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RESEARCH ARTICLE

Conference of MSc Students Abstracts of the best contributions

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Application of melt electrospinning in pharmaceutical technology

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There is a continuously increasing demand for application of continouos processes in the pharmaceutical industry. Electrospinning is a promising technology to create fibrous drug delivery systems with controlled dissolution of the active pharmaceutical ingredient. Fibers at submicron diameters can be formed both from polimer solutions and melts under the drawing force of electrostatic field.

Electrospinning of nanofibers from drug-containing polymer solutions is a technique capable of enhancing the dissolution rate of poorly water soluble drugs due to the formation of an amorphous solid solution and the huge surface area produced.

Melt electrospinning has several advantages over solution electrospinning. There is no need to dissolve the fiber forming polymer, there is no need for expensive solvent recovery, there is no risk of solvent explosion, there is no residual solvent in the fibers and a quantitative yield is achievable. Another important technological advantage of melt electrospinning is that it can be coupled with melt extrusion. To my knowledge, no paper has yet been published which deals with drug loaded melt electrospun fibers.

The aim of my work was to investigate the applicability of melt electrospinning to improve the dissolution of a model drug with poor water solubility (carvedilol) by preparing fast dissolving formulations containing different polymers and comparing them to solution electrospun and melt extruded formulations. I have designed and built a melt electrospinning equipment for laboratory use. The melt electrospinning equipment has two temperature-controlled zones to minimize degradation by heat and a programmable feeder both for melts and solutions, the throghput can be accurately controlled from low mass flows.

Based on the obtained results from XRD, FTIR and DSC, carvedilol was tranformed to fully to amorphous form and no detectable decomposition occured in the samples. The dissolution profiles showed that melt-electrospun samples had significantly faster drug dissolution rate than the pure crystalline carvedilol, and in the case of melt-electrospun samples the initial rate of release was faster than the solvent-based electrospun sample with a large specific surface area.

Determination of vitamin D3 in smoked bacon using liquid chromatography–tandem mass spectrometry

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It has been shown in the past decade, that vitamin D not only preserves the healthy bone system, but also plays an important role in the immune system and it's deficiency can cause cardivascular diseases and certain types of tumor. Although vitamin D is primarily produced endogenously when ultraviolet rays from sunlight strike the skin, it is important to know the amount of vitamin D in food, because it can be an alternative vitamin D source. It is probable, that in animal tissues the quantity of vitamin D depends on to what extent was the animal exposed to sunlight.

The goal of our research was to find out whether the smoked bacon contains any vitamin D, furthermore whether the bacon of a swine that was raised outdoors contains more vitamin D, then that of a swine raised in a pen, or not. For this purpose, we have developed an analytical method to measure vitamin D3 in fatty

smoked bacon. The method was based on high performance liquid chromatograpic-tandem mass spectrometry (HPLC-MS-MS) measurement, because the vitamin D concentration is very low in tissues.

A sample preparation method, based on saponification and liquid-liquid extraction with heaxne has been developed which was more simple than official method of the relevant Hungarian Standard and used smaller sample volumes. Vitamin D2 was used as an internal standard. Pretreated samples were separated by reversed phase chromatography on a core-shell C18 column and detected by positive atmospheric pressure chemical ionization tandem mass spectrometry.

The method was validated and was proven to be linear through 3 decades, the detection limit and the quantitation limit being 5 and 10 ppb, respectively. The recovery of vitamin D spiked water samples was around 90%, however from spiked, smoked bacon samples it was only around 34%.

The vitamin D content of bacon samples originating from swines that were kept outdoors and in closed pens were measured and compared. The smoked bacon of a swine, that was kept in closed pens contained 833 ng/g vitamin D3, which makes it a rich source of vitamin D. The other sample did not contain any measurable quantity of vitamin D3, probably because it was deteriorated during a two year storage.

Enzymatic hydrolysis of agricultural by-products and kinetic model of the process

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A remarkable advantage of the second-generation bioethanol-processing is that it uses lignocellulosic biomass, such as wood, grass, corn stover or wheat straw instead of food crops. Lignocellulose consists of cellulose, hemicellulose and lignin, from which the cellulose is suitable for bioethanol production. There are four main consecutive steps in the process: pretreatment, hydrolysis, fermentation and ethanol recovery. The enzymatic hydrolysis of cellulose to glucose by cellulases is one of the major steps in the conversion of lignocellulosic biomass. This is a heterogeneous process, and in order to make it more effective, it is inevitable to optimise the reaction conditions. Kinetic modelling of enzymatic saccharification can be helpful in the design of processes for sugar production. It can be used to reduce the number of experiments and it is a useful predicting tool as well. In the recent years several studies on the kinetics of the enzymatic hydrolysis of cellulose have been published, but the exact mechanism of cellulases on lignocellulosic biomass is not understood yet, or the models have some limitations. They need to be tested with further experiments, so they can be improved.

