The 14th Annual Symposium of Physical Pharmacy
Macromolecules and Particle Engineering

22.-23.1. 2003 Kuopio, Finland

Plenary Lectures
Posters

Society of Physical Pharmacy

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Program and abstracts

Editor:
Hanna Kortejärvi

Society of Physical Pharmacy

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PROGRAM

Wednesday

9:00		*Registration and Coffee (Posters must be placed by 9:30)*
10:00	Ossi Korhonen	Opening
10:05	Tomi Järvinen	Introduction of Mediteknia
10:30	Robert Forbes	Approaches towards perfecting protein particles
11:30		*Lunch and Poster presentation*
13:00	Heike Bunjes	Physicochemical aspects of lipid nanoparticles
13:45		Poster presentators
14:15		*Coffee*
14:45	Eero Suihko	Effect of water on lysozyme – sugar interactions
15:05	Leena Peltonen	Polymeric nanoparticles
15:25	Mikko Koivisto	Applications of isothermal microcalorimetry from the pharmaceutical point of view
15:45	Bert van Veen	The mutual effect of initial particle size and addition of another compound on tablet strength
16:05		Conclusion
16:10		ANNUAL MEETING OF THE SOCIETY OF PHYSICAL PHARMACY, FINLAND (members only!)
19:00		Poster award
19:10		*Buffet*

The 14th Annual Symposium of Physical Pharmacy Macromolecules and Particle Engineering 22-23.1.2003, Kuopio, Finland

Thursday

9:00	Arto Urtti	Gene delivery: challenge for physical pharmacy and biopharmaceutics
9:45	Marika Ruponen	The role of exogenous and cell surface glycosaminoglycans in non-viral gene delivery
10:05		*Break*
10:20	Michael Pikal	Challenges of protein formulation
11:05		*Lunch*
12:00	Anne Juppo	Microparticles in pharmaceutical products
12:45	Marja Savolainen	Controlled-release felodipine microparticles
13:05	Zanna Hyvönen	Novel cationic amphiphilic 1,4-dihydropyridine derivatives for DNA delivery
13:25		Conclusion
13:30		*Coffee*

LECTURES

The 14th Annual Symposium of Physical Pharmacy Macromolecules and Particle Engineering, 22.-23.1. 2003, Mediteknia, Kuopio

APPROACHES TO PRODUCING PERFECT PROTEIN PARTICLES

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Maintaining the chemical and physical stability of a protein therapeutic agent before processing, during processing and during shelf-life storage are major concerns in its formulation development. Whilst an aqueous liquid formulation intrinsically seems advantageous to maintain a protein in its native conformation, chemical instability often dictates that the protein must be dried to obtain a shelf-stable product. Formulation of biopharmaceuticals requires a detailed understanding of how the production process influences the properties of the bioactive component, and how biomolecule/excipient interactions influence biopolmyer stability and condition the solid-state properties of the formulation. For example, the design of particles containing proteins for inhalation must take into account the need to maintain higher order structural stability in addition to the conventional requirements of controlling inter-particle interactions and the optimisation of production/handling properties, transport/mechanical robustness, and physical/chemical stability. This lecture will give an overview of the strategies that have been employed at Bradford to design and produce protein particles that meet the requirements of modern drug delivery.

After a brief introduction to the formulation challenges that proteins present, stabilisation strategies will be discussed. The utility of sugars and polyols will be followed by highlighting our data on the use of novel excipients for solution and solid-state stabilisation. The lecture will then address opportunities to improve upon the means of producing protein particles. Whilst lyophilisation is typically employed to produce solid protein, the advantages of spray-drying and the use of supercritical fluid technology to produce particles in a more controlled manner, such as directing size and shape will be illustrated.

Two basic methods have considered the use of compressed or supercritical carbon dioxide to produce solid particles. The first method involves spraying a solution of the material in a mixture of CO_2 and organic solvent into air. This process has been termed rapid expansion of supercritical solutions (RESS). A limitation of the RESS process is the low solubility of most bioactives in CO_2. In the second method, organic bioactive solutions are atomised into a vessel containing compressed CO_2. Both this method, known as the supercritical fluid anti-solvent technique (SAS), and the RESS method have the limitation that they necessitate the use of organic solvents. Since such solvents are potential denaturants, their use with proteins is not ideal. York and co-workers have developed a new supercritical fluid technique which has the potential advantage of leaving the bioactive in a more favorable aqueous environment until transient time of passage into compressed CO_2 to form particles. A general schematic showing the SEDS process is presented in Figure 1. Briefly, a flow of supercritical carbon dioxide disperses and mixes streams of an aqueous protein solution and an organic solvent (e.g. ethanol) through a specially designed nozzle into a particle formation vessel under stable temperature and pressure. The ethanol precipitates the protein from the aqueous solution feed and the ethanol/water mixture is rapidly extracted by the supercritical carbon dioxide leaving dried protein particles. Changes in the working conditions (pressure, temperature and flow rates) allow control of the size, shape and morphology of the particles.

Interwoven in the presentation is the third strand to perfecting protein particles; namely, through the use of combinations of high-resolution analytical techniques. Hence a further aim of the presentation is to demonstrate the utility of high sensitivity DSC and FT-Raman

spectroscopy amongst others to aid formulation and process optimisation, including excipient selection.

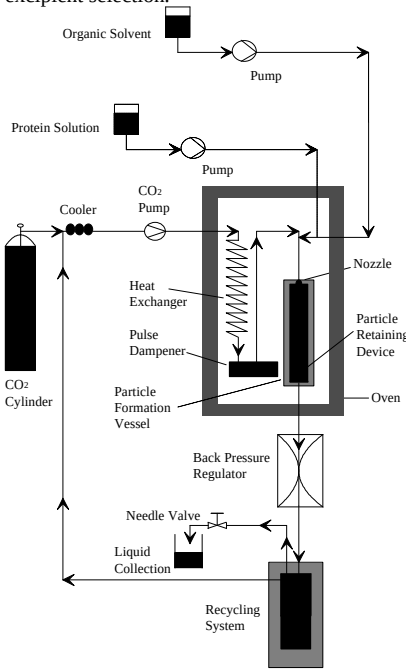

Figure 1. Schematic diagram of the SEDS experimental set-up.

Further Reading:

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PHYSICOCHEMICAL ASPECTS OF LIPID NANOPARTICLES

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Introduction

Among the wide variety of colloidal lipid-based drug carrier systems solid lipid nanoparticles represent a rather recent development. These particles are prepared from solid lipids such as crystalline triglycerides and can, e.g., be obtained by high-pressure homogenization of the molten lipid in an aqueous phase with the aid of adequate emulsifiers. After emulsification, the dispersion is cooled below the crystallization temperature of the dispersed lipid to yield a suspension of solid particles. The use of crystalline, usually polymorphic matrix lipids and their processing via the melt leads to complex systems not only with respect to their disperse state but also concerning thermal behavior and polymorphic transitions. Some relevant parameters with respect to these physicochemical aspects are discussed for nanoparticles prepared from saturated monoacid triglycerides (trilaurin (C12), trimyristin (C14), tripalmitin (C16), tristearin (C18)) and hard fats stabilized with different ionic and nonionic emulsifiers (e.g., sodium glycocholate, tyloxapol, poloxamer, Cremophor EL, polysorbates) and their combination with phospholipids. The dispersions were characterized by photon correlation spectroscopy (PCS), differential scanning calorimetry (DSC), X-ray diffraction and electron microscopy.

