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Full contact information appears alongside each article. Contributing companies would be delighted to hear from interested readers directly. Frederick Furness Publishing would also be very pleased to pass on to authors, or answer as appropriate, any queries you might have in relation to this publication or others in the series.

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"Injectable Drug Delivery 2010: Formulations Focus" is published by Frederick Furness Publishing.

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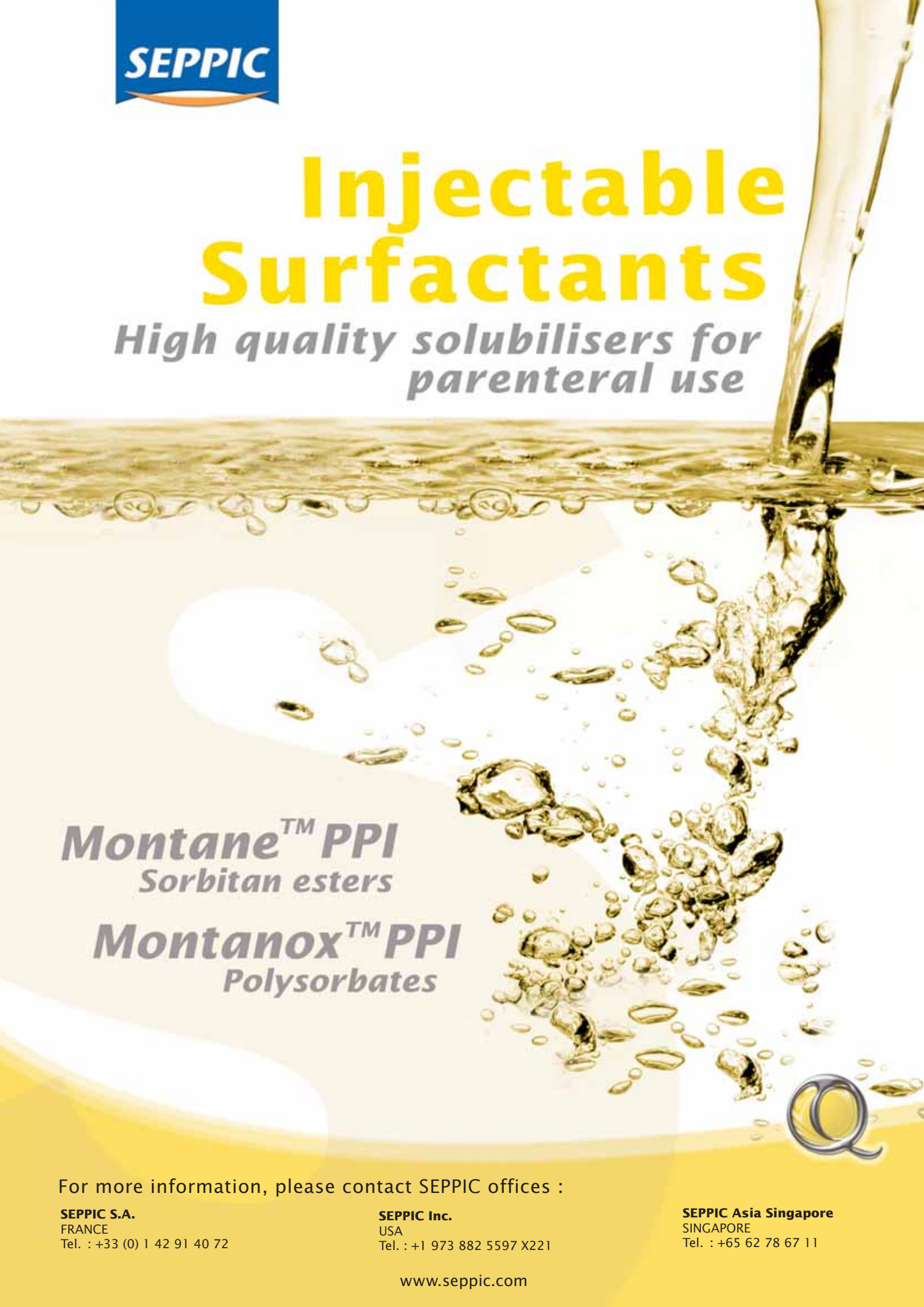

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INTRODUCTION

PROTEIN PHARMACEUTICALS – RECENT ACHIEVEMENTS AND TRENDS FOR FORMULATION AND DELIVERY

In this introductory article to the Injectable Drug Delivery (Formulations Focus) issue of ONdrugDelivery, we will focus on the delivery of protein therapeutics. The application of delivery technologies to formulate protein therapeutics in order to optimise or enable their development as viable pharmaceutical products is one of most important and fastest-growing areas of injectable drug delivery nowadays. However, this has not always been the case.

Personally, we remember from our own time as undergraduate students that certain professors declared protein pharmaceuticals to be "a temporarily existing hype that will go away".

The so-called hype has not only gone on to become one of the most active and intense periods of pharmaceutical R&D to date, but also one of the fastest growing business segments, today almost matching the more conventional small-molecule market in terms of revenues.

Protein pharmaceuticals have become a mainstay in the pharmaceutical product pipeline and today even the most traditional Schools of Pharmacy cannot ignore them. There have also been some very dramatic events with rises and falls of small, promising biotech start-ups in the late-1990s/early-2000s period that perhaps even Edgar Allan Poe could not plot in better words. Many lessons have been learnt both from the scientific perspective and in the financial world too. And probably, the learning curve still has to yet reach its apex.

In this transitional process we can look back and state: Yes, it is feasible to develop, and safely and economically to manufacture, most biopharmaceuticals for therapeutic use.

Challenges are still represented by the process to include the patient's perspective in terms of convenience of administration, and with regard to the tax payer, who wishes to have all therapeutic options available without paying too much for health insurance cover.

From the patient's perspective, much is being accomplished. As the first biopharmaceuticals put on the market are almost exclusively bound to needle-based routes of administration to be able to enter the systemic circulation, the second generation is aiming to reduce injection frequency (by using PEGylation strategies and depot technologies, for example), targeting the drug more efficiently to the site of action to reduce unwanted side-effects or to switch the administration from intravenous (requiring ambulant administration) to auto-injectors for subcutaneous or intramuscular administration to be handled by the patient him/herself.

After the recent withdrawal of Exubera™ insulin, the only systemically acting protein drug that had been made available as a pulmonary formulation, it may be another decade until serious attempts outside the parenteral route will reach the submission stage for market authorisation.

For the purpose of this editorial we will review a few examples of recent progresses in the field of parenteral protein delivery.

These are also discussed in much more detail in the recently published book: "*Protein Pharmaceuticals – Formulation, Analytics and Delivery*" [1], made possible through the strong support of the European non-profit organisation, APV (www.apv-mainz.de), which has also organised a series of seminars related to this same topic over the past ten years.

PEGYLATION

PEGylation, the covalent attachment of one or more polyethylene-glycol (PEG) molecules to a protein drug, was invented in the 1970s. Major players in the field of PEGylation today are, among others: Enzon Pharmaceuticals, Inc (Bridgewater, NJ, US); Nektar Therapeutics, Inc (San Carlos, CA, US); Mountain View Pharmaceuticals, Inc (Menlo Park, CA, US); Celltech (now UCB SA, Brussels, Belgium); Amgen, Inc (Thousand Oaks, CA, US), F. Hoffmann-La Roche Ltd (Basel, Switzerland); Schering-Plough (now Merk & Co, Inc, Whitehouse Station, NJ, US); and Eyetech, Inc (Palm Beach Gardens, FL, US).

So far, nine PEGylated products are on the market (see Figure 1). With the exception of Pfizer/Eyetech's Macugen, which is a PEGylated aptamer based on an oligonucleotide backbone, all marketed products are proteins.

The application of therapeutic proteins is facing several challenges, among which are:

- the mandatory application by injection
- low enzymatic resistance
- rapid renal elimination and resulting low t½ and AUC
- potential immunogenicity

PEGylation is posed to improve on these properties through shielding the protein against immunological recognition and enzymatic attack by steric hindrance, and reducing renal filtration by increasing the overall molecular weight, with 40kDa signifying the threshold molecular weight for PEGylated compounds. Reduction of local irritation or immune reactions at the injection site have also been reported after PEGylation.

The observation that PEGylated compounds or delivery systems accumulate preferably in tumor tissues or in joints under inflammatory conditions has caused the group of Maeda *et al* [2] to postulate the enhanced permeation and retention (EPR) effect. Overall, PEGylation results in a significant increase in retention time of the API in the systemic circulation, passive targeting by the EPR effect, reduction of immunological side effects, reduction in application frequency and thus general enhancement of patient compliance and adherence to therapy.

However, PEGylation also faces several challenges. Early N-hydroxysuccinimide chemistries involving attachment of PEG to amino functions did result in random PEGylation. This resulted in considerable challenges to production uniformity and quality assurance. In addition, PEG sites may be closely located to the reactive or binding site of the protein, which may impair its activity or binding affinity to the API's receptor. This, however, may not be true in every case.