The aim of my experimental work is to develop a kinetic model for enzymatic saccharification based on previous and own experimental data. The hydrolysis experiments were carried out at different substrate (alkali-extruded wheat straw) concentrations and enzyme dosages (Cellulosic Ethanol Enzyme Kit - Cellulase complex NS22086) at constant temperature (50 ˚C) and stirring (150 rpm) for 72 hours in 0.05 M acetate buffer solution (pH 5). After centrifuging the samples (10 min, 13000 rpm), the reducing sugar content of the supernatant was measured using 3,5-dinitrosalicylic acid (DNS). The concentrations of the glucose produced were quantified by high-performance liquid chromatography. These values were used for the kinetic modelling, where the independent variable is the hydrolysis time, the dependent variable is the glucose concentration, while the substrate concentration and the enzyme dosage are the inputs of the model. The three constants were regressed using Berkeley Madonna and Matlab 7.1.

The model was applied for previous experimental data on corn stover as well. The adequacy of fit was assessed with the R^2-values which were compared with the values of a previous model in the literature (Zhang et al., Bioresource Technology, 2010, 101, 8261-8266). In the case of corn stover the new model has a higher accuracy than the examined one. The model performed well on the description of the enzymatic saccharification, thus it is able to predict the glucose concentration, and it can contribute to the development of the process. However, with further work it can be improved and expanded.

Quantification of real cross links in polyaspartic acid gels

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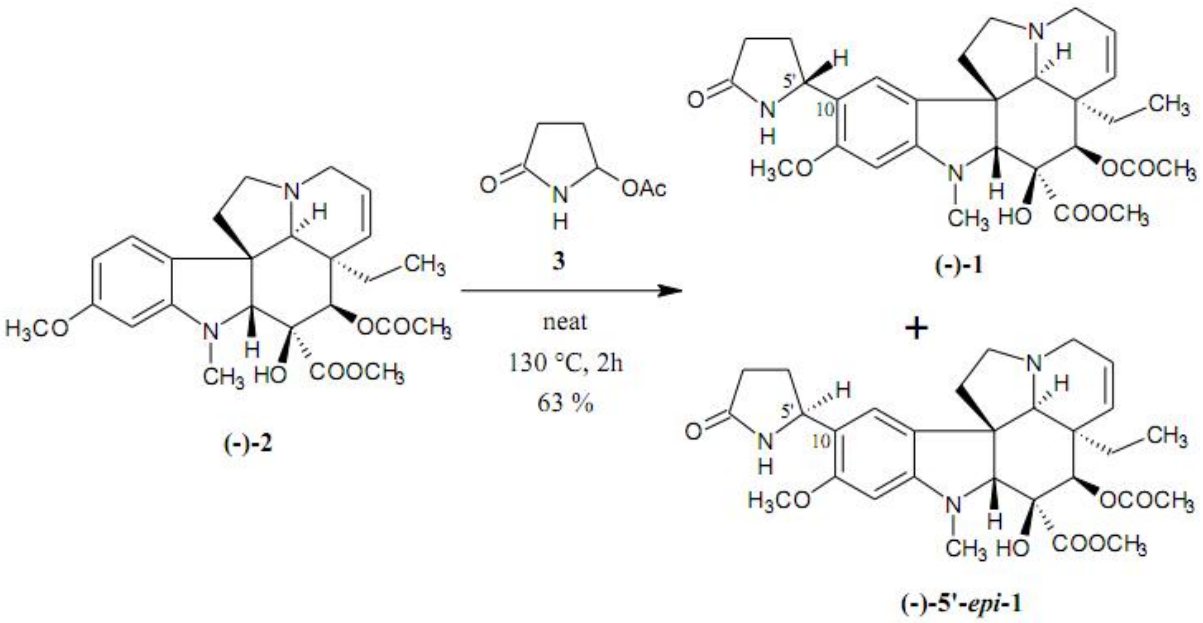
In recent years there has been increased focus on polymer based hydrogels. Biocompatible and biodegradable polymer networks can be prepared that change the physical and chemical properties – usually their volume - in a given response to the altering of environmental parameters e.g. temperature or pH. The properties of the polymer gels are determined by the chemical composition and the molecular weight of the polymer backbone, as well as its physical and chemical interactions between the

Fig. 1.



chains. The number of physical net points is statistically constant but the segmental mobility of polymers causes continuous moving of physical net points. In contrast to the physical net points, the covalently bonded cross linking points are immobile.