Results and Discussion

The type of matrix material determines the thermal and polymorphic behavior of the colloidal matrix lipid to a large extent [1, 2]. While the melting temperature of the nanoparticles - except for very small particles - is only slightly reduced compared to the corresponding bulk material a large depression of the crystallization temperature can be observed for nanoparticles based on saturated monoacid triglycerides (Fig. 1). In contrast to tristearin (C18) and tripalmitin (C16) nanoparticles which crystallize when cooled to room temperature after melt-homogenization, trimyristin (C14) and trilaurin (C12) nanoparticles remain in the liquid, supercooled state under these conditions. Trimyristin particles can be crystallized by cooling to refrigerator temperature but trilaurin nanoparticles usually require subzero temperatures for crystallization. The nanoparticles from the shorter chain triglycerides can remain in the liquid state for months and even years when stored sufficiently above their crystallization temperature. Since trimyristin nanoparticles may be obtained in the solid or liquid state depending on the preparation protocol they are interesting model systems to compare the properties of lipid nanoparticles in the solid and liquid state. Due to their mixed fatty acid chain composition, hard fat nanoparticles have a lower supercooling tendency than nanoparticles from monoacid triglycerides. The transition rate from the metastable α- to the stable β-polymorph increases with decreasing chain length of saturated monoacid triglycerides and is much faster in the nanoparticles than in the bulk. Some hard fats seem to obtain a more stable modification in the colloidal state than observed in the bulk material.

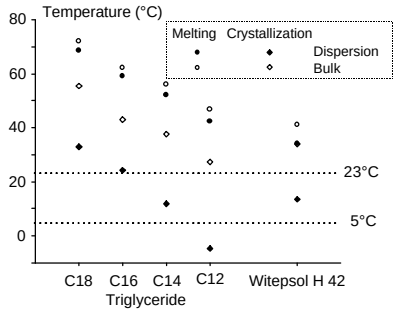

Fig. 1: DSC melting and crystallization temperatures of different triglycerides in the bulk and in colloidal dispersion.

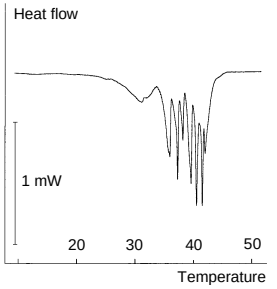

Fig. 2: Melting transition (DSC) of nanoparticles in a trilaurin dispersion of about 100 nm mean PCS particle size.

The melting behavior of triglyceride nanoparticles strongly depends on their particle size [3]. While the melting temperature of larger nanoparticles is only slightly reduced compared to the bulk material disintegration into very small particles leads to broadening of the melting transition and its shift to lower temperatures. For particle suspensions based on monoacid triglycerides with a mean size in the lower nanometer range the melting transition shows several distinct maxima which are not related to polymorphic transitions (Fig. 2). Instead, this structured transition is caused by the melting of fractions of particles characterized by a different thickness of the platelet-shaped particles.

The presence of the highly hydrophobic model drug ubidecarenone leads to a concentration dependent depression of the crystallization temperature of, e.g., tripalmitin nanoparticles. Incorporation of very high amounts of drug may thus favor the formation of supercooled, liquid particles. At lower drug concentrations, the crystallization temperature is not affected so much but the presence of drug still has a major impact on the particle properties by increasing the rate of polymorphic transitions. In contrast to the situation in bulk material, there are no indications for drug crytallization in the nanoparticles even at very high drug concentrations (e.g., 50 % related to the lipid matrix). Instead, the excess of drug adheres in liquid form to the solid nanoparticle matrix after crystallization of the triglyceride [4].

The crystallization and polymorphic behavior of triglyceride nanoparticles can also be influenced by the type of emulsifier used. There is a critical lower limit for the crystallization temperature of a given triglyceride. The presence of hydrophilic emulsifiers with long saturated alkyl chains (e.g., polysorbate 40 or 60) tends to increase the crystallization temperature [5] which is particularly interesting for crystallization enhancement in low crystallizing triglyceride particles. There are indications for a structure formation in the nanoparticle interface during cooling to the crystallization temperature for some triglyceride/emulsifier combinations with increased crystallization temperature which may be related to crystallization in the emulsifier layer prior to solidification of the triglyceride core.

Conclusions

The physicochemical properties of solid triglycerides in the the colloidal state may differ significantly from those in the bulk and depend on the matrix material, particle size as well as the presence of drugs and emulsifiers. Such nanoparticle dispersions thus require very careful characterization during their development into pharmaceutical carrier systems. On the other hand, the unique material properties of colloidally dispersed glycerides may also open up new possibilites for drug delivery purposes.

Acknowldgement

The author thanks M.H.J. Koch, M. Drechsler and K. Westesen † for their contributions to the work related to this paper and the EC for support of X-ray diffraction studies at the EMBL, Hamburg outstation.

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EFFECT OF WATER ON PROTEIN-SUGAR INTERACTIONS

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Aim of this study was to evaluate moisture-induced changes in physical form of co-spray dried trehalose-lysozyme and sucrose-lysozyme formulations. Co-spray dried trehalose-lysozyme and sucrose-lysozyme powders were stored at 0 % RH and 75 % RH and molecular mobility, and crystallinity of sugar component in the co-spray dried formulations, were determined by using solid state NMR and X-ray powder diffractometry, respectively. Also enzymatic activity of the lysozyme formulations was measured.

Trehalose-lysozyme and sucrose-lysozyme solutions (2 % w/v) in 1:9, 1:1 and 9:1 w/w sugar:protein ratios were spray-dried (Büchi 190 Mini Spray Dryer, Büchi Labortechnik AG, Switzerland) and the prepared materials were stored at 0 % RH or 75 % RH for 5-6 days prior to experiments. Solid-state NMR spectroscopy was performed using a Varian Unity Inova spectrometer (Palo Alto, CA, USA) operating at 299.947 MHz for ^{1}H. Spin-lattice relaxation time, T_1, measurements were used to determine dynamics on the higher frequency (MHz) time scale, associated with motions of the protein side chains and surface groups. T_1 was estimated by non-linear least-squares regression analysis. Powder X-ray diffraction (XRD) patterns were obtained using a Siemens D5000 Diffraktometer (Siemens, Germany). Enzymatic activity was measured using an assay based upon the lytic action of lysozyme on *M. lysodeikticus* cells.

Solid-state NMR results showed that increasing sugar content in the co-spray dried formulations stored at 0 % RH increased T_1 relaxation times suggesting decrease in molecular mobility (Table 1.). T_1 values for 1:9 sugar:lysozyme decreased when these co-spray dried formulations were stored at 75 % RH similarly to the behaviour of lysozyme.[1] This indicates a close association of the protein and sugar. Exposure of 1:1 and 9:1 sugar:protein formulations to moisture led to increase in T_1 values. In addition, the T_1 values for these samples were two-component, whereas the T_1 values were single-component for all the other samples (results not shown). The two-component behaviour of T_1 values might be an indication of separation of sugar and protein phases in the co-spray dried of 1:1 and 9:1 sugar:protein formulations stored at 75 % RH.

Table 1. Averaged proton (^{1}H) spin-lattice relaxation time constants (T_1) for co-spray dried lysozyme:sucrose and lysozyme:trehalose formulations after storage at 0 % RH and 75 % RH.

Material	T_1 (seconds)	
	0 % RH	75 % RH
Lysozyme	1.0	n.d.
Sucrose:lysozyme 1:9	1.1	0.6
Sucrose:lysozyme 1:1	2.3	8.8
Sucrose:lysozyme 9:1	5.8	6.7
Sucrose:lysozyme pm 1:1	9.8	n.d.
Trehalose:lysozyme 1:9	1.2	0.7
Trehalose:lysozyme 1:1	1.9	7.5
Trehalose:lysozyme 9:1	4.2	4.5
Trehalose:lysozyme pm 1:1	3.3	n.d.

