMH) derivatives of 2-arachidonoyl glycerol (2-AG) and 2-arachidonyl glyceryl ether (2-AGE), and (iv) α-methylated derivatives of 2-AG. The study consisted of three main parts. Firstly, effective synthesis and purification methods for the novel CB1 ligands were developed. Secondly, the cannabinergic activity of the synthesized compounds was determined in vitro by a $[^{35}S]GTP\gamma S$ binding assay (efficacy E_{max} and potency $-logEC_{50}$). Thirdly, the chemical and enzymatic stabilities of the novel CB1 ligands were studied in rat brain homogenate and membrane-free buffer.

The series of the ester, carbamate and carbonate derivatives of arachidonyl alcohol were evaluated, and it was observed that these kinds of compounds are not potent ligands for the CB1 receptor. In addition, the compounds were difficult to synthesize and handle due to their degradation, polymerization, and high liphophilicity. The important finding emerging from the second series of compounds was that, in contrast to the previous belief, the reversed amide derivatives of endogenous AEA are able to activate both CB1 and CB2 receptors. The study also revealed that the reversed amides exhibit significant metabolic stability under conditions where AEA is almost completely degraded. The study of DMH derivatives of 2-AG and 2-AGE showed that unlike the case of the AEA-type compounds and classical cannabinoids, the activity properties of 2-AG and 2-AGE cannot be improved by the replacement of the end pentyl chain with the DMH structure. Finally, a study with the a-methylated derivatives of 2-AG indicated that even though the stereochemistry of the α-position of 2-AG does not play any role in its affinity for the CB1 receptor, it has a significant role in G-protein activation. The study also indicated that the a-methylation can provide protection against the enzymatic degradation, and therefore, a prolonged duration of action for these compounds is to be expected.

National Library of Medicine Classification: QV 744, QV 38, QV 77.7, QV 126

Medical Subject Headings: chemistry, pharmaceutical; receptors, drug; neurotransmitter agents; cannabinoids; endocannabinoids; receptor, cannabinoid, CB1; ligands / chemical synthesis; structure-activity relationship; drug design

DESIGN, SYNTHESIS AND CHARACTERIZATION OF NOVEL WATER-SOLUBLE CHITOSAN DERIVATIVES

Holappa Jukka K.

Kuopio University Publications A. Pharmaceutical Sciences 88.2006.114 p.

Chitosan (poly-l,4-β-D-glucosamine) is a cationic biopolymer. Recently its applications in pharmacy and medicine, food science, agriculture, pulp and paper industry, cosmetics, water purification and the textile industry have been the focus of considerable interest. Chitosan is industrially prepared by N-deacetylation from chitin (poly-l,4-β-N-acetyl-D-glucosamine), which is the second most ubiquitous biopolymer after cellulose. Chitin is the main component of crustacean shells and it can also be found in some insects and fungi.

Chitosan has attracted considerable attention in the pharmaceutical and biomedical fields not only because of its unique activity properties, but also due to its biocompatibility, biodegradability and mucoadhesivity. Chitosan has been used in many conventional formulation applications, e.g., due to its ability to form films and gels. Novel applications of chitosan include the delivery of peptides, vaccines and genes. One well explored biological property of chitosan is its antimicrobial activity.

The polycationic properties of chitosan are thought to be responsible for most of its observed activities. The amino groups in chitosan are only partially protonized at physiological pH 7.4, and the major drawback of chitosan, if it is to be used as a pharmaceutical excipient, is its poor aqueous solubility. Chitosan is soluble in dilute aqueous acids, when its amino groups become protonated. The poor solubility in organic solvents hinders especially the processability of chitosan materials.

Quaternary ammonium derivatives of chitosan are interesting in view of pharmaceutical applications. These derivatives have two major advantages over the parent chitosan; 1) they are water-soluble at physiological pH and 2) they possess a permanent positive charge on the polysaccharide backbone. Quaternary chitosan derivatives have various potential pharmaceutical applications, e.g., as antimicrobials, as permeation enhancers and as gene delivery systems.

In the present study, two types of water-soluble quaternary chitosan derivatives were designed and synthesized, i.e., (1) chitosan N-betainates and (2) mono- and diquaternary piperazine derivatives of chitosan. A convenient synthetic method was also discovered for the preparation of mono- and diquaternary piperazinium acids, which were then attached to chitosan. In addition, organo-soluble N-chloroacyl-O-triphenylmethylchitosans were synthesized. These are very useful intermediates in the selective synthetic modification of chitosan.

Products were prepared with various degrees of substitution. In the synthesis of chitosan N-betainates, also starting materials with different molecular weights were used to obtain chitosan N-betainates with a range of molecular weights.

The chemical structures of end products were confirmed by ^{1}H NMR, ^{13}C NMR and by various 2D NMR techniques. Solid state ^{13}C CP/MAS NMR, FT-IR and elemental analysis were used to characterize some of the products. The molecular weights and molecular weight distributions of the end products were determined with the GPC-system attached to a light scattering detector.

Chitosan N-betainates possessed low antimicrobial activity under neutral conditions. However, the antimicrobial activity of chitosan-N-betainates increased with decreasing degree of substitution in acidic conditions, which suggests that for efficient antimicrobial action, the positive charge should be situated in the amino group of chitosan.

The properties of these novel water-soluble chitosan derivatives will be tested in various pharmaceutical applications in the future. For example, permeation enhancer properties, mucoadhesive properties, gene delivery properties, and antimicrobial properties will be tested in vitro, and hopefully some in vivo studies can be carried out with the most promising derivatives. The toxicity of these novel quaternary chitosan derivatives will also be tested.

Medical Subject Headings: biopolymers; drug delivery systems; excipients; chitin; chitosan/analogs & derivatives; chitosan/chemical synthesis; chitosan/chemistry; solubility; water; molecular structure; molecular weight; betaine; piperazines; anti-infective agents

MOLECULAR MODELING OF THE ENDOGENOUS CANNABINOID SYSTEM - USABILITY OF MODELING RESULTS IN DRUG DESIGN

Salo, Outi M.H.

Kuopio University Publications A. Pharmaceutical Sciences 87. 2006. 95 p.

The endogenous cannabinoid system (ECS) comprises receptors (CB1 and CB2), endogenous ligands (e.g., N-arachidonoylethanolamide, AEA; sn-2-arachidonoylglycerol, 2-AG) and ligand-metabolizing enzymes (e.g., fatty acid amide hydrolase, FAAH; monoacylglycerol lipase, MGL). Since this system is involved in various physiological and pathological processes, it represents an intriguing target for current drug design and development. Cannabinergic ligands (e.g., receptor agonists/antagonists or enzyme inhibitors) have therapeutic potential, for example in the management of nausea, pain, obesity, anxiety, neurological disorders, glaucoma, and cancer.

Modern drug design projects require a concerted effort from a multidisciplinary team if they are to be successful in producing novel compounds for clinical use. Computer-aided drug design can play a central role in facilitating the decision making, identifying the right lead candidates for the right target protein. Different molecular modeling strategies can be used, depending on the available knowledge about the target structures and/or ligands.

The general objective of the present study was to aid in design of novel therapeutically active molecules targeted at the ECS by using both ligand-based and structure-based molecular modeling strategies. Specifically, comparative models of the CB1 and CB2 receptors as well as the MGL enzyme were constructed and utilized in virtual screening or examining the putative binding site and the molecular interactions between the ligands and the protein. Three-dimensional quantitative structure-activity relationships (3D QSAR) of CB1 receptor agonists were studied using the potency of G-protein activation as biological data.

Molecular docking results at the CB1 model confirmed, for example, the importance of lysine K3.28(192) in the binding of well-known CB1 ligands. The CB2 model was successfully utilized in the virtual screening of novel CB2 compounds, resulting in the discovery of a selective CB2 agonist that can serve as a lead molecule for further optimization. Modeling the MGL enzyme provided insight into the possible structure and location of the substrate binding site. Both manual and docking-based alignments of classical and endocannabinoid-type CB 1 agonists produced statistically significant 3D-QSAR models. The alignments support the hypothesis that these structurally diverse molecules overlap only partially within the CB1 receptor binding site.

The usability of the modeling results in the present drug design project was evaluated. In general, a good model should be helpful in lead discovery and/or optimization. Future application of the present models will ultimately prove their full potential in facilitating the design of novel cannabinergic drugs.

National Library of Medicine Classification: QV 744, QV 77.7, QU 34

Medical Subject Headings: drug design; cannabinoids; endocannabinoids; monoacylglycerol lipases; models, molecular; protein structure, tertiary; binding sites; receptors, cannabinoid; ligands; enzyme inhibitors; quantitative structure-activity relationship; GTP-binding proteins

ENZYMATIC HYDROLYSIS OF THE ENDOCANNABINOID 2-ARACHIDONOYLGLYCEROL -CHARACTERIZATION AND INHIBITION IN RAT BRAIN MEMBRANES AND HOMOGENATES

Saario, Susanna M.

Kuopio University Publications A. Pharmaceutical Sciences 94. 2006. 96 p.