While PEG-aldehyde chemistry allowed targeting of the N-terminus more specifically by variation of the reaction pH, more advanced thiol and maleimide chemistries allowed the specific PEGylation of free sulfhydryl moieties.

PEGylation can lead to a reduction of *in vitro* activity of the modified molecule, which does not necessarily correspond to a loss in biological activity *in vivo*. It is thought that biopharmaceuticals may have a higher binding affinity to their targets than

needed for cellular activity, and that this affinity is only partially reduced by PEGylation.[3] In general, a poor *in vitro*/*in vivo* correlation is observed for PEGylated compounds, making candidate selection processes tedious and time-consuming.

New avenues for permanent PEGylation have been developed by companies such as Neose (now in liquidation) and Polytherics (London, UK). The former has developed technology to glycosylate proteins expressed in Escherichia coli, or optimise glycosylation patterns by specific enzymatic GalNAc glycosylation of serine and threonine. The technology has been successfully applied to E. coli-expressed G-CSF, interferon-alpha, and GM-CSF[4] The glycopegylation technology was sold to Ratiopharm subsidiary BioGeneriX AG, in 2008.

Another PEGylation approach, developed by PolyTherics, includes the reduction of intramolecular bonds in disulfide bridges and successive annealing of these bonds by using a spacer molecule, which is itself attached to PEG. Studies in the PEGylation of interferon alpha-2b [5] show that PEGylation occurs completely site specifically, at 'accessible' disulfides only, and that the native protein conformation is maintained, the company states. In March 2010, PolyTherics entered into a research collaboration with Zealand Pharma (Copenhagen, Denmark) on the PEGylation of peptide therapeutics.

Disadvantages of the PEGylation process, such as possible reduction in bioactivity, may be overcome by a new class of linker molecules, binding PEG reversibly to the protein, virtually creating a pro drug from which the active principle is released by hydrolysis or enzymatic activity over a prolonged period of time.[6] An advantage of this technology, which is considered as the next PEG linker generation, is that the native compound is recovered, and may have better access to compartments within the body than the high-molecular-weight PEGylated compound. In addition, releasable linkers can be designed to show a certain release profile, or even site-specific cleavage.

Since its inception in the 1970s, PEGylation has matured into a technology that offers the opportunity to improve on the properties of the full spectrum of peptide-, protein- and oligonucleotide-based drugs. Although not a trivial feat, PEGylation using permanent linkers is generally regarded as a commodity. Permanent linker chemistries – useful or not – have been vastly patented, leaving little room for new IP, with the technologies developed by Neose Technologies and Polytherics being among the few exceptions.

The development of releasable linker chemistries appears as a step forward, possibly offering the opportunity to create true biogenerics, as the API is regenerated from the PEG linkage, regaining its specific pharmacokinetic and bioactivity profile. Though a few "releasables" have been developed, the proof of concept still needs to be shown in clinical trials.

Company	Product	API	Indication
Amgen	Neulasta	PEG-GCSF	Neutropenia
Enzon	Adagen	PEG-aminodeaminase	Severe Combined Immuno Deficiency (SCID)
Enzon	Oncaspar	PEG-asparaginase	Acute Lymphoblastic Leukemia (ALL)
tEyetech/Pfizer	Macugen	PEG-aptanib	Age-related Macular Degeneration (AMD)
Pfizer	Somavert	PEG-hGH antagonist	Acromegaly
Roche	PEGasys	PEG-interferon-alfa-2a	Hepatitis C
Roche	Mircera	PEG-epoeitin beta	Chronic renal failure
Schering-Plough	PEG-Intron	PEG-interferon-alfa-2b	Hepatitis C
UCB	Cimzia	Certolizumab pegol	Crohn's disease

Figure 1: Overview of marketed PEGylated products

In conclusion, the lessons learned in PEGylation technology are:

- An ideal PEG reagent is derived from simple, proven, straightforward chemistry that produces linkages at predictable sites, contains non-immunogenic and non-toxic spacers or linkers and produces reaction byproducts that are innocuous.
- *In vitro* activity of PEG products is not predictive of their biological activity.
- PEGylation at or near binding domains may not necessarily result in loss of biological activity.
- Site-specific PEGylation of antibodies or antibody fragments alleviates loss of antigen binding usually seen for random PEGylation and maintains binding affinity.
- Releasable PEGylation offers the opportunity to develop conjugates releasing the original API in a sustained-release pattern.

LIPID TECHNOLOGIES

Although delivery of proteins in connection with non-covalently associated lipids, such as liposomes, is still in its infancy, several remarkable attempts have been made in recent years.

Not only scientifically remarkable, but also from a financial point of view, is the ApoA1Milano story. The background for the discovery of this variant of the HDL-bound ApoA1 is from an observation of a family living close to Milan, Italy, who have an unusually high life expectancy without any noticeable cardiovascular diseases. The company Pharmacia (now Pfizer), before merging with Upjohn, found the Milano variant and started initial development. Later it was spun-out to a new biotech company in the late 1990s, called Esperion Therapeutics, Inc (Plymouth, MI, US).

What makes the molecule so special? Lipoproteins circulate in the bloodstream in the form of natural, lipid-containing nanoparticles such as high- and low-density lipoprotein (HDL and LDL, respectively). Natural HDL particles can have either a spherical or discoidal shape and contain about 50% protein (predominantly ApoA1 and ApoA2), phospholipids, 25% cholesterol (of which 70% is esterified) and 5% triglyceride. Small discoidal HDL contains primarily ApoA1, and a lipid monolayer consisting of phospholipids and free cholesterol.[7] ApoA1Milano is a rare variant of ApoA1, which is associated with high HDL-cholesterol blood levels without increased risk for atherosclerosis. Administration of ApoA1Milano (apoA-Im) halted plaque formation in animal models and resulted in more efficient efflux of cholesterol from existing atherosclerotic plaques.[8,9,10]

Interestingly, Esperion was bought in 2003 by Pfizer in a breathtaking deal for US$1.3 billion. In 2008, Esperion Therapeutics regained independence through a financing round of US$22.75 million, buying back the product rights from Pfizer [11] (which retains an undisclosed stake in the company).

MICROPARTICULATE DEPOT TECHNOLOGIES

Also remarkable are the up and downs of microparticulate protein depot systems. After considerable research efforts and probably a still more embracing regulatory environment the first depot formulation, containing human growth hormone (hGH), was brought to the market by Genentech (South San Francisco, CA, US) as Nutropin™ Depot in the late 1990s. However, after about five unsuccessful years on the market it was withdrawn for several reasons. Up to now, Nutropin™ Depot was the only protein depot product to have reached the pharmaceutical market place.

What lessons have been learned and what are the new trends?

To provide an answer, two aspects must be considered closely.

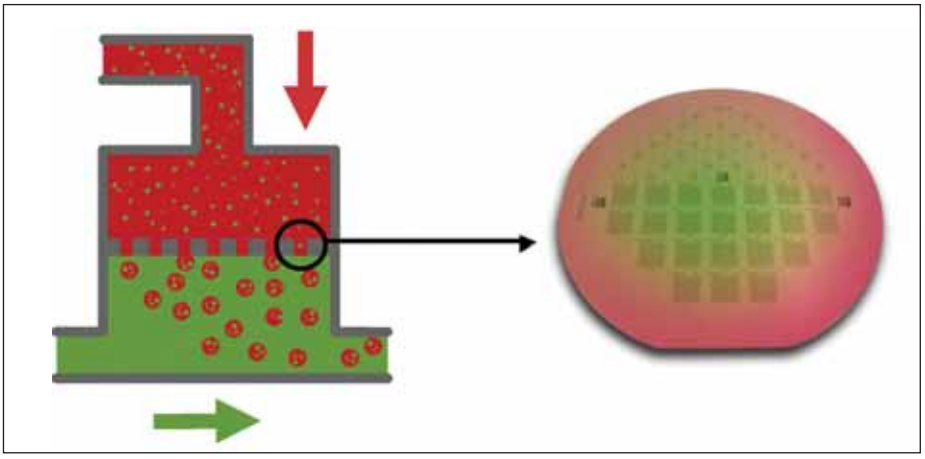

Figure 2: Schematic representation of the microsieve™ emulsification process. The dark red liquid (for example a w/o emulsion of the water-soluble drug in the polymer/solvent phase) is continuously processed through the silicon membrane (microsieve™) with defined pores into a medium (e.g. water) that does not mix with processed liquid. With this method the same w/o/w emulsions can be processed in a continuous fashion in contrast to bulky w/o/w batch productions as described previously.

The first is the polymer itself and the second is the process by which the protein is formulated into PLA/PLGA microparticles. The polymer is a highly lipophilic macromolecule, which requires particular organic solvents to dissolve adequately. Proteins, on the other hand, are mostly amphiphilic with a defined three-dimensional structure which is maintained by intramolecular Van der Waals forces and interaction between polar groups. If such a molecule is forced to interact with a highly lipophilic polymer structure it may irreversibly alter its conformation, for exampe, to a conformation which may not refold adequately after being released back into an aqueous environment.