Pm = physical mixture, n.d. = not determined

All studied powders were amorphous after spray drying and subsequent storage at 0 % RH. During storage at 75 % RH sucrose and trehalose crystallized in co-spray dried 9:1 and 1:1

sugar:lyzosyme formulations. Both co-spray dried 1:9 sugar:lysozyme remained amorphous even at 75 % RH.
Differences in relative enzymatic activity of lysozyme between studied formulations were not statistically significant.
In conclusion, solid state NMR results indicated strong interaction between sugars and protein, and decreased molecular mobility when sugar content is increased, in co-spray dried trehalose:lysozyme and sucrose:lysozyme formulations stored at 0 % RH. X-ray powder diffraction showed that storage at 75 % RH induces crystallisation of sugars, i.e., phase separation of sugar and protein components, in co-spray dried 1:1 and 9:1 sugar:protein formulations. This was also shown as an increase in averaged T_1 relaxation times that were a weighed average of two T_1 components indicating that sugar and protein phases were separated in these formulations. This water induced phase separation of sugar and protein components in co-spray dried formulations did not have significant effect on enzymatic activity of lysozyme.

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POLYMERIC NANOPARTICLES

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Controlled release micro- and nanoparticle formulations of biodegradable polylactide polymers have been formulated with various methods, one of which is the nanoprecipitation method invented by Fessi and co-workers in the late 1980's (Fessi *et al.* 1989). Since that time, lipophilic drug substances have been successfully capsulated inside the nanocapsules by the nanoprecipitation method. Moreover, efficient loading of hydrophilic drug substances inside the polymer capsules by the nanoprecipitation is very difficult, due to the very low affinity of the hydrophilic drug to the hydrophobic polymer.

In this study the entrapment efficiency of a hydrophilic drug, sodium cromoglycate, into poly(l)lactide (PLA) nanoparticles has been analyzed. Sodium cromoglycate loaded PLA nanoparticles were formulated by a modified nanoprecipitation process (Peltonen *et al.* 2002). In order to increase the amount of entrapped drug substance, the effect of changes in the pH value of the outer and inner phases during the particle formation process were determined as well as the effect of electrolyte addition to the aqueous phases. Sodium chloride was used as the electrolyte and changes in pH-values were studied by the drop-by-drop addition of crude HCl.

As a polymer was used PLA 100 000 g/mol (Fluka, Switzerland). Organic solvents were dichloromethane (DCM), methanol (analytical grade, Riedel-deHaën, Switzerland) and ethanol (Ph. Eur., Primalco, Finland). Sodium cromoglycate (ICN Biomedicals Inc., USA) was used as a model drug, and propylene glycol (Ph. Eur., University Pharmacy, Finland) was used as a stabilizing agent. The surface morphology and the size of nanoparticles were studied by scanning electron microscopy (SEM, DSM 962, Zeiss, Germany).

The amount of drug inside the nanoparticles was calculated by subtracting the amount of drug in the aqueous phase of the suspension from the total amount of the drug in the nanoparticle suspension.

The addition of electrolyte (NaCl) improved only slightly (from 12 to 15 %) the entrapment efficiency of the drug substance. The best effect was achieved when the salt was added in both the inner and outer phases, although the salt concentration in the inner phase needed to be kept considerably low (<0.1 M) in order to avoid the precipitation of the sodium cromoglycate.

The pH changes in the outer phase seemed to have a more pronounced effect on the drug entrapment than the corresponding change in the inner phase. The pH of the outer phase was reduced by adding a few drops of crude HCl to the 96 % aqueous ethanol phase. The best results were achieved by adding only one drop of crude HCl: approximately 70 % (w/w) of the drug was entrapped inside the nanoparticles.

From these results it was obvious that also the entrapment of hydrophilic drug substances is feasible with high efficiency. By adjusting the pH of the microenvironment during the particle formation process, the entrapment efficiency could be raised to a level as high as approximately 70 %.

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Acknowledgements
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APPLICATIONS OF ISOTHERMAL MICROCALORIMETRY FROM THE PHARMACEUTICAL POINT OF VIEW

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General
Isothermal microcalorimetry is a technique, where the heat flow from the sample is monitored as a function of time. The sample is kept at constant temperature ($DT < \pm 10^{-4}$ K) and the temperature difference between the sample and the reference is measured with thermopiles. This arrangement enables to observe heat flows as low as 0.1 μW. This means that almost all heat producing or consuming reactions can be detected.
The sample size can be 1 – 1000 mg and the only requirement is that it can be placed into the sample vessel (ampoule). During measurement sample can be irritated for example with different solvents or light. The typical study is to examine the behavior of the sample in humid atmosphere. Measuring time varies from approximately four hours to several days.

Applications
Due to the accuracy and the unspecific nature of microcalorimetry it offers a wide range of applications in the pharmaceutical sciences. However, in order to avoid false conclusions the use of complementary methods is highly recommended. The differential scanning calorimetry (DSC) and x-ray powder diffraction (XRPD) are most common ones.
The typical pharmaceutical applications of microcalorimetry are various stability studies including compatibility measurements. Also the kinetics of the studied reactions can be investigated. It has been suggested that the reaction rate of one percent per year can be observed with microcalorimetry. In the stability studies the sample can be irritated with gas, moisture or light, for example. The degradation of the sample or possible phase transitions would yield an exo- or endothermic heat flow signal. In the compatibility examinations the heat flows from pure samples are measured. If the heat flow from the mixture differs from the sum of the heat flows of the pure samples it can mean incompatibility between the samples.

The other generally used applications of microcalorimetry are amorphicity measurements. The idea is to recrystallize the sample in the ampoule and measure the recrystallization energy. The calculation of amorphicity is presently based on the assumption that the recrystallization energy of a partly amorphous sample is directly proportional to the recrystallization energy of an amorphous sample. With this method very low amorphous contents (< 1%) can be detected and quantified.

Using so-called RH-units the adsorption and desorption properties of samples can be studied. Because these phenomena are related with the surface properties of the sample microcalorimetry can be used to characterize surfaces. With a novel method developed at the Laboratory of Industrial Physics the heat and the quantity of vapor sorption can be determined simultaneously [1]. This allows determining the adsorption energy unit J/mol.

Finally
Because the isothermal microcalorimeters response to almost all thermally active reactions they offer an effective tool for the pharmaceutical materials research. However, the scientist must be very careful when operating the device and when making conclusions. There is a wide range of applications and they are restricted mainly by the imagination of the user.

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THE INITIAL PARTICLE SIZE AND ADDITION OF ANOTHER COMPOUND: THE MUTUAL EFFECTS ON TABLET STRENGTH

B. van Veen, K. van der Voort Maarschalk, G.K. Bolhuis, E. Geelen, K. Zuurman, H.W. Frijlink

Abstract:

The main subject of this presentation is to describe the effect of particle size on the matrix strength in tablets compressed from pure sodium chloride and from blends containing starch as minor component. Tablets were prepared with a constant air fraction of 0.15 and their tensile strengths were studied as a function of the composition. Removal of the starch particles by a heat treatment made it possible to measure the percolation thresholds and strengths of the sodium chloride matrices in the tablet structure. The percolation threshold of sodium chloride is directly related to its mean particle size, whereas the percolation threshold of starch hardly changes at low starch volume fractions. Furthermore, starch particles cause an increase in median pore diameter in tablets compressed from blends. Alterations in tensile strength of these mixture tablets are related to the median pore diameter. A unique relation has been found between the median pore diameter and the tensile strength of these mixture tablets. This relation is independent of the sodium chloride mean particle size and sodium chloride volume fraction. A similar relation exists for tablets compressed from only sodium chloride particles, but higher tensile strengths were observed. The deviation between both relations is explained by a difference in pore shape. The pore shapes in the tablets compressed from blends are relatively long and sharp in contrast to the more spherical pores in the pure sodium chloride tablets.