The specific binding site of Δ^9-tetrahydrocannabinol (Δ^9-THC), the main active ingredient of Cannabis saliva L., was characterized from rat brain nearly 20 years ago, and several endogenous compounds and proteins forming the endocannabinoid system have been discovered since that time. To date, it has become evident that the endocannabinoid system consists at least of two cannabinoid receptors; i.e., the CB_1 and CB_2 receptors, in addition to several endogenous ligands (endocannabinoids) and enzymes involved in the biosynthesis and metabolism of the endocannabinoids. The two most abundant endocannabinoids, N-arachidonoylethanolamide (AEA) and 2-arachidonoylglycerol (2-AG), are produced by neurons on demand, act near to the site of their synthesis and are effectively metabolized by fatty acid amide hydrolase (FAAH) and monoglyceride lipase (MGL), respectively.

The main focus on the present study was on the endocannabinoid-metabolising enzymes, especially MGL. Enzymes, in general, are attractive targets for drug design, due to their catalytic properties; i.e., they produce and degrade specific chemical bonds via several intermediate states that can be exploited for potential drug interaction, to the endocannabinoid system, MGL and FAAH may serve as potential therapeutic targets for treating certain disease states, such as anxiety and inflammatory pain. One significant advantage of MGL and FAAH inhibition over direct cannabinoid agonists could be higher selectivity, as the intervention would only increase the activity of the endocannabinoid system at the sites where the endocannabinoids are produced and released. This hypothesis has been supported by animal studies in which the FAAH inhibitor URB597 elevated endocannabinoid tone and, unlike the nonselective cannabinoid agonists, did not produce any motor side effects, such as catalepsy.

The hydrolysis of endocannabinoids was investigated in rat cerebellar membranes where the enzymatic activity towards 2-AG was found to be 50-fold higher than that towards AEA. Furthermore, potent FAAH-inhibitors such as OL-53 and URB597 did not inhibit the hydrolysis of 2-AG, suggesting that 2-AG is inactivated in rat cerebellar membranes by an enzyme other than FAAH, most probably by a MGL-like enzyme.

Sulfhydryl-specific compounds were investigated for the inhibition of this MGL-like enzyme in rat cerebellar membranes. N-Arachidonylmaleimide (NAM), a substrate analogue of 2-AG, which synthesized in this study, was found to be a potent inhibitor of MGL-like enzyme activity (IC_{50} 140 nM), indicating that NAM binds to a sulfhydryl group within the enzymatic binding-site.

URB754 was earlier reported to be a putative MGL inhibitor (IC_{50} 200 nM). However, URB754 did not show any significant inhibitory effect on the hydrolysis of 2-AG in rat brain homogenates in the present study, which raises some doubts for the use of URB754 as a lead structure for further development of MGL specific inhibitors.

The novel hit molecules obtained from a virtual screening of the endocannabinoid system were tested for their inhibitory activity on FAAH and MGL-like enzyme. Nine novel compounds inhibited FAAH with IC_{50} values between 520 nM and 44 µM. to addition, one compound was found to inhibit MGL-like enzyme activity with an IC50 value of 31 µM.

In conclusion, the results of this work provide useful information on 2-AG hydrolyzing enzymatic activity and its inhibition in the rat brain, which can be exploited for the design and development of novel MGL inhibitors.

Medical Subject Headings: cannabinoids; endocannabinoids/metabolism; neurotransmitter agents/metabolism; arachidonic acids/metabolism; glycerides/metabolism; monoacylglycerol Upases; amidohydrolases; hydrolysis; enzyme inhibitors; cerebellum; rats; drug design

GRADUJEN TIIVISTELMÄT

SCALE-UP OF HIGH-SHEAR MIXERS- UNDERSTANDING OF WET GRANULATION PROCESS

Jaana Hautala

Farmasian teknologian osasto, Farmasian tiedekunta, Helsingin yliopisto

The purpose of the study was to evaluate, whether the rheology based method of dimensional analysis could be applied to the scale-up of wet granulation in a series of small fixed-bowl granulators. The methodology relies on the demand of geometric and dynamic similarity. Thus a deeper understanding of the wet agglomeration process needed to be established in order to evaluate the possible differences in material flow and mixing behavior when changing the scale. The aim was also to study, whether the scale-up methodology characterising the rheology of the wet masses, could be a tool for proper end-point determination.

The composition of microcrystalline cellulose (MCC) 78.5 % (w/w), mannitol 18.5 % and polyvinyl pyrrolidone (PVP) 3.0 % was used in granulations. The Diosna high-shear granulation apparatus with bowl sizes 1 L, 4 L and 6 L was examined. Two different experimental designs with different water levels (0.15 - 0.75 g/g) and impeller rotation speeds were applied for the studies.
The measurements for power consumption combined with the determination technique of changed temperature, and in-line near infrared spectrometry (NIR) were chosen in order to follow and control the wet granulation process. The wet bulk density, consistency and at-line NIR spectra were measured after the agglomerations. The granules were tray-dried. The granule size distribution and density, as well as pore size distribution and images with scanning electron microscopy were determined. Before tabletting, the granules were milled and lubricated. The tablet hardness of the Diosna granules was determined.

The granule density increased and porosity decreased when increasing the level of liquid saturation with added water, agitation and mixing time. The increased granule density was also seen in increased values of wet mass consistency and NIR water peaks, as well as growth in granule size and decreased compactability. The best tablets were achieved from granules within the plateau region confirming the assumption for granulation end-point within this area. Correlations for tablet hardness were studied with the properties of wet and dry granules, and the best correlations were found with granule porosity, density and at-line NIR. However, the wet granule consistency'was shown to be inadequate for the process end-point determination.

Though first considered minor, differences in geometrical similarity lead to changes in material flow in used Diosna bowls. This was seen as changes in granule compactability, though the dimensionless numbers were kept constant between different scales. Compared to other Diosna bowls, a more free material rotation in Diosna 6 L granulation bowl lead to denser granules, and thus weaker tablets. It could be therefore stated that though first claimed, the used Diosna bowls were not exactly geometrically similar and minor differences can affect the behavior of wet masses on the course of granulation.

The scale-up method of dimensional analysis is greatly dependent on used formulation. With swelling materials, like MCC, the master curve should be constructed within the area where the wet bulk density increases. With MCC, this was received within the plateau region revealed by the different power consumption profiles.

Keywords: dimensional analysis, scale-up, end-point, geometrical similarity, consistency, flow pattern

LÄMPÖHERKILLÄ POLYMEEREILLÄ PÄÄLLYSTETTYJEN POLYSTYREENIPARTIKKELIEN OMINAISUUDET, SYTOTOKSISUUS SEKÄ KIINNITTYMINEN RAW 264 - MAKROFAGEIHIN JA CACO-2 SOLUIHIN

Anna-Kaisa Marttila

Farmasian teknologian osasto, Farmasian tiedekunta, Helsingin yliopisto

Soluunotto tapahtuu joko passiivisesti tai aktiivisesti. Passiivinen kuljetus tapahtuu diffuusiona solukalvon läpi eikä kuljetus vaadi energiaa. Monet lääkeaineet kulkeutuvat passiivista kuljetusta useammin aktiivisella, energiaa vaativalla, kuljetuksella solun sisään. Aktiivinen kuljetus tapahtuu kalvossa olevien kuljettajaproteiinien avulla, endosytoosilla. Tunnetuimpia endosytoosimekanismeja ovat fagosytoosi ja pinosytoosi. Fagosytoosi on tunnettu makrofagien soluunottomekanismi. Fagosytoosilla otetaan soluun suuria yli 500 nm kokoisia partikkeleita. Pinosytoosilla soluun kulkeutuvat yleensa partikkelit, jotka ovat kooltaan pienempiä kuin 200 nm. Seka fagosytoosi että pinosytoosi tapahtuvat reseptorivälitteisesti mutta pinosytoosi voi tapahtua myös ilman ligandin spesifistä kiinnittymistä. Pinosytoosissa ligandit kuljetetaan solun sisään solukalvosta muodostuneen vesikkelin sisässä.

Lääkeaineen kulkeutumiseen elimistössä voidaan vaikuttaa päällystämälla lääkeaineyhdiste polymeerilla. Polymeeri muuttaa yhdisteen ominaisuuksia, kuten kokoa, pintavarausta seka hydrofobisuutta, ja vaikuttaa siten yhdisteen kulkeutumiseen ja kiinnittymiseen elimistön soluihin. Polymeeripäällystyksellä voidaan myös rajoittaa tai hidastaa lääkeaineen vapautumista. Ympäristön lämpötilaan reagoivien lämpöherkkien polymeerien käyttöä on tutkittu runsaasti kontrolloidusti tapahtuvassa lääkeaineiden annostelussa. Yksi tunnetuimmista lämpöherkistä polymeereistä on poly-(N-isopropyyliakryyliamidi) (PNIPAM). Myös poly-(N-vinyylikaprolaktaami) (PVCL) on käyttökelpoinen polymeeri terapeuttisiin tarkoituksiin. Seka PNIPAM:n etta PVCL:n alempi kriittinen liukoisuuslämpötila (LCST) on noin 32 °C. Tämän lämpötilan alapuolella polymeerit liukenevat veteen. Kun lämpötilaa nostetaan LCST-lampötilan yläpuolelle, tapahtuu faasimuutos, kokoonpainuminen.