Secondly, the process to make microspheres involves, for example, a double emulsion method in which, from the viewpoint of an amphiphilic protein, it is literally squeezed in-between two very different solvent phases and it will most likely choose to interact with its polar part with the aqueous phase and its lipophilic moieties with the polymer/organic solvent phase. When the solvents are in motion, forces may act on the protein molecule or the protein molecules may interact, both of which can impact protein stability. The stress situation is enhanced in the drying steps involving solvent evaporation and subsequently freeze-drying.

As can be concluded from the above, either alternatives to the PLA/PLGA polymer have to be considered, or the process has to be modified to a more protein-friendly method, preferably avoiding double emulsion techniques.

Concerning the choice of polymers, more amphiphilic structures are preferred apparently by the protein. Probably the most advanced, most promising product coming through the pipeline is Biolex Therapeutics' (Pittsboro, NC, US) Locteron™ for the treatment of hepatitis C infection. It contains Interferon-α2b encapsulated using the PolyActive™ technology from OctoPlus (Leiden, The Netherlands).

Recent interim Phase II data [12] demonstrated that, in comparison with PEG-Intron™, Locteron™ was able to reduce the PEG-Intron™ related side effects by 65% while maintaining an equivalent reduction of virus titers.

Interestingly, and commercially probably just as relevant, is the dosing every other week for Locteron™ instead of once-weekly with PEG-Intron™ and other PEGylated Interferon formulations currently on the market.

Considering the market for PEGylated interferons for HCV therapy is about US$1.4 billion, successful approval and launch of Locteron™ would encourage the many other companies engaged in development of protein depot formulations.

Another trend to be recognised is the move towards processing approaches that offer an alternative to double emulsion.

Several established *in situ* solidifying hydrogel approaches are in development. For example, the Atrigel™ delivery system, developed by QLT, Inc (Vancouver, BC, Canada) and now under development also by QLT spin-out Tolmar, Inc (Fort Collins, CO, US), was featured in the 2006 Safer Injections issue of ONdrugDelivery (http://www.ondrugdelivery.com/publications/safer_injections.html).

The ReGel™ system, initially developed by Protherics and Macromed and now owned by BTG PLC (London, UK), is being applied in oncology and ophthalmic indications, amongst others. It is a thermosetting biodegradable gel that solidifies when injected into the body and is designed to provide high local concentrations of a drug for a sustained period.

A third example is Durect's (Cupertino, CA, US) SABER™ delivery system. It uses a highly viscous base component, such as sucrose acetate isobutyrate, to provide controlled drug release. When the base component is combined with drug, biocompatible excipients and other additives, the formulation is liquid enough to inject easily with a standard needle and syringe. After injection, the excipients diffuse away, leaving a viscous depot.

We would also like to point to what is in our personal opinion a still hidden jewel. This concerns the microsieve™ emulsification technology of Nanomi (Oldenzaal, The Netherlands), which utilises silicon membranes with defined pore sizes and shapes that are made by photolithographic techniques widely used in the semiconductor industry. In the microsieve™ emulsification process, monodisperse droplets are generated by dispersing one fluid into a second immiscible fluid through millions of tiny pores, where every pore has the same size and shape (see Figure 2).

Since every pore is essentially the same, every droplet generated by the membrane appears to be similar, resulting in highly uniform, reproducible and size-controlled droplets or, after an appropriate solidification step, highly uniform, reproducible and size-controlled particles. A unique feature of the microsieve™ emulsification technology is that the droplet size is mostly independent of the precise formulation and the transmembrane pressure, and solely determined by the membrane design.

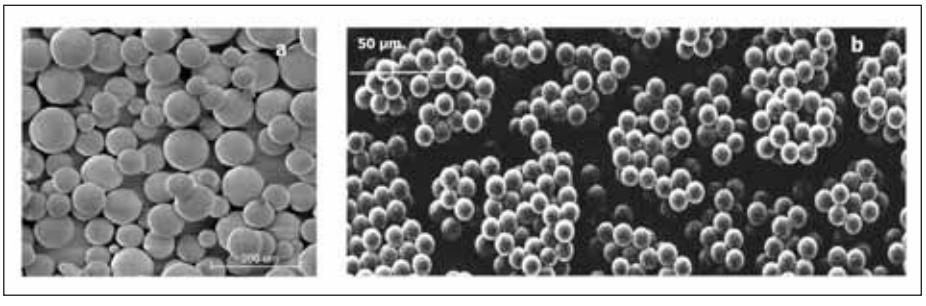

Figure 3: Microspheres for intramuscular delivery fabricated by a conventional method (a) compered with microspheres of the same formulation for intravascular delivery fabricated by microsieve emulsification (b). The latter allows increased monodispersity and the reduction of particle size (from 100-50 µm to around 6.5 µm).

A continuous process in which the size of the membrane and the number of membranes can be altered easily, the process appears to be readily scaled-up, compared with the established batch-controlled, double-emulsion process.

The derived particles are highly uniform and monodisperse (see Figure 2) which also opens the doors to vascular drug targeting or – in a more immediately practical sense – to improve syringeability compared with systems generating wider particle size distributions, since larger and smaller particles can be omitted.

CONCLUDING REMARKS

In this article, we could only take out short extracts on recent trends and developments in the field of protein drug delivery from our recently published book: *Protein Pharmaceuticals*. However, even this short piece shows clearly the R&D landscape in this field to be highly dynamic and colourful.

Many different disciplines of scientific research come together creatively to solve the challenges inherently connected when aiming to make the needle-based route of protein drug delivery more convenient, safer and more efficient for the therapy of the patient. With this in mind we are quite confident that the coming decade will bring exciting new developments and significant commercial success. It is clear that our jobs are in no danger of become boring at all!

Henrik L. Luessen, Tytonis BV, The Netherlands, & Gerrit Borchard, University of Geneva, Switzerland.

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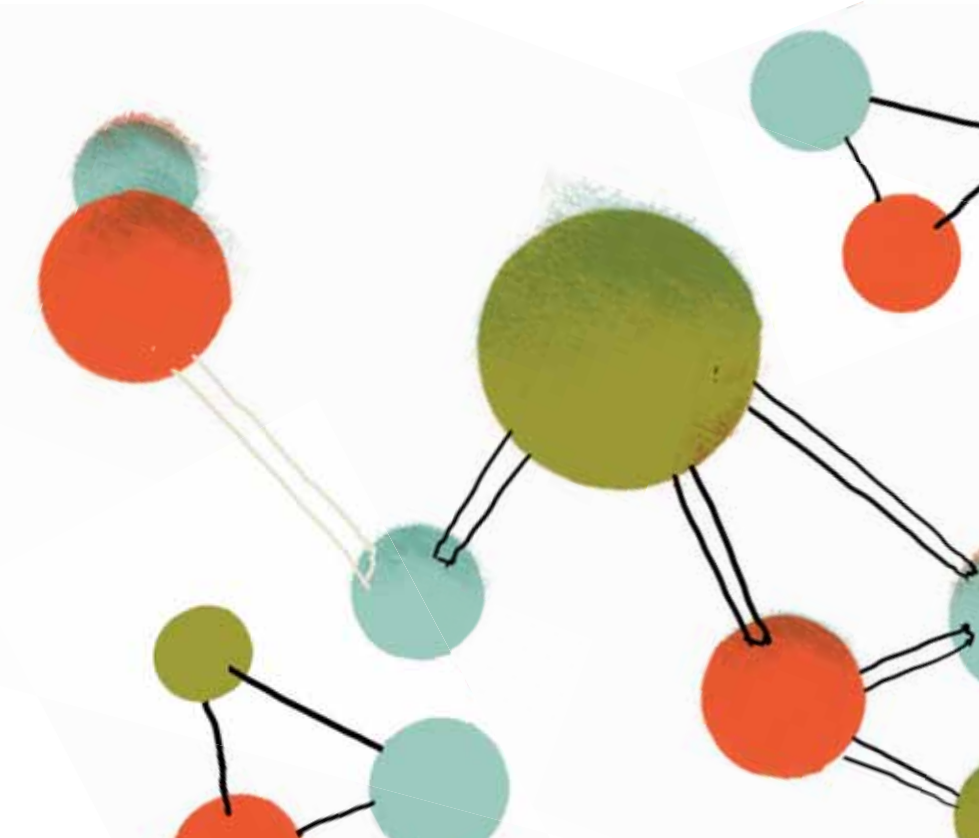

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LIPID LIQUID CRYSTALS FOR PARENTERAL SUSTAINED-RELEASE APPLICATIONS:

COMBINING EASE OF USE AND MANUFACTURING WITH CONSISTENT DRUG RELEASE CONTROL

Lipid liquid crystal phases are well-defined three-dimensional structures comprising co-existing lipophilic (lipid) and hydrophilic (aqueous) domains at the nanoscale. These are present either as an interconnected network or as isolated, discrete volumes. The dual chemical nature of LLC phases allows accommodation of a wide range of drug compounds, including peptides and small molecules. In this article, Professor Fredrik Tiberg, President and CEO of Camurus AB, and Dr Fredrik Joabsson, Director, Drug Delivery Systems and Technical Business Development, explain how, by exploiting the *in situ* self-assembly from lipid solution to LLC phase it is possible to create an adaptive drug delivery system (DDS) that combines ease of manufacturing and injectability, with consistent long-acting drug release.