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GENE DELIVERY: CHALLENGE FOR PHYSICAL PHARMACY AND BIOPHARMACEUTICS

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Gene therapy is a promising new treatment modality in medicine. Particularly the increasing knowledge about the links of the gene structure and diseases provides ever increasing number of medical targets that may be treated with gene therapy. Gene therapy is not limited to some rare genetic diseases, since various acquired diseases with altered level of gene expression are also possible targets of gene therapy (e.g. cancer).

In gene therapy exogenous DNA is transferred to the target cells that express the gene product. In principle any gene sequence can be transferred into the cell nucleus and the therapeutic protein will be produced. Problems of gene transfer (due to the large molecular weight and multiple negative charges of DNA) slow down the development of gene therapy for medical needs. Most gene transfer protocols use modified viruses for DNA transfer. The viral vectors have some problems related to their large scale production and safety. Therefore, chemical delivery systems are investigated as an alternative.

Chemical delivery systems should bring DNA into the target cells and deliver their cargo into the nucleus in active form. This is a difficult task that can be solved only by a combination of physical and biological studies. Traditional excipients of pharmaceutical technology are not adequate and new delivery systems and study methods are needed.

Cationic liposomes and cationic polymers are the most commonly used chemical delivery systems for DNA. Coding sequence of DNA (i.e. cDNA) is subcloned into plasmid DNA that may contain also DNA sequences that regulate the expression of cDNA. Plasmid DNA is complexed with cationic liposomes or cationic polymers in water solution. Upon complexation the negative charges of DNA are shielded and DNA is condensed. The complexes are often heterogeneous and their sizes are in the range of 20 nm – 2 micron. The complex size is determined usually by light scattering or electron microscopy (TEM). The organization of the carrier and DNA inside the complex particles is not well understood. It has been studied using TEM and SAXS. It is known that the polymers and liposomes show different complex morphology. DNA condensation and mobility of carrier molecules in the complex are usually investigated by fluorescence techniques (DNA intercalation of fluorophores, fluorescence anisotropy). The surface charge of the complex (i.e. zeta-potential) is highly dependent on the charge ratio (+/-) in the complex. The charges can be masked with steric stabilisation with polyethylene glycol. The aim is to generate monodisperse and pharmaceutically stable DNA particles.

DNA delivery system must be able to bring DNA into the target cells. Therefore, it should not fall apart or become inactivated by the extracellular components (e.g. albumin, collagen, glycosaminoglycans). Interactions with extracellular biomolecules are studied in in vitro coincubation experiments. Finally, experiments with cell cultures are used to measure cell uptake, mechanisms of cell uptake, intracellular distribution, and transfection efficacy. Fluorescence or radiolabelled DNA/carrier complexes are used in these experiments. Flow cytometry, fluorescence plate readers, confocal microscopy and liquid scintillation counting are typical methods. Most often marker genes are used in transfections. Genetically modified cells enable mechanistic biological studies. The cell uptake is not enough for activity, since DNA should be delivered into the nucleus.

Understanding the behaviour of the DNA delivery systems in the cells requires detailed physical pharmacy studies. The interactions between DNA, carrier and biological components (extracellular, intracellular) must be understood and properly mastered before reproducible and efficient gene therapy with chemical systems becomes clinical reality.

THE ROLE OF GLYCOSAMINOGLYCANS IN NON-VIRAL GENE THERAPY

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Cationic polymers and liposomes are widely used as DNA carriers in non-viral gene therapy. Although these vectors work in cell cultures, they are inefficient *in vivo*. Therefore, it is crucial to understand the mechanisms and identify the limiting factors of non-viral gene delivery. Glycosaminoglycans (GAGs) are highly anionic polysaccharides and can be found in the extracellular space and on the surface of cells, often as a part of proteoglycans (PGs). Because of their abundance and polyanionic nature, PGs and GAGs are potential barriers in non-viral gene therapy *in vivo*. The purpose of our study was to clarify the role of extracellular and cell surface GAGs in non-viral gene therapy.

Following carriers were tested: polyethyleneimine of 25 kDa (PEI), poly-L-lysine of 200 kDa (PLL), N-[1-(2,3-dioleoyloxy)propyl]-N, N, N-trimethyl ammonium methylsulfate (DOTAP) and DOTAP/DOPE (1,2-dioleyl-3-phosphatidylethanolamine) at molar ratio 1:1. After preparing complexes between carrier and plasmid DNA at charge ratio +/- 4, 3-fold anionic excess of exogenous hyaluronan (HA) or heparan sulfate (HS) was added to complexes. The effect of exogenous HA and HS on physicochemical properties, cell uptake and transfection efficiency of complexes was studied.

Exogenous HS blocked both the cell uptake and transfection efficiency of PEI complexes. This can be explained by premature release of DNA from the PEI carrier by HS. Exogenous HS clearly decreased also the transfection efficiency of other complexes but this cannot be explained by neither altered physicochemical properties nor decreased cellular uptake of complexes. In contrast, exogenous HA increased the transfection efficiency of PLL, DOTAP and DOTAP/DOPE complexes, although the cell uptake of these complexes remained at the level of control. Furthermore, exogenous HA did not release or relax DNA in any complex. In the case of PEI, the decreased gene expression by exogenous HA can partially be explained by decreased cell uptake. The interactions between GAGs and complexes are dependent on the structure of both the carrier and GAG. In addition, this data suggests that exogenous GAGs may alter the cell uptake route and intracellular behavior of the DNA complexes.

The role of cell surface GAGs in non-viral gene delivery was identified by using CHO cells mutated in GAG synthesis. The molar quantities of HS, chondroitin sulfate (CS) and HA on cell surface were correlated with the cellular uptake and gene expression level of DNA complexes. Two different CHO cell mutants unable to produce HS or CS showed enhancement both in cellular uptake and in gene expression, as compared with the wild type cells. Another CHO mutant with increased cell surface CS in the absence of HS, showed increased cellular uptake, but reduced gene expression. Taken together, cell surface GAGs inhibit the transfection efficiency of the complexes, probably by routing them into intracellular compartments that do not support transcription.

CHALLENGES OF PROTEIN FORMULATION: PROBLEMS WITH STABILITY

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Since many therapeutic proteins, do not have sufficient storage stability in aqueous, they are dried in an effort to improve stability, normally by freeze drying. However, a protein is exposed to a number of thermodynamic “stresses” during freeze drying, and some labile systems suffer degradation during the process. Moreover, storage stability of the dried product is not always satisfactory. Stability issues are addressed by optimization of formulation and process. Here, we focus on formulation!

Protein instability in the solid state frequently follows “stretched exponential” kinetics, likely a reflection of the fact that individual configurations are not in thermal equilibrium in the glassy state. Both physical and chemical stability are extremely formulation sensitive. For example, stability of human growth hormone (hGH) in the glassy state varies by nearly two orders of magnitude, depending upon specific excipient effects. Structure is important. Spectroscopic studies have shown that proteins often suffer extensive conformational changes during freeze drying, with the changes being sensitive to formulation details. Storage stability will be decreased if the altered conformations are more reactive than the native conformation. With hGH, storage stability does correlate with structure as measured by FTIR. Molecular mobility is also important. A major objective in drying is to produce a solid, where the molecular mobility required to support chemical and physical change is restricted. However, glassy does not necessarily mean stable, and large differences in stability between glassy formulations of the same drug are observed.