Työn kokeellisessa osuudessa tutkittiin fluoresoivien polystyreenipartikkelien (FPS) sekä lampöherkillä PNIPAM- ja PVCL-polymeereillä päällystettyjen FPS-partikkelien ominaisuuksia ja interaktioita RAW 264-makrofagien ja Caco-2 solujen kanssa. Kokeet suoritettiin myös FPS-partikkeleilla, jotka oli oksastettu amfifiilisella poly(etyleenioksidi)-makromonomeerilla (PEO-makromonomeeri), $MAC_{11}EO_{42}$. Polymeeripartikkelit kiinnittyivät makrofageihin paremmin kuin Caco-2 soluihin. Runsaslukuiset proteiinit mahdollistivat polymeeripartikkelien kiinnittymisen makrofagien pintaan. Polymeeripartikkelien soluunottoa ei ollut havaittavissa.

Polymeeripartikkelien soluadheesioon vaikuttivat partikkelien koko, hydrofobisuus, pintavaraus sekä lämpötila, jossa kokeet suoritettiin. PVCL:llä päällystetyt FPS-partikkelit kiinnittyivät polymeeripartikkeleista parhaiten sekä RAW 264 -makrofageihin että Caco-2 soluihin. FPS-PNFPAM assosioitui soluihin hieman FPS-PVCL -partikkeleita heikommin. Sytotoksisuus osoittautui näiden polymeerien kohdalla pienillä pitoisuuksilla melko vähäiseksi. Sen sijaan, FPS-PEO osoittautui lämpöherkkiä polymeerejä toksisemmaksi ja sen kiinnittyminen soluihin oli heikkoa. PVCL:n ja PNIPAM:n käytettävyys lääkeaineiden kuljetuksessa ja yhteensopivuus elimistön kudosten kanssa sekä tässä työssä todettu kiinnittyminen soluihin luovat hyvät mahdollisuudet PVCL:llä ja PNIPAM:lla päällystetyjen partikkeleiden kehittämiseen ja käyttöön kontrolloidussa lääkeannostelussa.

Avainsanat: PVCL, PNIPAM, lämpöherkkä polymeeri, makrofagi, soluun kiinnittyminen, soluunotto

ADSORPTION OF SOME DRUGS ON MICROCRYSTALLINE CELLULOSE IN AQUEOUS SOLUTION

Ville Matilainen

Farmasian teknologian osasto, Farmasian tiedekunta, Helsingin yliopisto

Microcrystalline cellulose (MCC) is widely used as a pharmaceutical excipient, it is important to study also adsorption between MCC and drugs. Adsorption of caffeine, cichocaine HCl, ethacridin lactate, lidocaine HCl, procaine HCl, sodium salicylate and tetracaine HCl on MCC were tested in aqueous solution.

Results indicate that ethacridin lactate had strong adsorption on MCC. The shape of adsorption isotherm curve of ethacridin lactate was clear. All the hydrochlorides had also adsorption. Different chemical structure of these drugs didn't affect so much on adsorption process. Mostly adsorption of these drugs was due to hydrochloride group of the drug. In general the results, however, show the extend of adsorption and the similarity of the adsorption of hydrochlorides.

Keywords: adsorption, adsorption isotherm, microcrystalline cellulose, ethacridin lactate

PINTAKUVA-ANALYYSI RAKEIDEN PROSESSIKÄYTTÄYTYMISEN TARKASTELUSSA

Hanna-Maria Tervakangas

Farmasian teknologian osasto, Farmasian tiedekunta, Helsingin yliopisto

Partikkelikoon määrittäminen lääkkeenvalmistusprosessin eri vaiheissa on tärkeää. Partikkelikoko vaikuttaa lääkeaineen biologiseen hyväksikäytettävyyteen, stabiiliuteen, prosessoitavuuteen ja annostarkkuuteen lääkevalmisteissa. Jauhemaisen aineen ominaisuudet ja käyttäytyminen lääkkeenvalmistusprosessin eri vaiheissa riippuvat suurelta osin materiaalin partikkelikokojakaumasta.

Vapaasti valuvilla jauheilla ja rakeilla on taipumus segregoitua. Erot seoksessa olevien partikkelien fysikaalisissa ja mekaanisissa ominaisuuksissa kuten partikkelien koossa, muodossa, tiheydessä, koheesiossa ja pinnan ominaisuuksissa aiheuttavat jauheen segregoitumista. Prosessiolosuhteissa segregoitumista aiheuttavia tekijöitä ovat muun muassa värähtely, leijutus, jauheen kaataminen, jauheen syöttönopeus, kosteus ja sekoitus.

Tärkein segregoitumiseen vaikuttava tekijä on partikkelikoko ja partikkelikokojakauma. Pienempien partikkelien kyky sijoittua suurempien partikkelien väliin on segregoitumisen kannalta kriittinen ominaisuus. Partikkelikomponenttien välinen kokoero on segregoitumisen kannalta merkittävämpi tekijä kuin absoluuttinen partikkelikoko. Seos, joka koostuu erikokoisista partikkeleista voi segregoitua tabletoinnin aikana jauheen syöttösuppilossa tapahtuvan värähtelyn seurauksena. Kiinteiden lääkemuotojen valmistaminen edellyttää partikkelikoon määrittämistä ja kontrollointia siten, että lääke- ja apuaineet ovat homogeenisena seoksena.

Partikkelikoon määrittämiseen on olemassa useita eri tekniikoita. Tavallisimpia farmaseuttisten jauheiden ja rakeiden koon määrittämiseen käytettyjä menetelmiä ovat seula-analyysi, mikroskopia, laserdiffraktio ja kuva-analyysitekniikat. Uusi partikkelikoon määrittämiseen jauheen pinnasta perustuva kuva-analyysimenetelmä on kehitetty hiljattain. Jauheen pintakuvasta saatava informaatio muunnetaan partikkelikooksi pintakuvainformaation ja seula-analyysin välille muodostetun mallin avulla.

Pintakuva-analyysimenetelmää modifioitiin tätä tutkimusta varten näytteenvalmistuksen ja kuvauksen osalta. Erikoistyön tarkoituksena oli luoda uusi partikkelikoon mittaamiseen pintakuva-analyysillä käytettävä malli. Menetelmän soveltuvuutta testattiin myös värähtelyn aiheuttaman segregaation simuloimisessa. Työssä selvitettiin myös partikkelikokojakaumaltaan erilaisten rae-erien segregaatiokäyttäytymistä monitoroimalla partikkelikokojakaumaa pintakuva-analyysin avulla tabletointiprosessin aikana. Monimuuttuja-analyysiä käytettiin tutkimusaineiston visualisoimiseen.

Pintakuva-analyysin modifiointi näytteenvalmistuksen ja kuvauksen osalta paransi menetelmän luotettavuutta. Menetelmän avulla voitiin monitoroida värähtelyn aiheuttamaa segregaatiota. Sen avulla voitiin myös onnistuneesti tarkastella partikkelikooltaan erilaisten rae-erien segregaatiokäyttäytymistä tabletointiprosessin aikana. Rae-erillä, joilla suurin osa partikkeleista sijoittuu fraktioihin 355-500 µm ja 1000-2000 µm, oli suuri taipumus segregoitua. Pintakuva-analyysillä määritetty rakeiden tabletointiprosessin aikainen segregoituminen oli yhdistettävissä tablettien väliseen painonvaihteluun.

Avainsanat: partikkelikoko, pintakuva-analyysi, segregaatio, monimuuttuja-analyysi

PERMEATION MECHANISMS OF SALICYLIC ACID AND DERIVATES ACROSS CACO-2 MONOLAYERS: INVOLVMENT OF TRANSPORTER PROTEINS

Jakub Čierný*

Division of Pharmaceutical Technology, University of Helsinki, Finland
*Department of Pharmaceutical Technology, Charles University in Prague, Czech Republic

The aim of this work is to study properties of the transport of salicylic acid and its derivates across Caco-2 cell monolayers, an influence of possible inhibitors of cell transportation and a brief review of related issues. The influence of substance, which opens tight junctions between Caco-2 cells, so called absorption enhancers, is also examined. The main goal is to find out, what kind of transport takes the main part in salicylic acid transport and to compare the results to the data obtained using gentisic and 5-hydroxyisophthalic acid, and, at the same time, to study the influence of ethylenediaminetetraacetic acid as an absorption enhancer and to compare possible inhibitive effect of several compounds. It was assumed that both passive and active transport take part in the transport of salicylic acid.

The theoretical part of the thesis briefly summarizes chemical substances properties, which have an influence on their transport across cell membranes, introduces Caco-2 cells as an important instrument of drug absorption and distribution research, defines the individual types of cell transports and describes the types of transporter proteins which can be localized on the Caco-2 cell membrane.

The experimental part of the work describes the technique of Caco-2 cells cultivation and the process of transport experiments. Most of experiments were done at 37°C using a pH gradient - pH 5.5 on the apical side and pH 7.4 on the basolateral side of the membrane. The cell monolayer integrity was tested by measuring transepithelial electrical resistance and by using radio-labeled compound. The samples taken during experiment were analyzed by the high performance liquid chromatography. The amount of transported compound was quantified by the permeation coefficient and by the flux.

We can see from the results that both active and passive transport takes part in transport of salicylic acid and its derivates across the Caco-2 cell monolayer. The opening of tight junctions brought a noticeable increase of transfer of passively transported compounds; the difference was not so clear in salicylic acid results that support the hypothesis of transporter proteins involvement. The influence of inhibitive agents was obvious; the highest influence was achieved using 2-hydroxyisopropylbenzoic acid.