Lipid liquid crystal (LLC) phase formation is the basis of Camurus' FluidCrystal® injection depot, which is presented as a low-viscocity mixture of long-chain lipids – for example, a soy phosphatidyl choline (SPC) and glycerol dioleate (GDO), together with small amounts of solvent – which forms LLC phases. Upon contact with minute quantities of aqueous fluids present in the tissue, the FluidCrystal delivery system self-assembles in a controlled manner into one or more reverse LLC phases, thereby effectively encapsulating dissolved or dispersed active pharmaceutical ingredients (APIs) and restricting diffusive transport to the surrounding tissue.

"FLUIDCRYSTAL IS WELL SUITED FOR USE AS AN ENABLING DELIVERY SYSTEM FOR COMPUNDS WITH VERY SHORT HALF-LIVES"

The use of a two-component lipid system, where each component favours a different phase structure, means that it is possible to tune phase behaviour by composition in order to optimise release and compensate curvature effects of dissolved drug. By exploiting LLC phases that are thermodynamically stable in excess aqueous water, a persistent sustained-release reservoir is assured, which is slowly degraded *in vivo* with the assistance of endogenous enzymes, and releases the API.

LIPID LIQUID CRYSTAL PHASES

Lipids consist of a polar group chemically linked to one, two or three fatty hydrocarbon chains. Non-polar-lipids with relatively small head groups – triacylglycerols (triglycerides), for example – interact very weakly with water and do not form liquid crystal phases. Polar lipids on the other hand are amphiphilic by nature and interact more strongly with polar water molecules, and spontaneously organise (self-assemble) to form a wide range of nanostructured phases.

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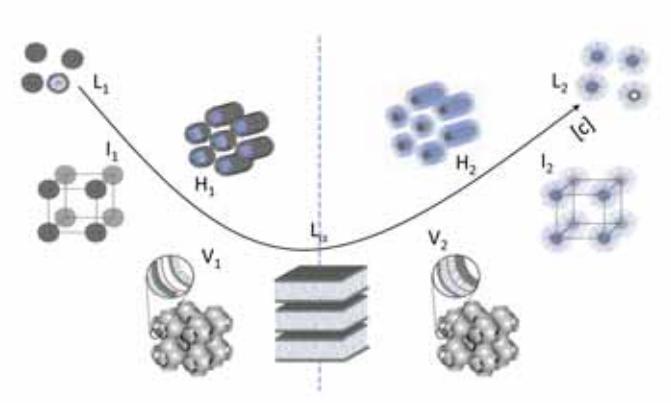

Figure 1: Common self-assembly structures of polar lipids in aqueous media. The lamellar liquid crystalline phase (L_α) can be regarded as the mirror plane (dashed line) between normal "oil-in-water" structures to the left and reversed "water-in-oil" structures to the right. On both sides, there are possibilities for forming cubic bi-continuous (V_1, V_2), hexagonal (H_1, H_2), cubic micellar (I_1, I_2), and micellar phases (L_1, L_2), as well as other intermediate phases.

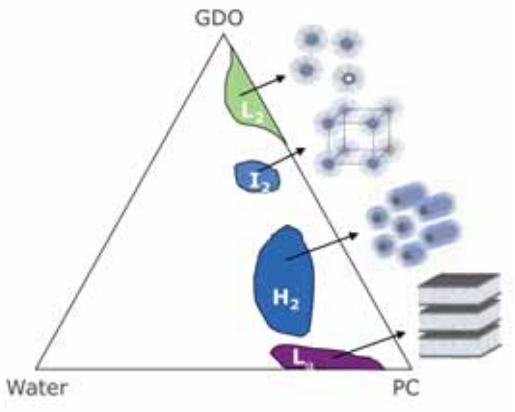

Figure 2: Schematic ternary phase diagram of glycerol dioleate (GDO), phosphatidyl choline (PC) and water. Four different water-swollen lipid phases are found which all are stable in excess water. They are reversed micellar (L_2), reversed micellar cubic (I_2), reversed hexagonal (H_2), and lamellar phase (L_α). The I2 and H2 phases in the GDO/PC/water system are the basis for the FluidCrystal Injection depot delivery system.

Figure 1 shows an idealised phase sequence from normal oil-in-water structures on the left-hand side, to reverse water-in-oil structures on the right-hand side of the planar lamellar structures in the mirror plane.

From a sustained-release perspective, the most interesting lipid phases are the reversed water-in-oil phases as they are stable in excess water and can effectively entrap various drug compounds. Lipids with long hydrocarbon chains are particularly interesting due to their low aqueous solubility providing resistance to fragmentation and lipid monomer formation during exposure to excess water present at the site of injection.

FluidCrystal depot formulations are based on liquid solutions of naturally occurring lipids; typically phosphatidyl choline (PC) and glycerol dioleate (GDO), but also other alternative combinations. It is important that the two lipids are fully miscible in the liquid state as this allows for easy manufacturing and handling.

Small amounts of co-solvent, such as ethanol or propylene glycol, may be added to reduce viscosity. When PC and GDO are mixed in around equal proportions and added to an aqueous environment, reversed cubic and/or reversed hexagonal LLC phases are formed which have been shown to be suitable for sustained-release applications (see Figure 2).

"THE FLUIDCRYSTAL SYSTEM ENABLES TRUE READY-TO-USE PRODUCTS, WHILE CURRENT MARKETED COMPETITORS REQUIRE RECONSTITUTION"

CONTROLLED-RELEASE MECHANISM AND PHARMACOKINETICS

An illustration of the evolution of the FluidCrystal Injection depot *in vivo* after subcutaneous injection is shown in Figure 3. The LLC phase develops immediately upon contact with aqueous media present in the tissue at the site of injection. As shown, the process progresses from the outside inwards, providing rapid encapsulation of the dissolved substance. Thereafter, the active substance is released by a combination of restricted diffusion and depot biodegradation.

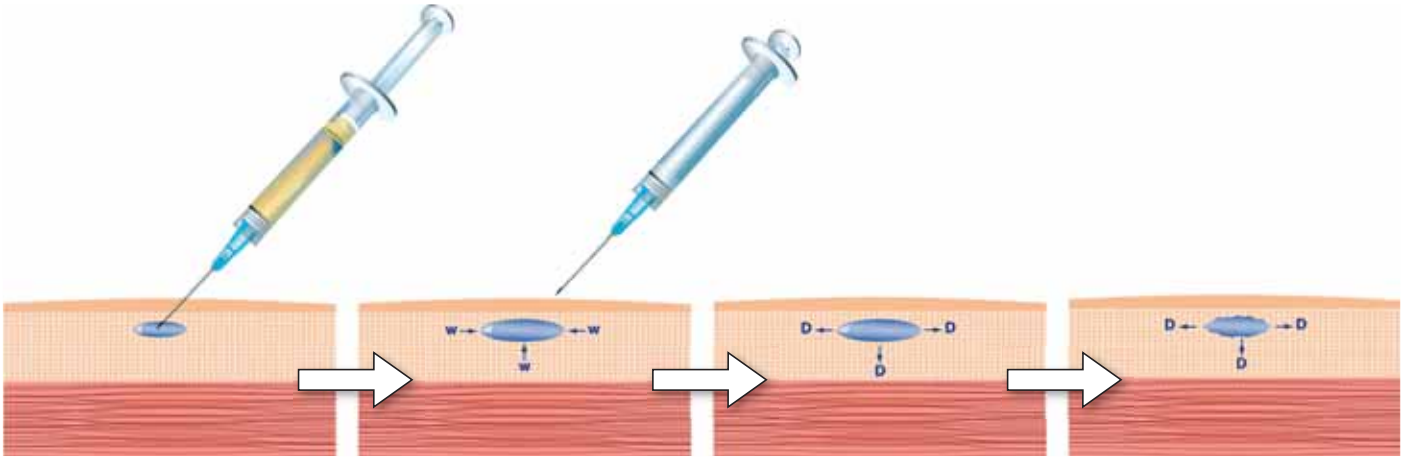

Figure 3: Sketch showing the evolution of a FluidCrystal Injection depot following a subcutaneous injection: absorption of ambient aqueous fluid and lipid self-assembly; and lipase-assisted degradation and erosion of the depot. W: water; D: drug.