Our hypothesis is that differences in dynamics in the glassy state, as reflected by differences in the structural relaxation time, are partially responsible for many of the formulation specific stability behavior of materials stored well below their T_g’s.

Some data suggest structural relaxation and stability are indeed correlated. The relative stability of two freeze dried cephalosporins, cefamandole sodium and cephalothin sodium, is in almost exact proportion to the relative structural relaxation times, stability of another cephalosporin (moxalactam) appears to be enhanced upon heat treatment or annealing, as expected from the theories of dynamics in the glassy state, stability in aspartame:disaccharide glasses correlates with relaxation time, and dimer formation in a small molecule system shows a rough correlation with relaxation dynamics. Finally, we observe a good correlation between stability and relaxation dynamics in glassy formulations for both hGH and an IgG1 antibody, although the correlations are not perfect.

MICROPARTICLES IN PHARMACEUTICAL PRODUCTS

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Introduction

The aim of this presentation is to give a review of the applications of microparticles in pharmaceutical products as well as techniques for their preparation. The pros and cons of different techniques are discussed. Here, microparticles are defined as solid particles with the size range 1-1000 µm. Some of the presented technologies can even be used for preparation of nanoparticles (1-1000 nm). The presentation is based on the literature in the area and practical examples from published articles and patent applications.

Applications

Microparticles (or microspheres, microcapsules, micropellets) have been used during/for several years for different purposes like organoleptic protection (taste masking) or improvement of drug stability by protecting the drug from oxygen, moisture or gastric fluids or by separating the drug from excipients. They are used to control the drug release and for diagnostics. The most common purpose for choosing microparticles today is to avoid harmful side effects as e.g. for cancer therapy or for drug targeting. Another application of microparticles is to utilise the gastrointestinal uptake of particles from villa, Peyer's patch and via enterocytes for poorly absorbed drugs like proteins and peptides where microparticles protect the drug for GI fluids. Microparticles may also be administered parenterally (im, as implantate, iv, sc) provided that the particle size is controlled as well as the sterility of the product. They can be administered intranasally, intraocularly, by inhalation, transdermally and most commonly orally. The products on market include e.g. ASA, theophylline, vitamins, progesterone, contraceptives, antihypertensives (Seloken® ZOC, AstraZeneca) and potassium chloride preparations. For cancer therapy, products like Enanton® Depot (Takeda) and Decapeptyl® Depot (Ferring) exist. According to literature Novartis has a parenteral microparticle product for parkinson disease, Parlodel®.

Techniques

Manufacturing techniques for microparticles can be divided into three main categories depending on their mechanism for forming the particles: emulsion, atomisation or crystallisation techniques. Emulsion methods like emulsion/solvent evaporation and emulsion/solvent extraction techniques are based on the homogenisation of two- or three-phase liquid system with surfactants where the macro- or microemulsion droplets are formed. The drug and carrier precipitates from these droplets into a particle. Homogenisation of the emulsion is the critical size-controlling step of these systems. According to literature e.g. Enanton® Depot microparticles are prepared with w/o/w emulsion solvent evaporation. The disadvantage is the use of organic solvents, relatively complicated processes and long aseptic process times. Atomisation techniques include spray drying, spray freezing, spray chilling and spray layering (fluidised bed coating). A spray is formed from a solution, dispersion or from a melt and the droplets are solidified to separate particles or several droplets form a particle (spray layering). The particle-size-controlling step for these techniques is the formation of spray in the nozzle. An example of products manufactured with spray drying is the Parlodel® NAR microparticles. More recent techniques include supercritical fluid crystallisation and spherical crystallisation. Here, particle precipitation is controlled by crystallisation kinetics. The supercritical fluid crystallisation method has several advantages compared with spray drying. Organic solvents can be avoided when supercritical carbon dioxide is used as solvent. Process temperatures are low and the removal of organic solvents with supercritical fluids is efficient. In spherical crystallisation, the mixture of solvents is used in order to produce crystal agglomerates with a method described

by Kawashima. Advantage with this method is that no excipient is needed to form a spherical particle. A disadvantage is the possible residual solvents in the agglomerates.

Excipients

The coating materials or matrix formers used in microparticles can be synthetic biodegradable polymers like polylactides PLA or poly(D,L-lactic-co-glycolides) PLGA, polyanhydrides, poly(ε-caprolactone) PCL or polyhydroxybutyrate PHB, other synthetic polymers like polymethacrylates or cellulose derivatives, or polymers of nature origin like albumin, chitosan or alginates. Other excipients can be e.g. plasticizers and emulgators.

Conclusion

Depending on the drug properties and the purpose of microparticles, there exist several techniques and carriers, which can be used to produce microparticles. Some of the processes are easy to scale up, like spray drying, others more difficult. Even aseptic processing and sterilisation is possible depending on the excipient and drug properties.

CONTROLLED-RELEASE FELODIPINE MICROPARTICLES

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Introduction

Solid dispersion techniques offer an interesting formulation approach for both enhancing drug solubility and controlling the release rate of the drug (1, 2). The advantage in solid dispersion controlled-release formulations is that a matrix type system is formed. Thus, the risk of dose dumping is avoided. Both polymers and lipids have been used as matrix forming materials.

In spray chilling a melted mass of drug and excipients is atomised into droplets, which quickly solidify in cool air. Spray chilling is a suitable technique for materials that are not temperature sensitive and have low melting points. Spray chilling has been used previously to prepare controlled-release particles of polar lipids. However, hydrophilic polymers have not been combined before with lipids at molten state to form controlled-release microparticles (3).

The aim of this study was to produce controlled-release tablets of a poorly water-soluble model drug, felodipine [Ethyl methyl 4-(2,3-dichlorophenyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridine-dicarboxylate], using various lipophilic and hydrophilic excipients as carriers. Before compression, the spray chilling technique was used to prepare a solid dispersion of the drug and the carriers.

Materials and methods

Felodipine was chosen as a model drug substance, because it is not temperature sensitive and it is poorly soluble in water. The lipophilic excipients chosen were cetanol, carnauba wax and Precirol® ATO 5, which are all polar lipids. The hydrophilic excipients were PEG 4000 and Pluronic® F127 (Poloxamer 407). The melting points of the excipients were between 50°C and 90°C, which made them suitable to use in the spray chilling process.

The microparticles were produced using spray chilling technique (4). After felodipine had dissolved into the molten lipid, hydrophilic excipient was added to the melt. The drug excipient ratio was 1:4 in the particles with only lipophilic excipients. In the particles were both lipophilic and hydrophilic excipients were used, the ratio drug, lipophilic-, hydrophilic polymer was 1:4:2, respectively.

The microparticles were characterized using scanning electron microscopy (SEM). The solid state of the drug in the solid dispersion was studied using x-ray powder diffractometry (XRPD), Raman spectroscopy and hot stage microscopy (HSM). FT-IR spectroscopy was used to obtain information about molecular interactions between the drug and the excipients, since molecular interactions are the basis for the formation of solid dispersions, and a requirement for the formation of a stable state. For dissolution studies the microparticles were mixed with microcrystalline cellulose and compressed to tablets. The tablet weight was 200 mg, and had a theoretical felodipine content of 10 mg.

Results

Spray chilling could be used to prepare felodipine microparticles using lipids and hydrophilic polymers as carriers, although the addition of hydrophilic excipient increased the amount of agglomerates. The produced particles were round and in the micrometer domain. The surface characteristics depended on the excipients: carnauba wax produced particles with smoothest surfaces and cetanol with the roughest.

FT-IR studies showed that hydrogen bonding existed between felodipine and all the excipients. The HSM supported the findings of XRPD, FT-Raman and FT-IR analysis that partial solid solutions were formed in all the samples. The crystallinity of felodipine had decreased in all the samples, even though the amount of crystalline felodipine varied significantly depending on the composition of the matrix.