KAHDEN TERMOANALYYTTISEN MENETELMÄN VERTAILU TUTKITTAESSA AMORFISEN LÄÄKEAINEEN KITEYTYMISKINETIIKKAA

Ville Alava

Fysiikan laitos, Turun yliopisto

Amorfisten aineiden ominaisuuksilla ja niiden määrittämisellä on tärkeä merkitys lääkeaineiden valmistuksessa. Amorfisen aineen määrä lääkeaineessa on määritettävä, koska tämä vaikuttaa lääkeaineen liukenevuuteen ja kiteytyminen voi tapahtua säilytyksen aikana aiheuttaen näin stabiilisuusongelman.

Aineen amorfinen muoto on termodynaamisesti epästabiili ja se pyrkii kiteytymään stabiiliin muotoon. Kiteisen ja amorfisen materiaalin koostumus molekyylitasolla on samanlainen, joten niiden terapeuttinen vaikutuskin on samanlainen. Näin ollen amorfisen aineen olisi oltava riittävän stabiili, jotta sitä voitaisiin säilyttää tarvittava aika ilman muutoksien syntymistä.

Farmaseuttisessa fysiikassa on monia termoanalyyttisiä tutkimusmenetelmiä, joista tässä tutkielmassa esitellään röntgendiffraktiomenetelmä (XRD), differentiaalinen pyyhkäisykalorimetria (DSC) ja isoterminen mikrokalorimetria (IMC).

Tässä tutkielmassa käytettiin aineena karbamatsepiinia, jota käytetään epilepsialääkkeenä ja hermosärkyjen hoidossa. Aine amorfisoitiin käyttämällä sulasammutusmenetelmää. Amorfisen karbamatsepiinin kiteytymiskinetiikkaa analysoitiin käyttämällä DSC:tä ja IMC:tä. Aineen amorfisuus varmistettiin käyttämällä XRD:tä.

Isotermisellä mikrokalorimetrilla tutkittiin aineen kiteytymistä viidellä eri lämpötilalla. Lämpövirtakäyristä havaittiin, että kiteytyminen tapahtuu sitä nopeammin, mitä korkeammassa lämpötilassa ainetta pidetään. Lämpövirtakäyriin sovitettiin Avramin yhtälöitä, joiden parametrin määritettiin tietokoneella.

Differentiaalisella pyyhkäisykalorimetrilla suoritettiin mittauksia kuudella eri lämmitysnopeudella. DSC-termogrammeista havaittiin, että lämmitysnopeudella oli vaikutusta kiteytymis- ja sulamispiikkien pinta-alaan. Havaittiin myös, että mitä suurempi lämmitysnopeus oli, sitä suuremmat olivat piikkien pinta-alat. Termogrammeista määritettiin myös lasitransitio-, kiteytymis- ja sulamislämpötilat.

Amorfinen karbamatsepiini kiteytyi I-muotoon. DSC:n ja IMC:n mittaustuloksista määritettiin aineelle aktivaatioenergia isokonversionaalisella menetelmällä, joka perustuu Arrhenius-yhtälöön.

Asiasanat: Amorfisuus, Karbamatsepiini, XRD, DSC, IMC, Avramin yhtälöt, Aktivaatioenergia

PARTIKKELIEN EROTTAMINEN DIELEKTROFOREESIN AVULLA

Antti Lehtonen

Fysiikan laitos, Turun yliopisto

Tässä tutkielmassa käsitellään partikkelien erottamista dielektroforeesin avulla. Tutkielman tarkoituksena on tutustua dielektroforeesiin ilmiönä ja antaa lukijalle kuva sen käytöstä partikkelien erottamiseen. Tutkielmassa esitetään erilaisia erotustapoja ja sovelluskohteita historiasta nykypäivään. Tutkielman kokeellisessa osassa julkaistaan mikrokokoluokan piipartikkeleilla tehtyjen erotuskokeiden tulokset.

Tutkielma on kirjoitettu käyttäen hyväksi kirjallisuutta sekä aiheeseen liittyviä artikkeleita. Tärkein lähdeteos on Cross, J. A. 1987 Electrostatics: principles, problems and applications. Tärkein lähdeartikkeli on L. Zhang, F. Tatar, P. Turmezei, J. Bastemeijer, J.R. Mollinger, and A. Bossche: Particle Separation by Dielectrophoresis. Sovelluskohteet on koottu eri julkaisuista.

Kun varaukseltaan neutraali partikkeli sijoitetaan epähomogeeniseen sähkökenttään, se polarisoituu ja siihen kohdistuu nettovoima. Voiman vaikutuksesta partikkeli kulkeutuu voimakkaampaan osaan kenttää. Tätä ilmiötä kutsutaan dielektroforeesiksi. Ehtona tälle on, että partikkelin ja väliaineen permittiivisyydet eroavat toisistaan. Kumpikin elektrodin polariteetti synnyttää dielektroforeesin. Kentän synnyttämiseen voidaan käyttää sekä vaihto- että tasajännitettä. Homogeenisessa sähkökentässä varaukseton partikkeli ei liiku.

Dielektroforeesin avulla partikkeleita voidaan erotella niiden dielektristen ominaisuuksien perusteella. On olemassa erilaisia, elektrodillisia ja elektrodittomia, tapoja erotella partikkeleita. Elektrodillisissa tavoissa elektrodia käytetään joko partikkelin vangitsemiseen tai nostamaan partikkeli nestevirran tai voiman kuljetettavaksi. Elektrodittomissa tavoissa partikkelit eivät ole kosketuksissa elektrodien kanssa. Dielektroforeesia voidaan käyttää myös eri kokoisten partikkelien erottamiseen.

Tutkimusten perusteella dielektroforeesi soveltuu partikkelien erottamiseen silloin, kun pienen partikkelikoon vuoksi silmämääräinen erottaminen on mahdotonta. Dielektroforeesilla on onnistuttu erottamaan halkaisijaltaan alle millimetrin olevia mineraalipartikkeleita sekä nanokokoluokkaa olevia biopartikkeleita.

Dielektroforeesin ja tutkimuksessa käytettyn koejärjestelyn avulla saatiin erotettua halkaisijaltaan alle 38 µm piipartikkelikokojakaumasta kapeampia kokojakaumia. Jännitteellä ja pumppausnopeudella todetttiin olevan vaikutusta erotuskykyyn.

Asiasanat: dielektroforeesi, partikkelien erotus, sähköstatiikka

SOLUUNOTTOMEKANISMIT JA NIIDEN TUTKIMINEN

Hänninen Raija S.

Farmaseuttinen tiedekunta, Kuopion yliopisto

Avainsanat: endosytoosi, kalvoliikenne, fluoresenssi kuvantaminen, kuljetusmekanismin inhiboiminen, soluunotto, bisfosfonaatit, doksorubisiini

Solunsisäisestä kalvoliikenteestä ja asiaan liittyvien soluelimien molekyylidynamiikasta tarvitaan tietämystä, jotta voidaan hahmottaa, kuinka lääkeaineet kulkeutuvat soluun endosyyttisellä mekanismilla. Koska lääkeaineiden toimintapaikka on yleensä organel-lin tietyllä alueella, halutaan endosytoosissa syntyvän kuljetusrakkulan pääsevän tiettyyn kohteeseensa. Lisääntynyt tietämys endosytoosimekanismeista ja sitä häiritsevistä tai manipuloivista välittäjistä aukaisevat uusia lähestymistapoja tehokkaampaan intra-sellulaariseen aineiden kuljetukseen. Endosyyttiset kuljetusmekanismit voidaan karkeasti luokitella kahteen kategoriaan, fagosytoosiin eli solusyöntiin ja pinosytoosiin eli solujuontiin. Pinosytoosia ilmentyy neljällä perusmekanismilla: makropinosytoosilla, klatriini- tai kaveolivälitteisellä endosytoosilla, sekä endosytoosilla, joka on riippumaton klatriinista tai kaveolista.

Elävien solujen kuvantamiseen sopivien fluoresoivien leimaamistekniikoiden käyttöönotto on muuttanut viime aikoina solubiologian tietämystä. Eläviä soluja pystytään kuvantamaan, koska GFP-leiman avulla voidaan reaaliajassa kuvata solun pysyvien osien muoto-oppia ja näiden osien muuntumista. Koska fagosytoosiin ja makropinosytoosiin liittyy solunulkoisen mediumin kapselointi solun pinnasta työntyvän membraanin sisään, fluoresoivia partikkeleita ja leimattuja mikroorganismeja voidaan käyttää merkkaamaan fagosomeja. Tiettyjen endosomaalisten organellien määrittäminen on ongelmallista, koska ne mm. ovat hyvin dynaamisia, lisääntyvät jatkuvasti ja siirtyvät paikasta toiseen. Yleisesti kuitenkin uskotaan, että kalvoliikenteen voi määrittää Rab-proteiinien avulla. Tietoja intrasellulaarista kuljetusreiteistä on saatu helposti estämällä yhden tai useamman kuljetusmekanismin toimintaa. Tämän vuoksi erilaisia inhibiitto-reita on käytetty endosyyttisten reittien tutkimisessa.