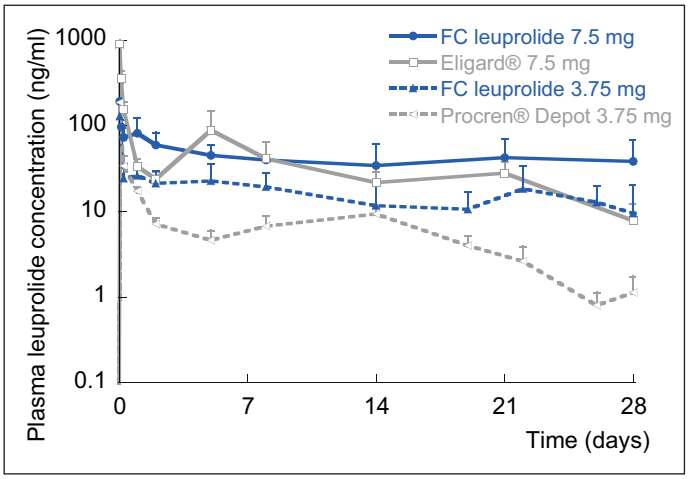

Figure 4: Mean plasma leuprolide concentration versus time (semi-log scale) after single subcutaneous injections of two FluidCrystal (FC) formulations containing 3.75 mg and 7.5 mg leuprolide, respectively, and the corresponding marketed Procren® Depot, analogue to Lupron® Depot in the US (Abbott) and Eligard® (Astellas Pharma) products in a rat model (n=6) (Camurus AB). All formulations and products tested are designed for a therapeutic duration of one month.

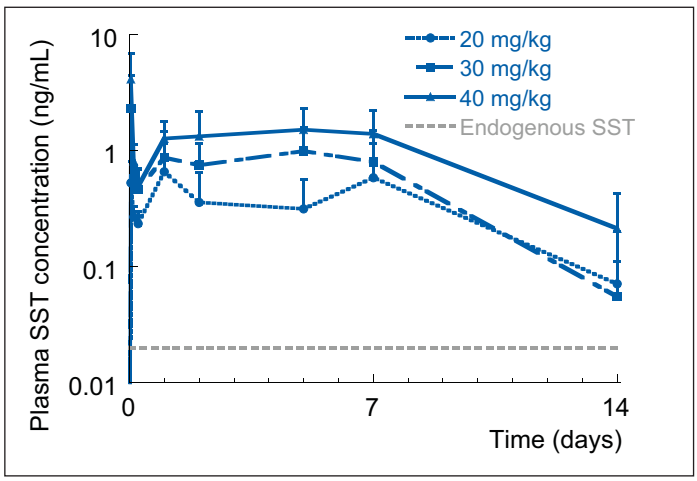

Figure 5. Mean plasma somatostatin (SST) concentration over 28 days (semi-log scale) after a single subcutaneous (filled circles) administration of FluidCrystal formulations with different doses of somatostatin(1-14) in a rat model (n=6).

An example of *in vivo* release data obtained for a FluidCrystal formulation of a peptide is provided in Figure 4. The data show the release of the peptide hormone leuprolide after single-dose subcutaneous injections. Corresponding release curves for marketed leuprolide products are also provided as reference.

Following injection, the FluidCrystal system gave stable and dose-dependent leuprolide plasma values for more than one month, after which the release decays rapidly as the depot degrades and becomes completely empty. Corresponding marketed PLA/PLGA-based microsphere and gel products show a more pronounced initial release/burst and thereafter lower and less stable plasma values over time.

As the long-term release from the FluidCrystal Injection depot is mainly controlled by the degradation of the lipid matrix itself, the shape of the release profile is largely independent of the half-life of the encapsulated API. This means that the FluidCrystal system is well suited for use as an enabling delivery system for compunds with very short half-lives, the therapeutic use of which would otherwise be limited. Figure 5 exemplifies this aspect by showing consistent release kinetics of three doses of a FluidCrystal somatostatin formulation for one week. In contrast, unformulated endogenous somatostatin has a half-life of only 1-2 minutes.

"FLUIDCRYSTAL IS A LIQUID COMPATIBLE WITH, FOR EXAMPLE, PREFILLED SYRINGES, ENSURING EASY HANDLING PRIOR TO ADMINISTRATION AND STRAIGHTFORWARD MANUFACTURING"

Besides extending the release of peptides and protein therapeutics, the FluidCrystal Injection depot is also being exploited for development of small-molecule therapeutics. Figure 6 shows the release profiles obtained for subcutaneously injected buprenorphine. After a relatively rapid increase of the plasma buprenorphine levels with maximum values after about a day, a very slow decay is observed with elevated levels being measured for at least 28 days. Depending on the administered dose and dose volume, C_{max} values of only 2-10 times those of C_{28days} are typical.

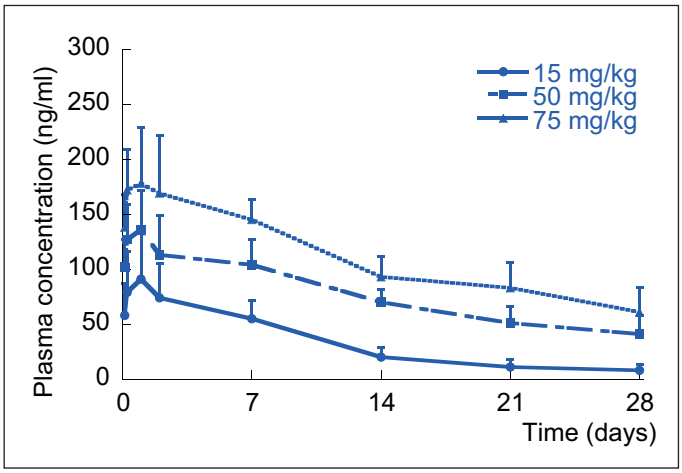

Figure 6. Mean plasma buprenorphine concentration over 28 days after a single subcutaneous administration of different doses of a buprenorphine FluidCrystal formulation in a rat model (n=6).

METHOD OF PREPARATION AND MANUFACTURING

Polymer-based depot systems, such as poly(lactic-co-glycolic acid) (PLGA) microspheres, have to be reconstituted in an aqueous medium before injection. In addition to the complex handling of microsphere-based products, manufacturing and process validation pose challenges which often require dedicated specialised manufacturing equipment or even complete dedicated manufacturing sites for the commercial production.

The FluidCrystal system is a liquid compatible with, for example, prefilled syringes, which ensures not only easy handling prior to administration but also straightforward manufacturing. The manufacturing process includes only a few standard steps: mixing and sterile filtration, followed by filling of vials, prefilled syringes, or cartridges for pen systems.

Figure 7 shows a comparison between the administration devices

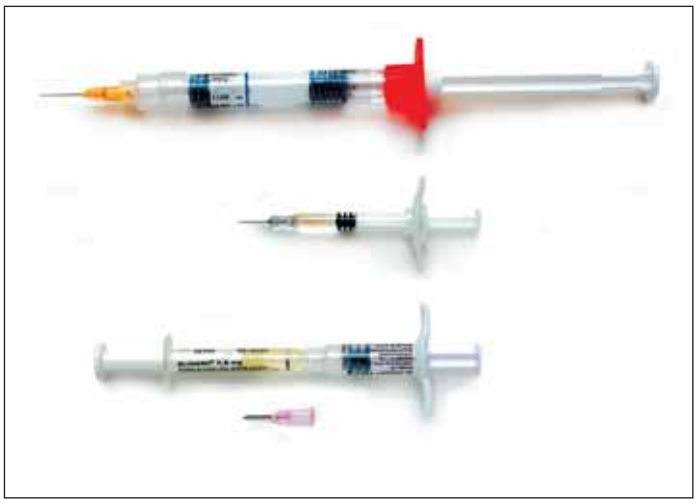

Figure 7. Comparison between the administration devices of the FluidCrystal based leuprolide product Prosenze® (middle) with its corresponding marketed products Procren® Depot (Lupron® in the US, above) and Eligard® (below). All the shown products are designed for a therapeutic duration of one month.

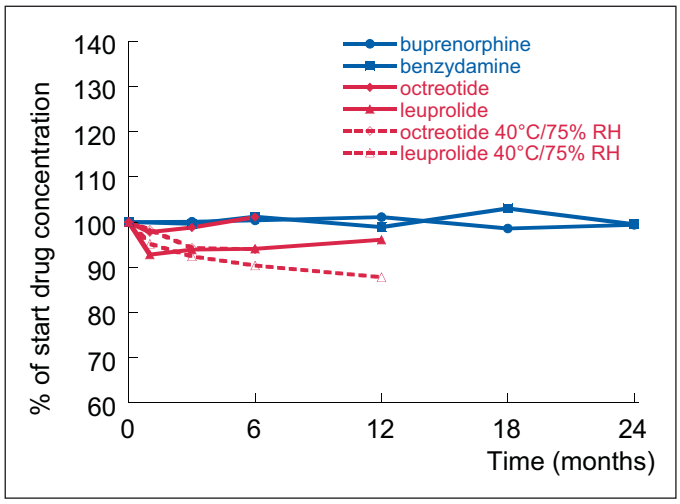

Figure 8. Change in the drug content of the small molecules buprenorphine and benzydamine (blue), and of the peptides octreotide and leuprolide (red) formulated in the FluidCrystal Injection depot. Data refer to samples stored for up to 24 months at 25°C/60% RH (full lines) and for octreotide and leuprolide also up to 6 months at 40°C/75% RH (dashed lines).

of Camurus' FluidCrystal leuprolide clinical-stage development product with the marketed products Procren® (same as Lupron®) and Eligard®. The FluidCrystal system enables true ready-to-use products, while current marketed competitors require reconstitution steps prior to administration.