The solid state of the drug in the particles and the dissolution rate (Fig. 1) is influenced by the choice of the carriers. Dissolution rate was slowest from tablets containing cetanol microparticles. These microparticles also had most crystalline felodipine in them. Addition of hydrophilic excipient could be used to adjust the dissolution rate. The most promising dissolution pattern was obtained from Precirol® ATO 5 + Pluronic® F127 tablets; felodipine release rate was constant and 45% of the felodipine was released after four hours.

Combining lipids and hydrophilic polymers in the molten state to form solid dispersion microparticles offers a promising method to produce a controlled-release formulation. However, the long-term stability of these solid dispersions should be studied to determine whether the noted molecular interactions are strong enough to maintain the solid state of the drug.

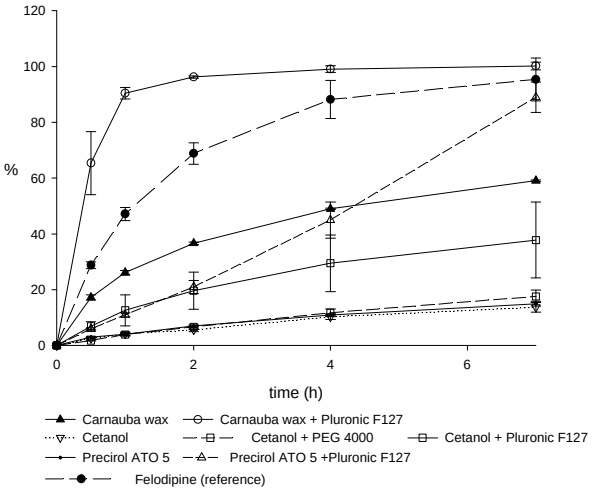

Fig. 1. Dissolution of felodipine from the tablets.

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NOVEL CATIONIC AMPHIPHILIC 1,4-DIHYDROPYRIDINE DERIVATIVES FOR DNA DELIVERY

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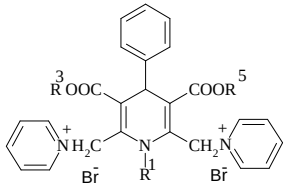
For successful gene transfer, delivery of DNA into the cells should be efficient and safe. Therefore increasing attention is focusing currently on the development of non-viral carriers, such as positively charged lipids and polymers. Despite of intensive use of cationic lipids as vectors, toxicity and low transfection efficacy still remain as problems. Although the physicochemical characteristics of carrier/DNA complexes (size, zeta-potential, etc) may affect transfection efficacy [1,2], the structural features of carriers for optimal gene delivery are not yet clear [3-5].
We have introduced novel non-viral gene delivery systems based on 1,4-dihydropyridine structure - double-charged amphiphiles with two quaternized nitrogens and different alkyl chain length at the positions 3 and 5 (Fig 1).



R^1	$R^3 = R^5$
H	$C_{10}H_{21}$
H	$C_{12}H_{25}$
H	$C_{14}H_{29}$
H	$C_{16}H_{33}$
CH_3	$C_{12}H_{25}$

Fig. 1. Structures of 1,4-dihydropyridine amphiphiles

We have examined some biophysical properties of the amphiphiles and their complexes with plasmid DNA, their cellular uptake and ability to transfect the cells. We compared 1,4-dihydropyridine's complexes with DOTAP/DNA complexes and polyethyleneimine of 25 kDa (PEI 25)/DNA complexes. DOTAP is a monovalent cationic lipid and PEI 25 – cationic polymer.
The amphiphiles formed liposomes in water with mean diameters in the range of 50-130 nm (S.D. up to 35-45%). The size of amphiphile/DNA complexes increased with decrease of +/- charge ratio (charge ratio < 4). At optimal charge ratio (+/- 4) the mean diameter of the complexes was in the range of 75 nm (S.D. up to 35-45%). Sizes of the liposomes and complexes were determined by quasielastic light scattering. 1,4-Dihydropyridine liposomes showed positive (25-49 mV) zeta-potentials. The zeta-potential of the complexes was dependent on the +/- charge ratio of DNA/carrier.
The ability to form complexes with DNA is necessary for good transfection and upon complexation DNA may be condensed. We evaluated the ability to condense DNA using EtBr displacement assay. The results show that amphiphiles efficiently condense DNA. The compound with two C_{12} alkyl chains (C_{12}) was the best in this respect, causing a maximal decrease of 56-60% in fluorescence intensity of EtBr. PEI 25 was able to decrease the fluorescence by 65-70% and DOTAP condensed DNA maximally by 33%.

According to titration results 1,4-dihydropyridine amphiphiles showed also buffering properties in the endosomal pH range (pH 5.0-7.4).
To evaluate the percentage of complexes taken up by cells, the cells were incubated with ethidium monoazide-labeled DNA/amphiphile complexes. GFP-coding plasmid was used to study gene expression. The uptake and gene expression were analyzed by fluorescence activated cell sorter (FACS). FACS results demonstrate that the gene expression is not proportional to cellular uptake. Although the amphiphiles C_{10}, C_{12} and C_{14} demonstrated similar, relatively high uptake levels, still their gene expression levels were different. Compound C_{12} was the most efficient derivative for transfecting the cells.
The effectiveness in vitro does not guarantee success in vivo, because the complexes may interact with various extracellular components in serum and tissues [1,6]. Our results showed that the presence of serum in transfection medium hampers transfection mediated by 1,4-DHP derivatives.
To improve transfection efficacy of amphiphiles in the presence of serum we combined them with the fusogenic lipid DOPE. C_{12}:DOPE formulations in serum-free medium had higher efficacies than C_{12} alone. C_{12}:DOPE formulations (1:1, 2:1,1:2) showed high transfection efficacy also in 10% serum-containing medium, whereas activity of C_{12} alone was completely blocked.
We conclude that 1,4-dihydropyridine derivatives are able to condense DNA and efficiently transfect DNA into the cells in vitro. The fusogenic lipid DOPE helps to maintain the transfection efficiency in the presence of serum.

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POSTERS

DETERMINATION OF AMORPHOUS CONTENT OF SPRAY DRIED LACTOSE BY SOLUTION CALORIMETRY

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In the present study, a solution calorimetry method was developed to determine the amorphous content of spray dried lactose. Lactose samples were prepared by spray drying of 15% (w/w) lactose suspension or solution with Büchi Mini-Spray Drier 190. The ratio of ethanol to water in the feed solution varied from 0:100 to 100:0 (w/w). Amorphous content of spray dried lactose varied from 0% to 100%, depending on the ratio of ethanol to water in the feed solution. Lactose spray dried from pure ethanol was 100% crystalline and lactose spray dried from pure water was 100% amorphous.

Amorphous content of the samples was determined by isothermal microcalorimetry.
Enthalpies of solution of lactose samples and enthalpies accompanied with immersion in a suspension of samples were determined by solution calorimetry (a Parr 1455 Solution Calorimeter). In order to measure the enthalpy of solution, the lactose samples (accurately 400 mg) were dissolved in water. The enthalpy accompanied with immersion in a suspension was determined by releasing the lactose samples into the saturated aqueous solutions. There was a good correlation both between the enthalpy of solution and amorphous content of the sample (R^2 = 0.9886) and between the enthalpy accompanied with immersion in a suspension and the amorphous content (R^2 = 0.9826). The enthalpy of solution (-53.28 J/g) and the enthalpy accompanied with immersion in a suspension (-68,65 J/g) of 100% amorphous lactose exhibited an exothermic reaction. In contrast, the enthalpy of solution (53,41 J/g) and the enthalpy accompanied with immersion in a suspension (4.16 J/g) of 100% crystalline lactose were endothermic. The present study shows that the amorphous content of the powder samples may be determined by measuring the enthalpy accompanied with immersion in a suspension of the sample.