Kokeellisessa osassa tutkittiin hiili-14-leimattujen lääkeaineiden, klodronaatin ja zole-dronaatin, soluunottoa MCF-7 ja MDA-MB-436 soluilla. Kokeessa käytetyt solulinjat valittiin niiden erilaisen bisfosfonaattien metaboliatuotteiden (IPP) ja aktiivisten yhdisteiden (ApppI) ilmentämisen vuoksi. Kokeen perusteella oli tarkoitus tutkia, onko soluun kulkeutuneen bisfosfonaatin määrä samansuuntainen kuin näiden solulinjojen ilmentämät ApppI/IPP määrä. Suoritetut kokeet eivät kuitenkaan antaneet selviä vastauksia tähän kysymykseen. MCF-7 solut, jotka ilmentävät ApppI:a ja IPP:a, ottivat sekä zoledronaattia että klodronaattia huomattavasti heikommin sisäänsä kuin MDA-MB-436 solut. Kokeen peusteella voi päätellä että zoledronaattia kulkeutuu MDA-MB-436 soluihin paremmin kuin MCF-7 soluihin. Toisessa osiossa tutkittiin MCF-7 rintasyöpäsolujen ja J774-makrofaagisolujen avulla, johtuuko doksorubisiinin ja zoledro-naatin synergia bisfosfonaatin lisääntyneestä soluunotosta vai onko synergian takana jokin muu mekanismi. Suoritetuissa doksorubisiinikokeissa ei pystytty osoittamaan pitävästi, että doksorubisiini edesauttaisi zoledronaatin soluunottoa.

ANTIBIOOTTISET PEPTIDIT ELIMISTÖN PUOLUSTUSMEKANISMINA

Koikkalainen Tero

Farmaseuttinen tiedekunta, Kuopion yliopisto

Avainasanat: antibioottiset peptidit, defensiinit, katelisidiinit, histatiinit

Epäspesifinen synnynnäinen immuniteetti kehittyi 2,6 miljardia vuotta sitten. Sitä seurasi kehittyneemmillä lajeilla adaptiivisen immuniteetin kehittyminen, joka lisäsi monimuotoisuutta, spesifisyyttä ja muistia taistelussa mikrobihaasteita vastaan. Synnynnäinen immuniteetti on tarvittaessa nopea, tappava ja monikykyinen, ja sen antimikrobiaalinen toimintaa ohjaa osittain pienet kationiset peptidit, joilla on potentiaalista antimikrobiaalista aktiivisuutta gram-negatiivisia ja -positiivisia bakteereja, sieniä, parasiitteja ja joitain viruksia kohtaan. Patogeenien nopean tappamisen perusmekanismi johtaa mikrobin solumembraanin häiriintymiseen, mutta tietämys mekanismeista on vielä vajavaista, ja myös muita mekanismeja on ilmeisesti olemassa. Ihmisen antibioottiset peptidit (AMP:t), kuten defensiinit ja katelisidiinit (LL-37) sijaitsevat leukosyyteissä. Lisäksi niitä eritetään erilaisista epiteeleistä, kuten iholta ja limakalvoilta ja myös silmän pinnalta. Antimikrobiaalisen roolin lisäksi AMP:t toimivat tärkeinä molekyyleinä tulehduksessa, immuuniaktivaatiossa ja haavan paranemisessa ja syövässä.

Kokeellisessa osassa tutkittiin rotansuoliston tuottamia antibioottisia peptidejä. Tavoitteena oli kehittää HPLC-menetelmä, jolla pystyttäisiin eristämään ja tunnistamaan antibioottisia peptidejä rotansuolistonäytteistä, sekä saamaan peptidien tunnistus ja aktiivisuusmittaus toimimaan yhdessä. Proteiininäytteiden ongelmana on yleensä näytteiden sisältämien epäpuhtauksien määrä. Täten näytteiden puhdistaminen ja rikastaminen on välttämätöntä tunnistettaessa 3-6 kDa:n kokoisia peptidej ä massaspektometrialaitteistolla.

Uusien analyysimenetelmien myötä uusien antibioottisten peptidien löytäminen on mahdollista.

PROTEIINIEN FYSIKAALISET OLOMUODOT LÄÄKEVALMISTEISSA JA NIIDEN STABIILIUS

Leivonen Katri

Farmasian teknologian ja biofarmasian laitos, Farmaseuttinen tiedekunta, Kuopion yliopisto

Avainsanat: proteiini, amorfinen, kiteinen, apuaineet, kylmäkuivaus, sumukuivaus, jäännöskosteus, stabiilius, lääkevalmiste

Proteiinilääkkeiden määrä on kasvamassa sekä uusien käyttöindikaatioiden että valmistusmenetelmien myötä. Proteiinin työstäminen lääkevalmisteeksi on kuitenkin ongelmallista, sillä proteiinit ovat kooltaan suuria. Proteiinin aktiivinen rakenne on järjestäytynyt useassa tasossa lopulliseen kolmiulotteiseen muotoon. Muutokset missä tahansa rakennetasossa saattaa johtaa aktiivisuuden menetykseen. Proteiinit eivät nykytekniikalla imeydy ruoansulatuskanavasta, joten ne on annosteltava pääsääntöisesti parenteraalisesti.

Tutkielman kirjallisessa osassa on käsitelty proteiinin valmistukseen liittyviä seikkoja. Proteiinit ovat aktiivisia liuosmuodossa, mutta niiden hajoamiskinetiikka on tällöin nopeaa. Kiinteälle proteiinille on useita valmistusmenetelmiä, joista kylmäkuivaus on yleisimmin käytetty. Siinä formulaatio kuivataan alhaisessa lämpötilassa ja paineessa. Kylmäkuivatun valmisteen jäännöskosteus on hyvin matala, mutta kuivausprosessi on kallis ja hidas. Kuivauksen aikana proteiinit altistuvat sekä äärimäisille lämpötiloille että veden haihtumisesta johtuville rasitteille, jotka saattavat hajottaa proteiinin. Stabiloivilla apuaineilla voidaan vähentää edellä mainittuja muutoksia ja muodostaa proteiinin rakennetta suojaava amorfinen matriisi. Proteiinin kiteyttäminen on yleensä vaikea, johtuen proteiinin kolmiulotteisesta rakenteesta. Kiteinen muoto on yleensä amorfista muotoa stabiilimpi. Markkinoilla olevat lääkevalmisteet ovat lähinnä kuivattuja proteiinijauheita, jotka uudelleen liuotetaan käyttöä varten.

Tutkielman kokeellisessa osassa pyrittiin selvittämään optimaalista apuainepitoisuutta kylmäkuivattaville proteiineille. Formulaatioita oli 12 erilaista, joissa proteiinin osuus oli 100-25 % (m/m). Kuivauksesta johtuvia muutoksia tutkittiin termodynaamisesti ja erilaisin spektroskooppisin menetelmin vertaamalla näytettä käsittelemättömään proteiiniin. Lisäksi tutkittiin formulaatioiden kiteytymiskinetiikkaa. Käytetyillä apuaineilla havaittiin olevan proteiinin rakennetta stabiloiva vaikutus, mutta optimaalista pitoisuutta ei pystytty täysin määrittämään. Stabiloiva teho kasvoi sokeripitoisuuden noustessa, kunnes saavutti kynnysarvon ja tasoittui. Samankaltaisten apuaineiden, sukroosin ja trehaloosin, tehossa suojata proteiinin rakennetta oli eroja, joiden syytä ei vielä täysin tunneta.

MIKROEMULSIOT IHOLÄÄKINNÄSSÄ

Maaranto Helena K.

Farmaseuttinen tiedekunta, Kuopion yliopisto

Avainsanat: mikroemulsio, iholääkintä, imeytymisen ediste

Mikroemulsiot ovat termodynaamisesti stabiileja kolloidisia valmisteita, joilla on matala viskositeetti ja pieni palloskoko (<0,2μm). Ne koostuvat vedestä, öljystä ja surfaktanteista. Suuren surfantanttipitoisuuden vuoksi mikroemulsioiden faasien välinen rajapintajännitys on alhainen ja sen ansioista mikroemulsiot muodostuvat spontaanisti ilman ulkoista energiaa sekoitettaessa käytettäviä ainesosia oikeissa suhteissa. Iho on potentiaalinen annostelupaikka useille lääkeaineille, vaikka ihon sarveiskerros toimiikin tehokkaana esteenä vierasaineiden imeytymiselle. Mikroemulsioiden mahdollisuuksia iholääkintään on tutkittu sekä in vitro- että in vivo- kokeissa ja mikroemulsioiden on todettu parantavan lääkeaineiden ihonläpäisyä konventionaalisiin valmisteisiin verrattuna. Mikroemulsiot liuottavat hyvin sekä rasva- että vesiliukoisia lääkeaineita ja lääkeaineiden lisääntynyt liukoisuus onkin tärkeimpiä mekanismeja ihon läpäisyn parantamisessa. Mikroemulsion ainesosilla on myös suuri merkitys imeytymisen lisäämisessä, koska ainesosat, kuten surfaktantit, voivat itsessään toimia ihon läpäisyn edisteenä. Lääkeaineiden imeytymiseen vaikuttaa myös mikroemulsion rakenne, joka tekee mahdolliseksi lääkeaineiden nopean diffuusion mikroemulsioissa. Pienen pisarakoon vuoksi mikroemulsiot pääsevät hyvin kontaktiin ihon pinnan kanssa, jolloin lääkeaineen konsentraatio ihon pinnalla kasvaa ja näin imeytyminen paranee.