In my present work I developed a new analytical method to quantify the chemical net points of polyaspartic acid hydrogels cross-linked with diamines [1]. The main goal of my present work was to determine the number of cross-linking agents which does not react or just one of its functional groups reacts during the gelation process. 2,4,6-trinitrobenzenesulfonic acid (TNBS) reagent was used. The TNBS reacts with primary amine groups selectively under mild alkaline conditions. This SN_2Ar type reaction gives a yellow product which is measurable by UV-VIS spectroscopy. Beside the main reaction, TNBS hydrolyses to picric acid which has absorption maxima at the same wavelengths as trinitrophenilated amine. The hydrolysis of TNBS is slow and does not interfere with the determination of amines in case of small molecules [2].

The number of unreacted cross-linking agents was measured directly in trinitrophenilated form, after they had been washed out of the gel. The non-reacted, pendant amine groups inside the gel were measured by indirect method. The conventional method was improved by optimizing the composition of reaction medium. Formation of by-product could be excluded thus the new method is applicable to determine amines of low reactivity, even in heterogeneous reaction e.g. on solid surfaces.

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The first synthesis of (-)-bannucine and (-)-5'-epibannucine. A simple method for C-C bond formation.

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Within the Research Group for Alkaloid Chemistry operating at the Department of Organic Chemistry, BME (present name: Department of Organic Chemistry and Technology), reseach in the field of synthesizing Aspidosperma alkaloids and related compounds has been conducted for decades. The topic of my experimental work is closely connected to this area: the elaboration of the first synthesis of the alkaloid bannucine **((-)-1)**. This natural compound is derived from the alkaloid vindoline having a 2-pyrrolidone moiety attached at position C-10. During our work we have accomplished the simple, one-step synthesis of the aforementioned alkaloid starting from (-)-vindoline **((-)-2)**. We applied a reagent containing a hemiamidal functionality **(3)**, from which the in situ generated cyclic *N*-acyliminium species served as an electrophile in an aromatic substitution reaction with vindoline, giving the target compound **(-)-1** and its 5'-epimer **((-)-5'-epi-1)** in good yield (Fig. 1).

Deformation mechanism and impact properties of three component PP/wood/elastomer composites

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Fillers are often used to reduce price of commodity polymer or to improve mechanical properties. Mineral fillers are still used for a wide range of applications, but they are replaced by natural reinforcements (like wood flour) more and more frequently. The addition of filler influences the mechanical properties of the composite. The stiffness is increased almost in every case but impact resistance is usually reduced which is important property of structural materials. This latter effect can be compensated by the addition of elastomers to the system. Former studies proved that these three component systems can have different structures. On one hand the elastomer can encapsulate the filler resulting in embedded structure. On the other the elastomer and the filler can form separate dispersion in the matrix. Not only the developing structure but also the deformation mechanisms influence the macroscopic properties of composites. Thus the aim of this study was to find correlation between the structure of the composites, the deformation processes and the properties.

Composites were prepared from several wood fibers and elastomers in polypropylene by extrusion and injection molding. Tensile and impact tests were used to characterize composites properties and deformation processes were identified with the help of acoustic emission. The structure of the composites was proved with scanning electron microscopy.

The results showed that structure of the composites can be tailored with the use of appropriate elastomers. The application of functionalized elastomer (MAEPDM) led to partially embedded structure while non-functionalized elastomer (EPR) and coupling agent (MAPP) created separate dispersion. In spite of the different structure, the impact properties of these composites were nearly the same. The acoustic emission measurements showed that the deformation mechanism changes when the rate of the deformation is increased. At higher deformation rate, the dominant micromechanical deformation mechanism turned into fiber fracture which led to fast catastrophic failure and low impact resistance irrespectively from the characteristics of the used natural reinforcement.