CHARACTERIZATION OF CHANGES IN THE TEXTURE OF LACTOSE AND MANNITOL UNDER CRYSTALLIZATION, POLYMORPHIC TRANSFORMATION AND COMPRESSION

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Many polycrystalline substances have macroscopic properties which are anisotropic, i.e. they depend on the orientation of the crystallites. In the present study this **preferred orientation of crystallites** is referred to as **texture**, as opposed to the commonly used meaning of for example the roughness or patterns of particle surfaces. Usually the only way to quantify the texture of a sample is to use crystallographic methods such as X-Ray diffraction (XRD). Sample (for example a pharmaceutical powder substance compressed to a tablet) is described as to being textured when certain crystal planes are orientated to the same direction. Normally all samples are textured to at least some degree.

The effect of textures on pharmaceuticals is still largely unstudied. In pharmaceutical substances textures may affect for example the dissolution properties, breaking strengths and hardness of tablets. Also the lamination and cap forming of tablets can be affected. Texture measurements may provide valuable non-destructive information of tablet integrity.

A sample may be textured in several ways depending on the handling and processing. For this study changes in the textures of lactose samples (20, 25, 40 and 100 % crystalline) and δ-mannitol were characterized under crystallization (lactose), polymorphic transformation (mannitol) and compression (100 % crystalline lactose and mannitol). Measurements were done on a X-Ray diffractometer equipped with a ATC-3 texture goniometer and software (Philips).

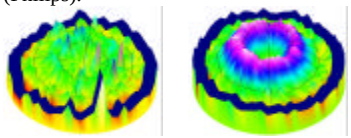

Figure. Textures of δ-mannitol uncompressed powder sample (left) and compressed tablet (right).

The results show that the compression process produces clear and different textures to both lactose and mannitol tablets [Figure] when compared with the uncompressed powder samples. The uncompressed lactose samples have a slight texture, which is strengthened by the compression process. The crystallization of lactose samples did not show distinct changes in the textures although some smaller changes were visible. Further studies are carried out to produce a complete picture of different types of textures from all reflections (directions) and to determine if for example there is some relation between tablet integrity and textures.

RELATIONSHIP BETWEEN AMORPHOUS CONTENT OF LACTOSE AND ITS HEAT OF SOLVATION IN METHANOL

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In the present study, a solution calorimetry method was developed to determine the amorphous content of spray dried lactose.

Lactose samples were prepared by spray drying of 15% (w/w) lactose suspension or solution with Büchi Mini-Spray Drier 190. The ratio of ethanol to water in the feed solution varied from 0:100 to 100:0 (w/w). Lactose spray dried from pure ethanol was 100% crystalline and lactose spray dried from pure water was 100% amorphous. Physical mixtures (w/w) were made by mixing the 100% amorphous lactose and the 100% crystalline lactose, and they were used when the correlation between heat of solvation and the amorphous content of lactose samples was measured.

Heat of solvation of lactose samples was determined by solution calorimetry (a Parr 1455 Solution Calorimeter) in saturated methanol. There was an excellent linear correlation between the heat of solvation and the amorphous content of lactose samples (r=0,99997). Thus it is possible to determine the amorphous content of the spray dried lactose sample by using this standard curve. Amorphous content of spray dried lactose varied from 0% to 100%, depending on the ratio of ethanol to water in the feed solution.

The present study shows that the amorphous content of the powder samples may be determined by measuring the heat of solvation in methanol. Usually solubility of drug in water is high. Thus, compared with measurement of heat of solvation in saturated aqueous solution much less sample is required when methanol is used in studies.

A NOVEL OPTICAL TECHNIQUE FOR AT-LINE PARTICLE SIZE ANALYSIS IN FLUIDIZED-BED GRANULATION

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Purpose. The purpose of this study was to investigate suitability of a recently introduced optical technique in at-line particle size measurement in a fluidized bed granulation process.

Methods. In total 33 granulations were made with a fluidized bed granulator using varying process conditions. Approximately 20 samples from each batch were taken during processing with 1.5-minute intervals. Each sample was analyzed using a novel instrument with a CCD camera with optics and illumination units with stabilized collimated light beams. Images of the surface of each granule sample were taken and the received image information was transformed to a particle size distribution.

Results. The results indicate that the introduced optical method provides a reliable at-line method for particle size analysis during a granulation process. The technique was suitable in the measurement of granule samples during all process phases, therefore it was possible to measure both dry and wet samples with a wide particle size range (100-1500 μm). The method is fast, the instrument is easy to operate and the sample size required can be very small (less than 0.5 grams). The analyzed sample also remains undamaged. This study confirms the results from earlier studies that information from surface images of pharmaceutical powders contains substantial data for particle size analysis.

Conclusions. The study proves that the new optical method for at-line particle size analysis provides rapid analysis of powder samples in a wide particle size range. The technique is a proper tool in process control of pharmaceutical solids and powerful in measurement of granule growth kinetics.

OPTIMIZING THE PROCESS PARAMETERS OF INJECTION MOULDING TO MINIMIZE THE STATIC CHARGE OF POLYPROPYLENE TEST ROD

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Abstract
In the injection moulding process the manufactured plastic components may carry a significant amount of charge which has undesirable consequences in some applications e.g. in dry powder inhalers (DPI's). In high resistivity polymers, the charge may lie in the bulk of the material which prevents typical neutralizing procedures such as use of ionizers. Instead of neutralizing the injection moulded components, the process parameters were adjusted to minimize the charge of the melt. Electrostatic charges on manufactured polypropylene test rods were measured using a Faraday cup. By adjusting several process parameters such as feeding cylinder and mould temperatures, cooling time, injection speed and holding pressure the generated charge on the polypropylene test rod could be reduced ten times compared to the charge values obtained with the initial process parameters. Taguchi matrix was used in assessing the effect of separate process parameters on the generated charge. Electric field measurements showed that the samples were not polarized and that the charge was uniformly distributed. Charge decay measurements showed that after storage some samples with high initial charges still carried higher charges than did initially samples which were produced with optimized parameters. Together with electrostatic measurements, physical dimensions of the samples were also monitored to ensure the usability of the products. Also, samples were characterized by differential scanning calorimetry. Results prove that a significant reduction in the static charge was achieved by optimizing the process parameters using the Taguchi matrix.

MEASUREMENT OF THE ELECTROSTATIC CHARGE OF PHARMACEUTICAL AEROSOLS

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Electrostatic charge of aerosols has a great effect on lung deposition, the function of an inhaler and the separation of drug particles from carrier particles. In this study we measured the charging of pharmaceutical aerosols using different measurement devices: a charge separator, a Grid-probe[1], an ordinary Faraday cup, an inverse Faraday cup and a Faraday cup specially designed for aerosols. The aerosols which were measured are typically used in inhalers as carriers. It was also studied how some of their properties affect the charging: the homogenous powders were characterised by the diameter of particles and the two-component powders by the concentrations of their components.

The measurement devices were connected to an electrometer, which was used to measure the charge of the aerosol. Measurements using different devices showed quite similar results. From the results it was concluded that the calculated charge to mass ratio of homogenous powders increased as the diameter of the particles decreased (Fig. 1). However, the concentration of the individual components of a two-component powder affects the polarity of charge (Fig. 2).