Mikroemulsiolla on monia etuja verrattuna konventionaalisiin valmisteisiin. Ne ovat mm. helppoja ja edullisia valmistaa sekä ne säilyvät hyvin. Ongelmana on kuitenkin monien mikroemulsion ainesosien sopimattomuus iholääkintään joko toksisuuden tai ihoärsyttävyyden takia. Myös mikroemulsion nestemäinen koostumus voi aiheuttaa vaikeuksia annosteluvaiheessa. Iholääkintään tarkoitetuille mikroemulsioformulaatioille on haettu runsaasti patentteja ja tulevaisuudessa mikroemulsiot tulevat varmasti olemaan myös kliinisessä käytössä yhtenä iholääkinnän annosmuotona. Formulointi- ja toksisuusongelmat on kuitenkin ratkaistava sitä ennen ja saatava enemmän tietoa in vivo- tutkimuksista ihmisillä.

SYKLODEKSTRIINIKOMPLEKSOITUMISTA TEHOSTAVAT MENETELMÄT

Näsi Elina P.

Farmaseuttinen tiedekunta, Kuopion yliopisto

Avainsanat: syklodekstriini, inkluusiokompleksi, kompleksoitumistehokkuus, siklosporiini, inhalaatiojauhe

Syklodekstriinit (CD) ovat rengasmaisia, katkaistua kartiota muistuttavia oligosakkarideja, jotka koostuvat kuudesta (α-CD), seitsemästä (β-CD) tai kahdeksasta (γ-CD) glukopyranoosiyksiköstä. Syklodekstriinimolekyylien ulkopinta on hydrofiilinen, joten useimmat Syklodekstriinit liukenevat hyvin veteen. Syklodekstriinien sisäpinta on lipofiilinen ja muodostaa onkalon, jonka sisään lipofiiliset lääkeainemolekyylit tai molekyylien osat voivat kompleksoitua. Syklodekstriinikompleksoinnin avulla voidaan vaikuttaa suotuisasti useisiin lääkeaineiden fysikaalisiin, kemiallisiin tai biologisiin ominaisuuksiin, kuten vesiliukoisuuteen, liukenemisnopeuteen, stabiiliuteen ja biologiseen hyötyosuuteen.

Lääkevalmisteissa tulisi olla mahdollisimman vähän syklodekstriiniä, koska ylimääräinen syklodekstriini muun muassa pienentää lääkeaineen biologista hyötyosuutta, heikentää säilytysaineiden tehoa ja kasvattaa valmisteen massaa. Syklodekstriinikompleksoituminen on usein tehotonta, jolloin pienen lääkeainemäärän kompleksointiin tarvitaan suuri määrä syklodekstriiniä. Kompleksoitumista voidaan tehostaa apuaineilla, jotka lisäävät lääkeaineen ominaisliukoisuutta, kohottavat kompleksin stabiiliusvakiota tai vaikuttavat molempiin samanaikaisesti. Lääkeaineiden ominaisliukoisuutta voidaan lisätä muun muassa liuoksen pH:ta säätämällä, keraliuottimia lisäämällä tai muodostamalla lääkeaineesta suoloja. Kompleksien stabiiliusvakioihin voidaan vaikuttaa muun muassa hydroksihappojen, aminohappojen, keraliuottimien ja vesiliukoisten polymeerien avulla.

Opinnäytetutkielman kokeellisessa osassa tutkittiin Syklodekstriinikompleksoinnin vaikutusta siklosporiinin vesiliukoisuuteen. Lisäksi selvitettiin kompleksoinnin vaikutusta siklosporiinin inhalaatio-ominaisuuksiin tutkimalla Taifun®-kuivajauheinhalaattoriin pakatun kompleksin jakautumista in vitro Andersenin kaskadi-impaktoriin. Siklosporiinin vesiliukoisuutta voitiin parantaa Syklodekstriinikompleksoinnin avulla. Syklodekstriinikompleksointi vaikutti suotuisasti myös siklosporiinin jakautumiseen kaskadi-impaktorin alempiin osiin. Siklosporiinin syklodekstriinikompleksin ongelmana oli kuitenkin heikko valuvuus, jonka vuoksi annostarkkuus oli huonoja inhalaattorista ulos tuleva siklosporiiniannos oli pieni.

BIOHAJOAVISTA POLYMEEREISTÄ VALMISTETUT KIRURGISET IMPLANTIT ERILAISTEN KUDOSVAURIOIDEN HOITOON

Oinonen Noora

Farmasian teknologian ja biofarmasian laitos, Farmaseuttinen tiedekunta, Kuopion yliopisto

Avainsanat: biohajova, implantointi, implantti, polymeeri

Implantoinnin historia ulottuu yli 2000 vuoden taakse. Luiden ja nivelten tukemiseen on käytetty ortopedisiä implantteja 1800-luvun loppupuolelta lähtien. Materiaalien ja lääketieteen kehittyessä implanteilla on saatu aikaan yhä parempia hoitotuloksia erilaisten kudosvaurioiden hoidossa. Implanttien valmistusmenetelmien kehittyminen on osaltaan kasvattanut niiden rakenteellisten ja fysikaalisten ominaisuuksien hallintaa.

Ortopedisissä sovelluksissa implanttien pääasiallinen tehtävä on liittää yhteen murtuma-ja leikkauspintoja, sekä tukea vauriokohtaa paranemisprosessin aikana. Myös keinonivelet luetaan ortopedisiksi implanteiksi. Biohajoavat polymeeri-implantit soveltuvat hyvin paikalliseen lääkeannosteluun, sillä ne vapauttavat kontrolloidusti lääkeainetta kohdekudokseen pitkiäkin aikoja. Kudosteknologisissa sovelluksissa implanteilla on lähinnä kasvavaa solukkoa tukeva rooli. Polymeerin hajotessa sen tilalle muodostuu halutunlaista kudosta.

Implantit ovat kosketuksissa elimistön eri kudosten kanssa yleensä pitkiä ajanjaksoja. Tämän vuoksi implantin kudosyhteensopivuus on selvitettävä aina ennen kliinistä käyttöä. Kudosyhteensopivuuteen vaikuttavat monet eri tekijät ja suurin osa niistä liittyy implantoitavaan valmisteeseen. Tutkijoiden tavoitteena on kehittää menetelmiä, joilla kudosvauriot hoidetaan potilaiden omilla elävillä soluilla ja niistä kasvatetuilla kudoksilla.

Tämän opinnäytetyön kokeellisessa osassa tutkittiin vapautumisväliaineena olevan puskuriliuoksen vaikutusta lääkeaineen vapautumiseen poly(£-kaprolaktoni)-kalvosta. Tutkimuksessa käytettyjä lääkeaineita olivat natriumsalisylaatti, timololi ja deksametasoni, joiden vapautumista tutkittiin kuudessa eri puskuriliuoksessa 8 viikon ajan. Saatujen tulosten perusteella voitaisiin olettaa, että käytetyillä kuudella puskuriliuoksella ei ole toisistaan poikkeavaa vaikutusta pienimolekyylisten lääkeaineiden vapautumiseen.

IHON EPIDERMIKSEN SOLUMALLIEN RAKENNEMORFOLOGIA JA LIPIDIKOOSTUMUS

Peura Ville

Farmasian teknologian ja biofarmasian laitos, Farmaseuttinen tiedekunta, Kuopion yliopisto

Avainsanat: epidermiksen solumallit, morfologia, lipidikoostumus, ROC, farmakokineettinen mallitus

Ihon epidermiksen solumalleja käytetään lääke-ja vierasaineiden testaamiseen koe-eläinten sijasta. Epidermiksen uloin kerros, sarveiskerros on tärkein ihoa ja sisäelimiä vierasaineilta suojaava kerros. Sarveiskerros estää tehokkaasti transdermaalisesti annosteltujen lääkeaineiden pääsyä verenkiertoon, jonka vuoksi ihonkaltainen sarveiskerros on tärkein solumalleilta vaadittava kriteeri. Erilaisten solumallien läpäisevyys-, ärsytys-ja rakenneominaisuuksia on tutkittuja niitä on altistettu useille lääke-ja vierasaineille.

Työn kirjallisessa osassa keskityttiin kuvaamaan muutamien erilaisten epidermismallien rakenteen morfologiaa, lipidikoostumusta sekä lipidien järjestyneisyyttä sarveiskerroksessa. Ihosolujen eli keratinosyyttien kasvattaminen neste-ilma -rajapinnassa tuottaa rakenteeltaan läheisesti ihoa muistuttavan sarveiskerroksen, kun taas nestepinnan alla kasvatettuihin viljelmiin ei muodostu sarveiskerrosta. Monet neste-ilma -rajapinnassa kasvatetut epidermismallit ovat samankaltaisia ihoon verrattuna morfologialtaan, mutta niiden sarveiskerrokset ovat useita kertoja läpäisevämpiä kuin ihossa. Epidermismallien sarveiskerroksen ja elävän epidermiksen paksuuksissa sekä solurakenteessa on havaittu poikkeamia mallien välillä sekä ihoon nähden. Ihossa havaittavia sarveiskerroksen kehittymiseen vaadittavia erilaistumistekijöitä esiintyy myös epidermismalleissa, mutta niissä on havaittu myös sairastuneelle iholle ominaisia erilaistumistekijöitä. Lisäksi lipidikoostumuksessa ja -järjestyksessä, jotka vaikuttavat lääkeaineiden imeytymiseen sarveiskerroksen läpi, on monissa malleissa merkittäviä eroja ihoon verrattuna.