Development of a decision support system for molecular targeted personalized therapy of cancer patients

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In recent years it has become evident, that cancer is a genetic disease caused by somatic and germline mutations of approximately 460 genes. This offers new possibilities for oncology diagnostics and for the development of anti-cancer drugs. The goal is to find the molecular target which is mutated in the individual tumor, and correspondingly inhibit the unregulated signal transduction processes in the cell with targeted drugs. Drug sensitivity or resistance largely depends on the mutation site within the gene, which can be accurately identified with next generation sequencing techniques. Due to the early success of targeted therapy, the molecular datasets and number of clinical trials with new molecules have rapidly increased in the last decade, and now there are more than 400 drugs in clinical development. The number of mutations of cancer genes in the Catalogue of Somatic Mutations in Cancer (COSMIC) reached 540.000 today [1].

Our aim is to develop a decision support system for the oncologists, which predict the efficiency of targeted drugs based on the mutations found in the patient's tumor. The decision support system's core is the TARGETED DRUG SENSITIVITY DATABASE^{TM},which examines the effect of somatic mutations on drug sensitivity according to scientific literature. The database contains drug sensitivity data from mutant cell lines or enzymes compared to their wild type form. The constructed database is innovative, since there are no similar databases [2]. Based on the TARGETED DRUG SENSITIVITY DATABASE^{TM} and clinical evidences the TARGET-DRUG ASSOCIATION ANALYZER^{TM} decision-making system analyzes the biological relevance of the biomarker pattern found by the molecular diagnosis and identifies the positive or negative association with the drug efficacy. The decision support system contains over 17 proto-oncogenes as well as 1 tumor suppressor gene and 20 registered targeted drugs.

Future plans include the expansion of the database to a larger number of oncogenes and tumor suppressors, and drug sensitivity prediction with bioinformatics tools (protein structure mod-

eling and drug docking) where no experimental or clinical data are available.

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Modification of the responsive behaviour of poly(aspartic acid)

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Development of „controlled-release and targeted delivery" systems is a current key issue in modern pharmaceutical research. The main benefit of these drug delivery vehicles is the reduction of side effects by controlling drug concentration at effective levels over a desired period of time [1]. pH-sensitive pharmaceutical films based on acrylate copolymers (Eudragit®) developed by Evonik are the most important products in this field. The physicochemical properties of these copolymers can be controlled within wide limits by the composition of the films. These products offer a wide range of possible drug release profiles and provide good performance in enteric, protective and sustained release [2]. The general disadvantage of Eudragit® polymer films is the lack of biodegradability. This drawback can be overcome by the application of biodegradable and biocompatible poly(aspartic acid) (PASP) [1]. Its water solubility and the related physicochemical properties are strongly dependent of environmental pH due to its polyelectrolyte character. Consequently it can serve as a suitable alternative of acrylate based polymers.

The aim of my work was to design and synthesize PASP polymers with adjustable pH and temperature sensitive properties. Various mono- and diamines were used to modify polysuccinimide (PSI) in nucleophilic ring-opening reactions. The prepared PSI polymers were subsequently hydrolyzed to the corresponding PASP derivatives. Structure of the polymers and degree of modification were determined by ^{1}H NMR.

Modification of poly(aspartic acid) with monoamines resulted in polymers with increased hydrophobicity and a shift in the release profile was observed. pH-dependent solubility could be controlled in a programmable manner by changing the degree of modification and the length of hydrophobic side chains. pH-dependence of solubility could be inverted by introducing tertiary amines into the side chains of the polymer. The hydrophobic-hydrophilic balance thus the solubility of the modified polymers could be adjusted by the molar ratio and type of the applied mono- and diamines. A significant temperature responsive feature could be rendered to poly(aspartic acid) by the total aminolysis of the polymer by a certain combination of low molecular weight diamines. Inverse temperature-dependent solubility was confirmed by optical measurements as well as by differential scanning calorimetry. Phase transition temperature could be shifted to a pre-determined value by varying the modifying molecules.

It can be concluded that pH- and temperature-sensitive PASP polymers were synthesized by the alkaline hydrolysis of amine modified PSI polymers. Responsive properties of PASP derivates are proved to be programmable by the composition of polymers. These results suggested that the proposed polymers could be developed into controlled and targeted drug delivery devices.

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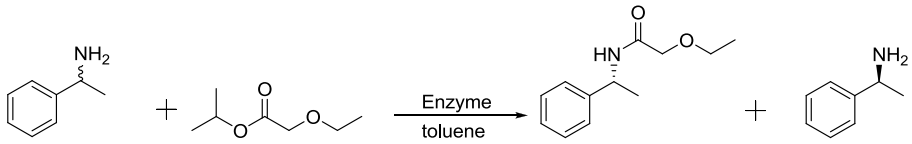
Fig. 2.