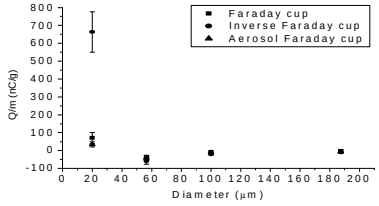

Fig. 1. Mass to charge ratio as a function of diameter using different devices.

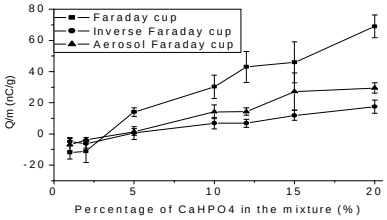

Fig. 2. Mass to charge ratio as a function of proportion of $CaHPO_4$ using different devices.

Additionally, the charge of the aerosol in the inverse Faraday cup and the potential difference caused by the aerosol in the Grid-probe were also measured as a function of time using a computer. The Grid-probe measurements indicated that drug and additive particles were emitted in succession; the positively charged smaller particles leave the inhaler before the particles of greater diameter and negative charge. This finding may have a significant effect on the future development of inhalers and drug dosage.

[1]M. Murtomaa, S. Strengell, E. Laine, A. Bailey, Proceedings ESA-IEJ Joint Meeting , 332-343, 2002.

CELLULAR UPTAKE OF STARCH-ACETATE MICROPARTICLES

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Biodegradable polymer microparticles (mps) have been investigated, e.g., for the intracellular drug delivery. In the present study, cellular uptake of calcein-containing starch-acetate (SA) mps (Fig. 1) by cultures of human retinal pigment epithelial (RPE) cells was examined. The average degree of acetyl substitution per glucose residue of starch was 2.6 (SA DS 2.6). Calcein (mw 623) was used as fluorescence label of mps.
SA DS 2.6 mps (Table 1) were prepared by water-in-oil-in-water double-emulsion technique. Cellular uptake of SA mps was analyzed by flow cytometry (10,000 events were collected) and confocal microscopy.
After 3-hours incubation, cellular uptake of SA DS 2.6 mps (64 µg /10,000 cells) was 8.1±0.3% (Fig. 2). No more SA DS 2.6 mps were taken into cells after 3-hours incubation. Cellular uptake of SA DS 2.6 mps was also confirmed by confocal microscopy images taken from living RPE-cells. The present results suggest that injectable biodegradable SA mps may be suitable for the intracellular drug delivery.

Table 1. Characteristics of calcein-containing starch-acetate DS 2.6 microparticles.

D10% µm	D50% µm	D90% µm	Calcein loading (%, w/w)	Encapsulation efficiency [a] %	Yield [b] %
5 ± 0	11 ± 0	22 ± 0	0.04	18	41

Theoretical calcein loading was 0.2%.
A Encapsulation efficiency % = (actual calcein loading / theoretical calcein loading) * 100%
B Yield % = [microparticles obtained / (total amount of calcein + total amount of polymer)] * 100%

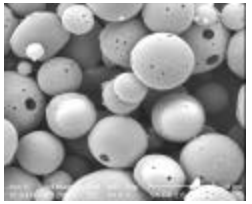

Figure 1. Scanning electron microphotograph (2000x) of calcein-containing starch-acetate DS 2.6 microparticles.

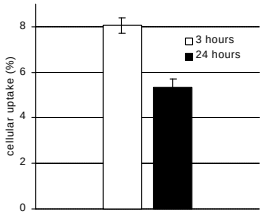

Figure 2. Cellular uptake of calcein-containing starch-acetate DS 2.6 microparticles by cultured RPE cells after 3- or 24-hours incubation (mean ± SEM, n=6).

GAS ADSORPTION MEASUREMENTS WITH ARGON AND NITROGEN – EFFECT OF WATER IN THE STRUCTURE OF POROUS SAMPLES

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Surface Area Measurements

The specific surface areas of several reference and test samples have been measured using both nitrogen and argon. New effective cross sectional areas of nitrogen and argon based on the reference measurements have been calculated [Table]. No correlation was found between the cross sectional areas of adsorbents and BET constants. The results of the surface area measurements got better after the new cross sectional values had been applied. The results also show that the condensation energy of argon is lower than a value of nitrogen, which means that argon would be better surface area measuring gas. Moreover, argon is less reactive gas than nitrogen. However, the cross sectional area of argon should be revised with extra measurements.

Table: The specific surface areas (SSA) of the reference samples and the corresponding cross sectional areas (CSA) of adsorbents. The notation "$CSA_{ref\ -\ Ar}$", for example, means that the cross sectional area of argon has been calculated from the reference value of the surface area

	SSA_{ref} [m^2/g]	$SSA_{N2}^{CSA=16.2Å}$ [m^2/g]	$SSA_{Ar}^{CSA=14.2Å}$ [m^2/g]	$CSA_{ref\ -\ N2}$ [$Å^2$]	$CSA_{ref\ -\ Ar}$ [$Å^2$]	$CSA_{N2\ -\ Ar}$ [$Å^2$]
REF1	0.44	0.420	0.345	17.0	18.1	17.3
REF2	2.3	1.90	1.56	19.6	20.9	17.3
REF3	5.3	4.74	3.86	18.1	19.5	17.4
REF4	15.9	15.22	13.07	16.9	17.3	16.5
average				17.9	19.0	17.1

Isotherms, Porosity and Effect of Water in the Structure of Porous Samples

The complete nitrogen and argon adsorption-desorption isotherms of dried and moisturized silica gel and silica-alumina were measured. The surface area and total pore volume of the moisturized samples were significantly lower than the values of the dry samples. Moreover, the nitrogen isotherms of moisturized samples have very anomalous form. Because the similar behavior was not observed in argon isotherms, some reaction between nitrogen and water could be doubted. This means that nitrogen shouldn't perhaps be used when measuring the surface areas of moist samples.

USING STEPSCAN DSC TO MEASURE THE AMORPHICITY OF SPRAY-DRIED LACTOSE SAMPLES

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StepScan differential scanning calorimetry (StepScan DSC) is a recently developed temperature modulated DSC technique of PerkinElmer Instruments. The StepScan DSC approach is based on conventional power compensated DSC. The heating program of StepScan DSC includes two different states. In the first state, the heating state, sample is heated small temperature increments (1 - 4 °C) by some known heating rate (e.g. 10 °C/min). The second state, the isothermal state, includes a short isothermal time interval (0.5 – 1.0 min), in which the sample reaches a thermal equilibrium. After that a new heating state starts.

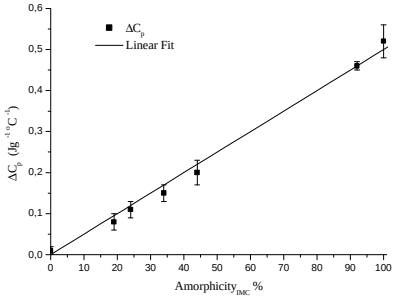

Figure. StepScan DSC results for DC_p at the glass transition are proportional to the amorphicity of lactose samples measured with IMC.

The StepScan DSC approach provides **Thermodynamic C_p** and **IsoK Baseline** data set. IsoK Baseline data set is calculated by choosing the last point in the isothermal state of each step and joining them together. This data set shows the irreversible or slow processes of sample. Thermodynamic C_p data set is computed from areas under the heating steps by taking the first point of a heating step as the initial point and then taking the last point of the heating step before the isothermal state. Thermodynamic C_p shows the reversible or fast processes, such as glass transitions.

A glass transition and recrystallization of several spray-dried lactose samples having different amorphicity have been studied with StepScan DSC. The results show that the change of the specific heat at glass transition is proportional to the amorphicity measured with isothermal microcalorimetry (IMC) [Figure]. This means that the StepScan DSC is a very potential method for determining the amorphicity of samples.