Kokeellisessa osassa määritettiin kortikosteronin läpäisevyys rotan ihosoluista kehitetyssä epidermismallissa (ROC). Kokeissa käytettiin imeytymisen edistäjiä parantamaan lääkeaineen läpäisyä ja samalla tutkittiin yhdisteiden ärsytysvaikutusta ihosoluissa. Läpäisykokeiden tulosten perusteella rakennettiin farmakokinetiikkaa ennustava Stella-®malli, jolla pyrittiin jäljittelemään koeolosuhteita. Läpäisykokeissa ainoastaan natriumlauryylisulfaatti paransi lääkeaineen imeytymistä havaittavasti ja se aiheutti testatuista aineista toiseksi eniten soluärsytystä. Kokeissa lääkeaineen pitoisuus luovuttajatilassa laski noudattaen epälineaarista kinetiikkaa, mikä heikentää tulosten luotettavuutta. Farmakokineettinen malli jäljitteli koeolosuhteita tyydyttävästi lukuunottamatta luovuttajatilan epälineaarisuutta.

SOLUKALVOLÄPÄISEVYYDEN ENNUSTAMMEN IN VITRO MENETELMIN; PAMPA, RP-HPLC JA IAM-KROMATOGRAFIA

Rousu Katja M.

Farmaseuttinen tiedekunta, Kuopion yliopisto

Avainsanat: PAMPA, parallel artificial membrane permeability assay, permeaatio, in vitro menetelmät, solukalvo läpäisevyys, IAM, retentiotekijä, RP-HPLC

Solukalvoläpäisevyyden ennustaminen lääkekehityksen mahdollisimman varhaisessa vaiheessa säästää resursseja ja nopeuttaa jatkotutkimuksiin valittavien molekyylien seulontaa. Runsaasti käytetty Caco-2 solumallimenetelmä on kallis ja aikaa vievä menetelmä alkuvaiheen tutkimuksiin.

RP-HPLC - kromatografia ja IAM - kromatografia ovat menetelmiä, joilla molekyylit saadaan nopeasti järjestettyä retentiotekijän avulla lipofiilisyyden mukaiseen järjestykseen, mutta nämä menetelmät eivät anna tarkkoja permeaatiotuloksia. RP-HPLC menetelmän käyttö permeaation ennustamisessa on nykyään vähäistä. IAM- kromatografiassa permeaation ennustaminen perustuu molekyylin jakautumiseen fosfolipidillä päällystetyn stationäärifaasin ja vesipohjaisen liikkuvan faasin välille. Tämä menetelmä on edelleen käytössä. Viimeaikoina IAM- kromatografiassa ori stationäärifaasiin liitetty hOCTl-transportteria sisältävää membraania, jota käytetään molekyylien transportteriin sitoutumishalukkuuden tutkimiseen.

PAMPA - menetelmä ennustaa suoraan permeaationopeutta ja on nopea, helppo ja toistettava menetelmä. PAMPA - menetelmällä voidaan luoda ruuansulatuskanavaa muistuttavat olosuhteet; valitsemalla kokeissa käytettävät pH:t fysiologiselta alueelta ja käyttämällä kuoppakohtaista magneettisekoitusta rasva/vesifaasin rajapintaan muodostuvan sekoittumattoman vesikerroksen ohentamiseen. Menetelmässä permeaatio tapahtuu ruuansulatuskanavan membraania muistuttavan fosfolipidikalvon läpi.

Tutkielman kokeellisessa osassa PAMPA - menetelmä testattiin ja saatuja tuloksia verrattiin useiden laboratorioiden keskiarvotuloksiin. Testausvaiheessa saavutettiin riittävä korrelaatio saatujen tulosten ja vertailutulosten välillä, joten menetelmä voitiin hyväksyä käyttöön. Seuraavassa vaiheessa PAMPA- ja Caco-2- menetelmien välille löydettiin hyvä korrelaatio transsellulaarisesti permeoituvien molekyylien permeaatiotuloksia vertailtaessa. Lisäksi PAMPA - menetelmää käytettiin IVIVC-projektin molekyylien tutkimiseen. Menetelmällä tutkittiin myös niukkaliukoisia molekyylejä keraliuotinta käyttäen.

DIMETYLOITUNEIDEN INDOLIALKYYLIAMIINIEN KÄYTTÖ ALKUPERÄISKANSOJEN KESKUUDESSA JA NYKYINEN STATUS

Tukiainen Mikko T.

Farmaseuttinen Tiedekunta, Farmaseuttisen Kemian Laitos, Kuopion yliopisto

Jurema on alkuperäiskansojen käyttamä juoma, joka valmistetaan Mimosa hostilis -kasvin juurista. Kyseisen juoman käyttöä tavataan edelleen Brasilian koillisosissa. Kasvi sisältää mm. bioaktiivisia tryptamiineja, jotka aiheuttavat valmisteelle tyypillisiä keskushermostovaikutuksia. Juomaa käytetään edelleen alkuperäiskansojen seremonioissa lääkitsemis-/ uskonnolliseen tarkoitukseen. Valmisteen vaikutusmekanismi on edelleen selvittämattä, koska juuressa esiintyvä alkaloidi dimetyylitryptamiini (DMT) ei aktivoi keskushermostoa oraalisen annostelun jälkeen, sillä elimistön katabolinen entsyymi monoamiinioksidaasi (MAO) hajottaa DMT:n ennen sen imeytymistä maha-suolikanavasta verenkiertoon. DMT:n ja sitä rakenteellisesti läheisesti muistuttavien analogien fysiologinen rooli on edelleen selvittämättä, mutta sisäsyntyisinä niitä on havaittu sekä terveissä että psyykkisesti sairaissa ihmisissä, joten ne voidaan lukea osaksi serotoniinijarjestelmää joko välittajäaineina tai niiden metaboliatuotteina. On esitetty hypoteesejä, jotka ehdottavat niille roolia psyykkisten sairauksien patogeneesissa, mutta pitoisuuksien seuranta virtsasta on osoittanut, etta nämä pitoisuudet eivät ole suoraan verrannollisia yksilön henkiseen tasapainoon.

Kokeellinen osuus sisältää uuden menetelmän Jurema-juoman oletetun aktiivisen ainesosan eristämiseksi ja tunnistamiseksi. Eristäminen suoritettiin miedosti happamissa olosuhteissa uuden aineen stabiloimiseksi. Kyseinen alkaloidi eristettiin ja puhdistettiin korkeapainenestekromatografilla (HPLC) ja kemiallinen rakenne tunnistettiin massa-spektrometrillä (MS) ja NMR-spektroskopisilla menetelmillä (NMR).

Tutkimuksen tuloksena vahvistettiin, etta M. hostilis sisaltää uudentyyppisen alkaloidin, jota ei aikaisemmin ole raportoitu. Tämän uuden alkaloidin voidaan olettaa ratkaisevan Jureman vaikutusmekanismin tulevaisuudessa, kun selvitetään sen kyky estää mahdollisesti MAO:a in vivo.

PROLYYLIOLIGOPEPTIDAASI-INHIBIITTORIEN LIPOFIILISYYDEN MÄÄRITTÄMINEN

Yli-Kokko Anna Leena

Farmaseuttisen kemian laitos, farmaseuttinen tiedekunta, Kuopion yliopisto

Avainsanat: lipofiilisyys, jakautumiskerroin, prolyylioligopeptidaasi-inhibiittori, logP

Prolyylioligopeptidaasi -entsyymin (POP) inhibiittorit estävät aivoissa esiintyvien proliinipitoisten peptidien pilkkoutumista. Prolyylioligopeptidaasi pilkkoo useita peptidejä, muun muassa erilaisia muistiin ja oppimiseen vaikuttavia polypeptidejä, proliinin karboksyylipuolelta. On esitetty, että näiden peptidien pilkkoutumisen estolla voitaisiin kohottaa peptidien pitoisuutta aivoissa ja siten vaikuttaa positiivisesti sellaisiin sairauksiin ja oireisiin, jotka vaikuttavat muistia heikentävästi. Yhdisteiden lipofiilisyyksien määrityksellä pyritään selvittämään Prolyylioligopeptidaasi-inhibiittorien soveltuvuutta lääkeainekandidaateiksi. Lipofiilisyys on yksi tärkeimmistä lääkkeen fysikaalisista ominaisuuksista, sillä se vaikuttaa yhdisteen imeytymiseen, jakautumiseen, solukalvon läpäisemiseen sekä yhdisteen affiniteettiin kohdeproteiiniin sitoutumisessa.

Tässä työssä tavoitteena oli määrittää sarjalle aiemmin syntetisoituja prolyylioligopeptidaasi-inhibiittoreita niiden jakautumiskertoimet (P). Työssä käytettiin neljää erilaista menetelmää. Ensin yhdisteille määritettiin laskennallisen menetelmän avulla jakautumiskerroin virherajoineen. Näin saatiin suuntaa antava lukuarvo varsinaisten menetelmien suunnittelun tueksi. Tämän jälkeen tarkoituksena oli määrittää ionisoitumattomille, tutkittaville yhdisteille retentioaikaan perustuva jakautumiskerroin korkean erotuskyvyn nestekromatografian (HPLC) avulla. Tässä ei kuitenkaan onnistuttu, sillä tutkittujen yhdisteiden taipumus muodostaa vetysidoksia laitteiston stationäärifaasin kanssa vääristi tuloksia. Näiden yhdisteiden jakautumiskerroin määritettiin lopulta perinteisen, "Shake-flask" -menetelmän avulla. Ionisoituvien yhdisteiden lipofiilisyys määritettiin potentiometriseen titraukseen perustuvalla menetelmällä erityisen, juuri tähän tarkoitukseen suunnitellun laitteiston avulla.