Investigation of the lipase activity from *Pseudozyma aphidis* in the enzymatic kinetic resolution of racemic amines

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The aim of my work was investigation of lipase activity of *Pseudozyma aphidis* (closely related to *Pseudozyma antarctica*=*Candida antarctica*) in the kinetic resolution of racemic 1-phenylethanamine. The lipases were adsorbed directly from various fermentation media onto in-house made surface functionalized silica gel supports. The resulting immobilized enzyme preparations were compared in enantioselective batch mode acylation reactions. Our perspective goal is perform such reactions in continuous-flow bioreactors as well.

1-Phenylethanamine is the simplest aromatic, chiral amine thereby it is optimal model substrate for enantioselective reactions. First, the efficiency of acyl donors were tested in the desired kinetic resolution with lipase B from *Candida antarctica* (CaLB). Ethyl and isopropyl acetates were commercially available, wheras the ethyl and isopropyl ethoxyacetates were synthesized from ethoxyacetic acid. These four esters were compared as acyl donors in the kinetic resolution of three racemic amines (1-phenylethanamine, 4phenylbutyl-2-amine, 1,2,3,4-tetrahydronaphthalen-1-amine) with differently immobilized forms of *Candida antarctica* lipase B. Because the isopropyl etoxyacetate proved to be the most active and selective acyl donor in the test reactions with CaLB (~20-fold activity enhancement compared to that with ethyl acetate), this acyl donor was used to investigate the lipase activity in various fermentations of *Pseudozyma aphidis* (Fig. 2).

Synthesis and characterization of poly(aspartic acid)/-poly(*N*)isopropylacrylamide conetwork hydrogels

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Hydrogels have been studied extensively because of their unique bulk and surface properties, as well as their responsiveness to external stimuli. Hydrogels play an important role in various biomedical applications, especially in the development of controlled drug delivery systems. Besides the stimuli responsive attribute, biocompatibility and biodegradability of the controlled drug delivery system is also important. Drug delivery systems aim to be compatible with the drug and control the rate of its release at the targeted biological disease site with little or no damage to the surrounding tissues [1]. Polymer gels based on polyaspartic acids [PASP] may fulfil these requirements. Furthermore PASP polymer containing pendant carboxylic groups thus it possesses sensitivity to the environmental pH. Poly(*N*-isopropylacrylamide) [PNIPAAm] is a well-known thermoresponsive polymer. In water PNIPAAm gel undergoes a volume phase transition at around 34 ˚C, close to the temperature of human body. PNIPAAm based cross-linked hydrogels have been widely applied to controlled drug delivery and biomedical materials. However, non-biodegradability of PNIPAAm may restrict the application in the biomedical field [2].

With a combination of PASP and PNIPAAm in one gel network we could get a multiresponsive conetwork gel with strong pH and temperature sensitivity as well as with biodegradability.

The use of aqueous medium in the preparation of a drug delivery matrix is important in view of biological application. We synthesized water soluble polysuccinimide (PSI) functionalised with 2-hydroxiethyl pendant groups. PSI was also modified with allylamine, these groups enabled the formation of PSI/-PNIPAAm conetwork hydrogels by using NIPAAm in a radical polymerisation reaction. The pH- and thermoresponsive PASP-/-PNIPAAm conetwork hydrogel was obtained after the hydrolysis of PSI to PASP.

In view of potential applications, the optimal hydrogel has to be able to give a considerable response in swelling degree to both pH and temperature change, and to have excellent mechanical properties. The main synthesis parameters (modification ratio by allylamine and ethanolamine to succinimide repeating units, the mass ratio of PASP to NIPAAm, overall concentration of polymers) were systematically changed during the optimization process in order to find the gel with optimal properties.

The swelling behaviour of conetwork hydrogels as a function of pH and temperature was investigated in detail. The PASP*l*PNIPAAm hydrogel showed non-linear volume change at a well-defined pH and temperature value. The structure of the multiresponsive hydrogel was studied by Fourier transformation infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR). The morphology was investigated by scanning electron microscopy (SEM). Using DiclofenacNa as a model drug in vitro drug release experiments were carried out and the release profiles were characterized. In conclusion the temperature and pH responsive PASP-*l*-PNIPAAm conetwork hydrogels could be exploited in controlled drug delivery.