STABILITY IN LIPID DELIVERY SYSTEMS

Stability is a key issue in any pharmaceutical product development, both in relation to storage and use. The ideal physical form of long-acting LLC products, including FluidCrystal, is in the form of a ready-to-use liquid with the drug substance dissolved. As these solutions are non-aqueous, they present a very different chemical environment compared with that of ordinary aqueous parenteral solutions or lyophilisates. Importantly, the solutions can also be designed to provide very good chemical stability of dissolved active compounds over time, as demonstrated by the data shown in Figure 8.

Due to the inherent instability of peptide and protein formulations, storage stability is an issue of considerable concern. Many of the underlying processes, such as hydrolysis, oxidation, and aggregation, are enhanced in aqueous environments, but significantly inhibited in water-free lipid systems. As an illustration of this, the FluidCrystal octreotide and leuprolide formulations included in Figure 8 demonstrate good stability and show potential as room-temperature-stable peptide drugs. Corresponding marketed peptide products, such as Sandostatin LAR® and Eligard®, require refrigerated storage.

CURRENT DEVELOPMENT STATUS OF FLUIDCRYSTAL INJECTION DEPOT

Camurus currently has three injectable products based on the FluidCrystal technology in clinical development: two long-acting peptide products for acromegaly and prostate cancer, respectively; and one buprenorphine product for the treatment of opiate addiction.

The preclinical and clinical documentation gathered to date, with a total exposure time in patients of more than 10 years and in animals of several hundred years, has demonstrated the FluidCrystal Injection depot to be safe and locally tolerable when administered subcutaneously and intramuscularly. All lipid excipients used are generally recognised as safe (GRAS) and have well characterised metabolic pathways.

PC is used in several parenteral products, while an in-house bridging toxicology program has been performed in regards to the parenteral use of GDO. With the expanding pool of pharmacokinetic, safety, local tolerability, CMC and regulatory data, the prospects for bringing forward new drug candidates using LLC delivery systems have become extremely favourable.

Ease of manufacturing, using only standard pharmaceutical processing steps and ready-to-use injection devices, distinguishes the FluidCrystal Injection depot from the currently marketed DDS technologies based on polymer microspheres and gels, which typically require specialised processing equipment as well as reconstitution before injection. High compatibility with standard devices, including prefilled syringes and cartridge pen systems, furthermore results in a favorable cost of goods and allows easy handling and injection by patients and healthcare professionals through thin needles, typically in the range 25-27 G.

ABOUT CAMURUS

Camurus provides innovative nanoscale drug-delivery systems for development of high-value therapeutics. The advanced delivery solutions range from long-acting depots to lipid nanocarriers designed for improved intravenous, transdermal and oral delivery. These are used in partner projects with biotech and pharma companies worldwide for enabling and improving delivery of a wide range of difficult drug compounds, including peptides, proteins, and insoluble small molecules.

Camurus also develops its own drug product candidates, which exploit the varied benefits of the proprietary FluidCrystal® and FluidCrystal® nanoparticle technologies. Camurus' in-house product portfolio addresses significant healthcare needs in areas of growth-hormone disorders, oncology, cancer supportive care, metabolic disease, and drug addiction – medicines that are innovative and effective and that offer added patient benefits, including improved safety and convenience of handling and administration.

Camurus is privately owned and based in Lund, Sweden. The company was founded in 1991 out of a long history of research on LLC structures at Lund University. To date, Camurus' technology is used in three marketed products. For more information, visit Camurus' web site at: www.camurus.com.

FluidCrystal® Injection depot – a new generation parenteral sustained release system.

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- Tunable duration from days to months
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COMPANY PROFILE - NANOMI

Nanomi develops microsphere-based drug delivery systems for the controlled release of small molecules, peptides, proteins and other therapeutic compounds. Nanomi's Monosphere™ technology, based on its proprietary microsieve™ emulsification process, enables the production of highly monodisperse microspheres in a robust, reproducible and cost-effective way.

Monosphere™ technology brings the precision and reliability of semiconductor technology to the process of microsphere manufacture. Silicon microsieves™ with uniform pores applied in an emulsification process provide microspheres of very uniform and predictable size. This total control of particle size together with its robustness, reproducibility and straightforward scalability make Monosphere™ a valuable asset for the development and manufacture of microspheres for injectable depots. This value is underlined by three of the "top ten big pharma" companies being among Nanomi's customers.

The control of particle size provided by the Monosphere™ technology allows for less painful injection through very thin needles (see Figure 1). The versatility and mild process conditions allow the encapsulation of a wide spectrum of compounds, for instance sensitive molecules like peptides/proteins, and at the same time enables the manufacture of microsphere formulations for parenteral drug delivery via the typical routes of IV, IM, SC, intra-articular, etc. New delivery strategies such as local delivery to an organ through capillary embolisation by injecting microspheres of a specific uniform size in the artery of that particular organ can also be accomplished.

Monodispersity is of particular interest in some therapies, such as cancer treatment. An example is tumor chemo-embolisation, where a dual mode therapeutic approach can be followed by injecting a monodisperse microsphere depot containing antineoplastic drugs into the tumour blood supply. On one side, targeted delivery is achieved by the release of the antineoplastic drug into the tumor and simultaneously, tumor growth is prevented and shrinkage enhanced by blocking the arteries that supply blood to the tumor.

In depot formulations, particle size is a crucial parameter that should be controlled when designing microsphere drug delivery systems. Size is extremely important to achieve the desired release behaviour but also in relation to the route of administration. The smaller the microspheres the better the injectability and the smaller the needle gauge required, which directly relates to patient comfort. Controlling the lower and upper size limits is essential to avoid large product losses by fractionation of the unwanted particles, activation of the immune system caused by too small particles and variations in the release profile.

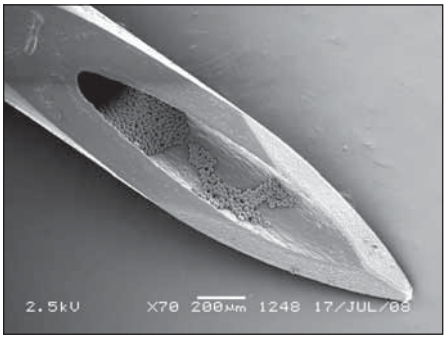

Figure 1: PLGA monodisperse microspheres (21 µm) in a 27 G needle

Therefore, Nanomi focuses its efforts on developing monosphere formulations with a specific size and tight size distribution (typically with a coefficient of variation of <5%), which can be chosen for optimal product performance, especially in pharmaceutical products with highly valuable active compounds.

Currently, microspheres in the range of 1-50 µm are routinely manufactured with narrow size distributions in the multiple gramme range. The process can be straightforwardly scaled up to >1 kg/day.

Nanomi is in the process of integrating its microsieve™ emulsification process in a GMP fill-and-finish facility, which is expected to be operational in 2011.

MONOSPHERE TECHNOLOGY

The heart of the Monosphere™ technology is the proprietary microsieve™ emulsification process. Microsieves™ are silicon membranes fabricated by precise and highly reproducible semiconductor technology in a cleanroom environment, with excellent uniformity of pore size and shape, in a highly reproducible way. The microsieves™ have very high chemical and thermal resistance.

Process compatibility:

- Solvent evaporation
- Melt emulsification
- Monomer emulsification
- O/W, W/O, W/O/W, S/W/O and S/O/W systems
- Large range of materials, solvents and active compounds (hydrophilic/hydrophobic)
- Aggressive cleaning agents and methods, including autoclaving

Compared with other particle production methods, Nanomi's technology offers total size control and a robust, uniform and predictable process to manufacture monodisperse microspheres. Particle size is independent of the formulation and is solely determined by the membrane design.

PARTNERSHIP OPPORTUNITIES

Nanomi develops its proprietary technology platform for the precise production of droplets and particles, and targets business-to-business partnerships with companies in the life sciences field that have a high value product/application that can strongly benefit from its technology. Nanomi does not distribute and market products on its own.

ABOUT NANOMI

Nanomi is an independent, privately owned Dutch company specialised in the formulation and development of microsphere-based drug delivery solutions. The company, which is located in the East of the Netherlands, was founded in 2004 and has been profitable from the outset. Nanomi has successfully entered into business agreements for the development of products for several top multinational companies.