Työssä onnistuttiin määrittämään yhdisteiden oktanoli-vesi —jakautumiskertoimet ($\log P_{o/w}$) erilaisia menetelmiä apuna käyttäen.

KIINTEIDEN GEENILÄÄKKEIDEN FORMULOINTI

Åhlman Anna

Farmaseuttinen tiedekunta, Kuopion yliopisto

Avainsanat: DNA/kantaja-kompleksi, stabiilius, kylmäkuivaus, apuaineet

Geeniterapia luo tulevaisuudessa uudenlaisen vaihtoehdon tautien hoitomenetelmäksi. Sen tarkoituksena on korjata taudin aiheuttaman proteiinin puute tai sen liian alhainen pitoisuus takaisin vaadittavalle tasolle. Geenin siirto vaatii toimiakseen kuljettimen, jolla taataan geenin pääsy kohdesolun tumaan. Geenin siirrossa on käytetty kahdenlaisia kuljettimia eli kantajia: virusvälitteisiä ja ei-virusvälitteisiä. Virusvälitteistä geenin siirtoa on tutkittu enemmän ja todettu tehokkaaksi, mutta se sisältää joitakin ongelmia valmistukseen ja turvallisuuteen liittyen. Ei-virusvälitteinen geenin siirto on sen sijaan turvallista, mutta in vivo tehokkuus on alhaisempaa.

Geenin siirto menetelmiä on kehitetty suhteellisen pitkälle, mutta ihmisille annosteltavan geenilääkkeen formulaation suunnittelu vaatii vielä paljon tutkimista. DNA/kantaja-kompleksien on todettu olevan kovin epästabiileja ja herkästi aggregoituvia liuosmuotoisessa formulaatiossa ja siten formulaation kehitys on suuntautunut kiinteisiin valmisteisiin. Kompleksien stabiiliutta on saatu parannettua jäädytyksellä, mutta jäädytetyn formulaation säilytys ja kuljetus vaativat erityisolosuhteet, jotka aiheuttavat paljon lisäkustannuksia. Sen sijaan kylmäkuivaus mahdollistaa valmisteen säilytyksen huoneenlämmössä ja nopean käyttövalmiuden. Kuivauksella on mahdollistettu stabiili kompleksiformulaatio, joka vaatii apuaineiden käyttöä. Lähinnä sokereilla on todettu olevan komplekseja kylmäkuivaukselta suojaava vaikutus ja kompleksien aggregoituminen on saatu estettyä. Apuaineiden vaikutusmekanismi, jolla ne säilyttävät kompleksien partikkelikoot entisellään kuivausprosessin aikana, ei ole vielä täysin selvitetty.

Kokeellisen osion tarkoituksena oli tutkia ei-virusvälitteisten DNA/kantaja-kompleksien stabiiliutta erilaisten apuaineiden (dekstraanit, trehaloosi, sakkaroosi, tweenit) avulla. Aluksi kompleksien partikkelikoot ja niiden aggregoitumisherkkyys määritettiin liuoksissa. Näistä parhaat formulaatiot kylmäkuivattiin ja liuotuksen jälkeen niiden koot määritettiin uudelleen. Tutkimuksessa löydettiin useita kompleksien aggregoitumista estävää apuaineformulaatiota. Erityisesti pinta-aktiiviset apuaineet Tweenit olivat lupaavia. Myös trehaloosia ja sakkaroosia sisältävät formulaatiot olivat varteenotettavia. Monet dekstraaniformulaatiot eivät kyenneet estämään kompleksien aggregoitumista. Kuitenkin pelkkien kokomittausten perusteella ei voida tehdä liian pitkälle meneviä johtopäätöksiä, vaan lisätutkimuksia kompleksiformulaation vaikutuksesta in vivo tehokkuuteen pitää tutkia.

KRYPTO

POLYMORFI				→	↴	■	■	■	■	■	■	■	■	■	■	■	■	■	■
				■	1	16	14	16	3	13	4	16	6	12	19	8	■	5	14
				■	12	6	13	19	13	4	8	4	■	3	8	5	■	16	2
				■	19	16	4	2	17	12	15	13	■	16	21	16	■	14	12
				■	18	19	8	3	16	15	16	4	■	7	■	4	13	16	17
■	↓	■	■	■	6	■	15	11	■	12	■	■	16	16	4	■	2	3	16
■	11	16	8	■	12	1	16	16	3	■	17	2	19	12	■	16	■	■	3
■	16	19	3	■	14	18	4	6	8	4	16	7	2	4	■	17	12	11	12
■	19	16	8	10	10	8	■	13	■	13	5	12	4	■	2	4	14	8	12
■	2	■	■	■	8	→	19	13	11	4	8	↴	■	1	3	8	8	■	21
■	4	16	3	16	■	6	13	4	16	15	12	19	8	■	16	8	4	16	16
■	4	16	16	19	16	■	19	■	15	16	4	8	5	13	■	5	■	1	■
■	2	14	16	19	■	17	2	14	16	■	■	1	12	■	21	8	16	15	16
■	16	8	14	8	■	18	■	20	■	11	■	8	14	■	16	11	19	13	15
■	↓	16	■	■	7	■	5	20	14	20	11	21	18	3	16	8	17	16	■
■	13	■	13	2	2	17	16	4	12	■	8	8	■	6	■	8	13	■	15
■	2	14	16	■	12	14	16	■	3	5	■	16	5	16	8	13	13	5	16
■	17	2	2	■	6	12	15	8	17	16	15	3	16	13	19	8	15	13	15
■	13	3	16	■	16	6	■	1	■	1	8	8	4	■	4	■	8	3	12
■	6	8	15	8	6	8	■	■	17	16	14	16	■	11	16	19	4	8	■
■	8	■	4	13	13	■	3	8	16	■	■	3	■	8	5	12	■	■	3
■	3	■	16	16	14	15	13	■	11	16	8	4	■	6	2	4	16	19	16
■	4	16	8	■	17	12	14	4	4	8	11	16	8	■	12	11	14	16	4
■	12	■	15	■	17	16	15	■	8	14	16	■	13	■	14	16	3	5	16
■	16	3	16	■	8	11	16	19	16	8	15	13	15	■	12	14	8	12	15

Kryptokirjaimet:

1	2	3	4	5	6
7	8	9	10	11	12
13	14	15	16	17	18
19	20	21			

Fysikaalisen farmasian XVIII symposium
OSALLISTUJAT

Mika Pulkkinen	Kuopion yliopisto
Sanni Matero	Kuopion yliopisto
Pekka Hoppu	Helsingin yliopisto
Teemu Heikkilä	Turun yliopisto
Marko Kuosmanen	Kuopion yliopisto
Tanja Lipsanen	Orion Pharma
Bert van Veen	Orion Pharma
Samuli Hirsjärvi	Helsingin yliopisto
Marja Savolainen	Helsingin yliopisto
Anna-Maija Lampi	Helsingin yliopisto
Reijo Käkelä	Helsingin yliopisto
Susanne Wiedmer	Helsingin yliopisto
Marjo Yliperttula	Helsingin yliopisto
Julia Lehtinen	Helsingin yliopisto
Zanna Hyvönen	Kuopion yliopisto
Vesa-Pekka Lehto	Turun yliopisto
Jarno Salonen	Turun yliopisto
Mikko Tenho	Turun yliopisto
Joakim Riikonen	Turun yliopisto
Olli-Pekka Hämäläi-nen	Turun yliopisto
Elina Näsi	Kuopion yliopisto
Kristiina Järvinen	Kuopion yliopisto
Juha Mönkäre	Kuopion yliopisto
Jussi Tervonen	Kuopion yliopisto
Sini Eskola	Suomen Proviisoriyhdistys ry
Johanna Kuokkanen	Kuopion yliopisto
Niina Kivikero	Helsingin yliopisto
Karin Kogermann	Helsingin yliopisto
Jouni Hirvonen	Helsingin yliopisto
Timo Korjamo	Kuopion yliopisto
Aki Heikkinen	Kuopion yliopisto
Pirjo Räsänen	Orion Pharma
Saara Mahlamäki	Orion Pharma
Tinna Lücke	Orion Pharma

POLYMORFI
PARASETAMOLI VR
OMELETIT SIV AU
LATUKONE ADA RO
YLISANAT J TEAK
M NH O AAT USA
HAI OPAAS KULO A S
ALS RYTMI TAJUT KOHO
LAIFFI E EVOT UTRIO
U I LEHTI PSII D
TASA METANOLI AITAA
TAALA L NATIVE V P
URAL KURA PO DIANA
AIRI Y Ä H I R AHLEN
A J VÄRÄHDYSAIKA
L LUUKATO I I M I E N
URA ORA CV AVAILEVA
KUU MONIKANSALLINEN
ESA AM P PIIT T I SO
MINI MI KARA HALTI
I TEE CIA S IVO S
S AARNE HAIT MUTALA
TAI KORTTIHAI OHRAT
O N KAN I RA E RASVA
ASA IHALAINEN ORION