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Study on the active constituents of saw palmetto in different extracts

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Saw palmetto (*Serenoa repens*, Bartram) is a native palm in the U.S. It is used especially in the treatment of benign prostatic hyperplasia, but it is also useful in other men's and women's health problems. It has become more popular, because of its safe application and its large amount in North America.

The extraction of saw palmetto can be carried out either with traditional solvents or supercritical fluid extraction. The ripe, dried berries are the source of the extract. I used n-pentane and 96% ethanol as solvents for the traditional Soxhlet-extraction and carbon dioxide for the supercritical fluid extraction (SFE).

The aims of my work were to analyze the fatty acid, the phytosterol and the carotenoid compounds of different extracts and to determine the external mass-transfer coefficient by modeling of SFE. Fatty acid and phytosterol composition were examined using GC. 25 types of fatty acids were detected and there were no qualitative differences among the extracts. Applying TLC, it is possible to separate the free phytosterols and methyl sterols, and they can be studied as pure fractions. Campesterol, stigmasterol, β-sitosterol and cycloartenol were the major constituents. One fatty acid (myristic acid) and one phytosterol (Δ5-avenasterol) were found in the extracts which are not mentioned in the literature.

The carotenoid content was measured by spectrophotometric method. The concentration of carotenoid is proportional to the absorbance at 450 nm. The extract obtained with ethanol solvent contains the highest amount of carotenoids.

The external mass-transfer coefficient was determined using the Sovova's model of supercritical fluid extraction [1]. It can be calculated with Sherwood-number. There are different equations for Sherwood-numbers in the literature, but they are established for special plants, therefore we can't utilize them universally. My aim was to select that Sherwood-equation which gives the best estimation for my system. The best correlation was published by Mongkholkhajornsilp et al. [2].

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Developing New Pharmaceutical Technologies to Formulate and Stabilize Solid Probiotics

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Nowadays biodrugs have a spectacular growing on the pharmaceutical market, thanks to the fast improving biotechnology

and the therapeutic advantages of them. These changes mean new challenges for the pharmaceutical scientists.

During the researches a probiotic bacteria (*Lactobacillus acidophilus*), which are prominent members of biodrugs, were investigated.

In current industrial practice the most commonly used drying technique is freeze drying, but it has many drawbacks. Besides the significant decreasing of activity, it is very time and energy consumptive and has a very long cleaning period due to the chance of contamination. In this work, I would like to present gentle technologies, which could be effective alternatives against freeze drying.

One of the examined technologies was the film coating. It's a commonly used pharmaceutical method for coating tablets. Using this process, we can produce bacteria-containing tablets in one step, so the damage caused by mechanic stress can be avoided.

The other applied technology was electrospinning that has been primarily developed in the textile/filtration industry and is a promising gentle and effective continuous way to formulate bacteria. Further advantage is, that the electrospinning can be scaled up easily, which is very important for industrial application.

During the research various stabilizers (trehalose, sucrose, skim milk) were added to protect the bacterias during the drying method and to provide long term stability for them.

A Membrane Protein in the Nucleus

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Prion and Shadoo proteins, which belong to the prion protein super family, are membrane proteins. They possess a secretion signal on their N-terminus that directs them to the secretory pathway. On their C-terminus they have a signal sequence for glycosylphosphatidylinositol (GPI) anchor attachment that tethers them to the outer leaflet of the cell membrane. While studying the subcellular localisation of these proteins with the use of fluorescent protein tags we found the Prion protein in the cell membrane of mammalian cells but, surprisingly, the Shadoo protein showed nuclear localisation in a fraction of cells in addition to the expected membrane localisation. My aim was to explore the reason for this duality.

I examined the localisation of several fusion fluorescent protein constructs in mammalian cells to reveal which part of the Shadoo protein is responsible for the unexpected localisation. There are particular segments in both proteins which are capable to direct them to the nucleus from the cytosol but the question remains: How can a secretory protein get out of the secretory pathway to the cytosol?

My results suggest that the different nature of the GPI anchor signals of the two proteins provide an explanation for their altered nuclear localisation: the presence of the Prion protein GPI signal decreases the cytosolic, and thus, the nuclear localisation of proteins which it was attached to. In contrast, the presence of the Shadoo protein GPI signal on the same protein results in a more intense nuclear localisation. Furthermore, our results suggest that there may be a pathway for proteins to transit from the secretory pathway to the cytosol. This may be of great importance concerning proteins which are involved in signal transduction or which have functions in both compartments (in the plasma membrane and the nucleus). Our results call for further examinations to reveal the exact mechanism of this transition.