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INTRATHECAL DELIVERY OF PROTEIN THERAPEUTICS TO TREAT GENETIC DISEASES INVOLVING THE CNS

Protein therapeutic delivery to the CNS presents extremely difficult challenges and is an underdeveloped area of research. Here, Dr Zahra Shahrokh, Senior Director, Pharmaceutical and Analytical Development, Dr Perry Calias, Senior Director, Nonclinical Development, and Dr Lawrence Charnas, Medical Director, all of Shire Human Genetic Therapies, Inc, describe some of the development hurdles that must be overcome in this area, and provide data on their most recent advances in intrathecal (IT) delivery of enzyme replacement therapies for CNS indications.

A focus on enzyme replacement therapies (ERT) for lysosomal storage diseases has led Shire Human Genetic Therapies, Inc (Shire HGT) to develop products for treating Fabry disease, Hunter syndrome, and type 1 Gaucher disease. These products are administered intravenously (IV) and are effective in treating the somatic symptoms of the disease.

Developing ERT for diseases involving the CNS is a challenge because IV administered enzyme does not adequately cross the blood-brain barrier (BBB) at a level needed for therapeutic effect. Moreover, formulations that are suitable for CNS administration are limited, posing a major challenge in generating stable products of adequate concentration.

By developing new methods and formulations to deliver enzymes to the CNS, Shire HGT is at the forefront of developing ERT for treating lysosomal storage diseases with CNS involvement. ERT for CNS symptoms in Hunter syndrome and Sanfilippo A syndrome are in Phase I clinical development, and efforts are ongoing in preclinical development for metachromatic leukodystrophy and globoid cell leukodystrophy.

STRATEGIES FOR CNS DELIVERY OF THERAPEUTICS:

The blood-brain barrier is a structural feature of endothelial cells which restricts the diffusion of microscopic objects (such as bacteria) and large or hydrophilic molecules (such as proteins) into the cerebrospinal fluid (CSF), while allowing the diffusion and active transport of selected small molecules.[1] This barrier creates a challenge in delivering adequate levels of protein therapeutics to the brain when administered by IV injection. Several strategies have been designed to overcome this hurdle for CNS drug delivery:[2]

- Receptor-Mediated Transport: Some large molecules essential for brain function are transported across the blood-brain barrier through active transport or transcytosis. These molecules or their mimetics can be used as vehicles for delivering peptides and other compounds to the brain. The transferrin uptake system has been well studied as a possible avenue into the brain following systemic administration. Covalent association of transferrin to a protein has resulted in enhanced delivery to the brain following intravenous administration.[2,3]
- Convection-Enhanced Delivery: This modality involves the stereotactic placement of several catheters into brain parenchyma through cranial burr holes and the subsequent infusion of agents via a microinfusion pump.[4] Convection-enhanced delivery uses a pressure gradient established at the tip of an infusion catheter to push a drug into the extracellular space. The intention is to distribute the drug more evenly, at higher concentrations, and over a larger area than when administered by diffusion alone. The placement and monitoring of these devices requires specialised technology which is limited to only a few centres across the globe. This factor, along with the invasive nature of the technique, has limited

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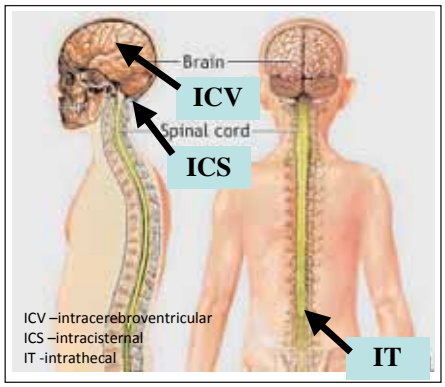

Figure 1: Routes of CNS Delivery

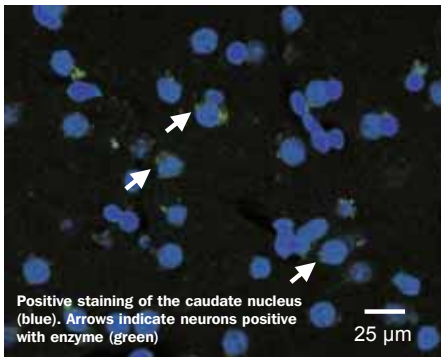

Figure 2: Distribution of a Lysosomal Enzyme into Neurons after an IT dose of 30 mg

the utility of this approach for the treatment of rare diseases.

- Transient Hyperosmotic Opening of BBB: Mannitol-induced transient opening of BBB has been shown to effectively deliver macromolecules to the brain.[5] However, risk of introducing bacteria and viruses outweighs the benefit of therapeutic delivery in paediatric genetic diseases.

The routes of CNS delivery are shown in Figure 1 and briefly described below:

- Intraventricular (ICV) Delivery: This route delivers drugs directly into the ventricle. ICV delivery can be facilitated via the Ommaya reservoir or other access port that is implanted in a pocket in between the scalp and the periosteum on top of the head, with a leading catheter placed directly into the ventricle. Clinical trials with GDNF were conducted with monthly administration of the protein for eight months using intraventricular cannula connected to an implanted access port.[6]
- Intracisternal (ICS) Delivery: Direct delivery to the CSF of the cerebellomedullary cistern (cisterna magna) is a procedure commonly used in animal species due to logistical ease compared with ICV or IT-Lumbar in smaller rodents. This route of administration is less commonly used in human studies.
- IT-Lumbar Delivery: IT administration is the most common route for direct administration

Solution	Na^+ mEq/L	K^+ mEq/L	Ca^{++} mEq/L	Mg^{++} mEq/L	$HCO3^-$ mEq/L	Cl^- mEq/L	pH	Phosphorous mg/L	Glucose mg/L
CSF	117-137	2.3	2.2	2.2	22.9	113-127	7.31	1.2-2.1	45-80
Elliotts B	149	2.6	2.7	2.4	22.6	132	6.0-7.5	2.3	80

Figure 3: Composition of CSF and Elliott's B Solution

of drugs into the CSF.[7] Procedurally, this is done by either lumbar puncture (slow bolus) or via port-catheter delivery systems (infusion or bolus). An implanted catheter is connected to a reservoir (for bolus) or an infusion pump, either implanted or external. The catheter is most commonly inserted between the laminae of the lumbar vertebrae and the tip is threaded up the thecal space to the desired level (generally L3-L4). IT delivery of ERTs via lumbar puncture has been described.[8-10]

INTRATHECAL DELIVERY CONSIDERATIONS

Distribution of a therapeutic following IT administration is primarily dependent on CSF flow and diffusion into the brain tissue.[11] The CSF is produced at 20 mL/hr in humans with a turnover of 3.7 times per day. CSF flow is initiated from its site of production (the choroid plexus) in all ventricles, enters cisterna magna via holes (foramina), circulates over the surface, and reabsorbs in arachnoid granulation. There is a bidirectional flow around the spinal chord which should facilitate diffusion of IT administered drugs towards the brain following lumbar puncture.

Protein delivery to the brain is typically diffusion limited. When nerve growth factor was administered as a polymer implant into the brain interstitium, it diffused into brain tissues only 1-3 mm over several days.[12] Simulation analyses indicated that this slow penetration is due to the protein's slow diffusion rate.[13]

Currently, CNS therapies requiring neuronal delivery of the drug are limited to small, hydrophobic molecules that enter cells by membrane diffusion or by modification of proteins to utilise active transport processes.[2, 14] In contrast, we have observed considerable brain tissue distribution following IT delivery of our lysosomal enzyme therapeutics without any protein modification (Figure 2).

Such unique brain distribution may be due to axonal transport [15] by way of the glycosylation structure that targets uptake into the target tissues and organelles. Mannose-6-phosphate (M6P) receptor-mediated uptake of M6P-containing glycoproteins targets our enzyme therapeutics to the cells and subsequently into the site of action in the lysosome. Neurons have been shown to contain M6P receptors [16] and internalise lysosomal enzymes.[17]

PROTEIN SOLUBILITY AND STABILITY CONSIDERATIONS

General considerations for formulations for CNS delivery were summarised by Grouls.[18] IT-Lumbar delivery is limited by the delicate

Drug Name	Method of IT Administration	Dose Volume	pH	Excipients
baclofen	device, bolus and infusion	2mL	5 to 7.0	NaCl, Water
bupivacaine	incremental doses per volume	2 to 20 mL	4.0 to 6.5	NaCl, Water
cytarabine	bolus injection	6 mL	7.4 to 7.7	NaCl, Water
Depocyt	bolus injection (1 to 5 minutes)	5 mL	5.5 to 8.5	NaCl, Water, Lipids
iohexol	bolus injection (1 to 2 minutes)	up to 17 mL	6.8 to 7.7	0.1 mg EDTA
morphine sulfate	bolus injection	1 to 2 mL	2.5 to 6.5	NaCl, Water
morphine sulfate	bolus injection	5 mL	5.0 to 8.0	NaCl, Water
penicillin G potassium buffered	bolus injection		6.0 to 8.5	6.8 mg NaCl, 65.6 mg KCl, buffered with Na citrate and citric acid
prialt	microinfusion device, long term	N/A	4.0 to 5.0	NaCl, L-methionine
sufentanil citrate	slow injection/infusion	0.2 to 1 mL	3.5 to 6.0	citric acid to adjust pH

Figure 4: List of Approved Intrathecal Formulations

Excipient/ Formulation	Drug Name	pH, volume, delivery mode/frequency	Species	References
100 mM sodium phosphate, 150 mM NaCl, 0.001% P 80	α -L-iduronidase	pH 5.8; 10x dilution in Eliott's B solution; Four weekly IT injection of 6.9 ml drug	Dogs	8
10 mM citrate, 150 mM NaCl	GDNF	µL/hr infusion (Medtronic pump) into lateral ventricle or putamen for up to 1 year	Monkey	19
10 mM citrate, 150 mM NaCl	GDNF	pH 5.0; 6 µL/hr infusion ICV via Medtronic pump	Human Phase I	20
5% dextran in saline (hyperbaric solution)	Neostigmine	in 2 mL normal saline, IT	Human Phase I	21
0.75% or 7.5% glucose	Tetracaine (0.5%)	7.5 – 7.8, Lumber puncture, 2-3 ml at 0.1 mL/s	Human	22
Paraben (methyl- and propylparabens) *	Neostigmine methyl sulfate	2 mL basic solution of drug in 0.5 N saline, 5% glucose; IT	Human	23
Glycerin (50%)	bupivacaine	Epidural, 5 mL, pH 6.0.	Human	24
Isotonic Mannitol* in normal saline	Adenosine	Single IT injection of 2 mL (backache due to mannitol)	Human Phase I	25
EDTA* (~ 0.3 mM)	2-chloroprocaine	low pH; 15-30 mL followed by 10 mL every 45 min.; epidural, 5 min injection (low back pain due to EDTA)	Human	26
Liposome administered in sterile saline	Cytarabine (DepoCyte)	7.4 - 7.7, 20 µ particles in saline ICV every two weeks	Human	27
DepoFoam®	Cytarabine	1.2mg/mL triolein, 5.7 mg/mL DOPC 1 mg/mL DPPG suspended in saline; ICV or IT every 2-3 weeks, 1.25-12.5 mL	Human	28
PEG suspension 2.5% PEG (3400)	Butamben	Five weeks epidural injections in dogs. Multiple epidural injections of 10% suspension in saline in 2 cancer patients	Dog Human	29
0.9% NaCl with 2.5% PEG and 0.025% polysorbate 80	5% Butamben	4 epidural injections of 15-25 mL in 4 days (discomfort at larger volumes)	Human	30

*not tolerated well

Figure 5: Examples of Clinical and Investigational Intrathecal Formulations

balance of CSF composition and intracranial pressure. Thus, without removal of CSF, the dose volume is limited to ≤3 mL in humans (and ≤1 mL in the adult cynomulgus monkey).

Dose volume limitation necessitates high concentration protein formulation (>10 mg/mL) when doses are in the several tens of milligrams. Several factors can impact protein solubility to achieve the desired concentration, including ionic strength, amino acid sequence and other co-solubilising agents.

Solution compositions used routinely for CNS administration are isotonic saline (unbuffered) or Elliott's B solution (artificial CSF) with a composition listed in Figure 3. Isotonic solutions may not render adequate solubility for some proteins. Additionally, Elliott's B solution contains a very low buffer concentration that may not provide adequate buffering capacity needed to stabilise protein formulations during long-term storage. The artificial CSF solution also contains various salts which are often not compatible with protein formulations. For example, calcium salts may mediate protein precipitation.

The most common approved CNS bolus formulation composition is saline (150 mM NaCl in water), as shown in Figure 4. Others have been tested (Figure 5), some with poor safety profiles. Proteins typically require controlled pH and specific excipients for their solubility and stability (see Figure 6), so we systematically investigated formulation components that would be suitable for our proteins for direct administration to the CSF.

Our initial investigation was targeted to a lysosomal enzyme that required a minimum of 15 mg/mL protein concentration for therapeutic effect. The optimal pH for stability of this protein was 6. With a pI range of 5.1-6.5, it showed enhanced solubility at higher pH (Figure 7, left). Solubility also increased with increasing ionic strength (Figure 7, right), from approximately 10 mg/mL in 50 mM ionic strength to 34 mg/mL in 300 mM NaCl. Given these findings, an isotonic phosphate buffered formulation at pH 7.5 was selected. While this composition rendered suitable solubility and stability for the enzyme, it was not well tolerated *in vivo* (as described below).

IN VIVO TOLERABILITY

As mentioned, saline and phosphate buffered saline are the most commonly used vehicles for formulating or diluting drugs for direct CNS delivery as well as for flushing the delivery system before and after dose administration. We have discovered that small differences in buffer concentration and pH have a very large impact on *in vivo* safety and tolerability of the administered solution. A preliminary study in adult cynomulgus monkeys was conducted to evaluate the toxicology and safety pharmacology of repeated IT-lumbar doses of our enzyme. Each animal was implanted with a port-catheter system to facilitate an every-other-week dosing regimen.

The device control animals received phosphate buffered saline at pH 7.2. The vehicle-control group was dosed with an aqueous solution of 20 mM sodium phosphate, 130 mM NaCl, and 0.005% polysorbate 20 at pH 7.5. This formulation was evaluated as it rendered adequate solubility and stability to the protein.

Clinical signs were observed during and immediately after dosing; the incidence was comparable between the control groups (device control and/or vehicle-dosed group) and enzyme-dosed groups, with no evidence of a dose response. Consequently, the study was terminated after the second dose. A representative view of the histopathology is given in Figure 8.

These clinical observations, which occurred in all animals including the vehicle-dosed animals, prompted a series of toxicology studies

Parameter	Typical Range/Type	Rationale
pH	5 to 7.5	For stability and sometimes solubility
Buffer type	acetate, succinate, citrate, histidine, phosphate or Tris	To maintain optimal pH. May also affect stability
Buffer concentration	5-50 mM	To maintain pH. May also stabilise or add ionic strength
Tonicifier	NaCl, sugars, mannitol	To render iso-osmotic or isotonic solution
Surfactant	Polysorbate 20, polysorbate 80	To stabilise against interfaces and shear
Other	Amino acids (e.g. arginine) at tens to hundreds of mM	For enhanced solubility or stability

Figure 6: Most Common Parenteral Protein Formulation Constituents

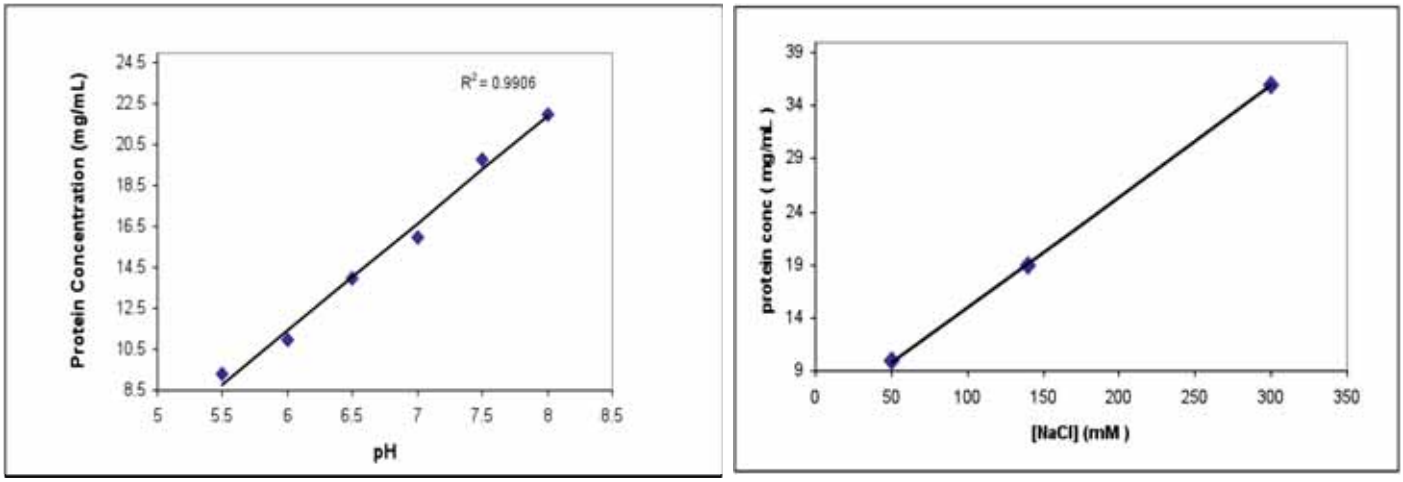

Figure 7: Increasing Solubility of An Enzyme with Increasing pH (left) and Ionic Strength (Right)

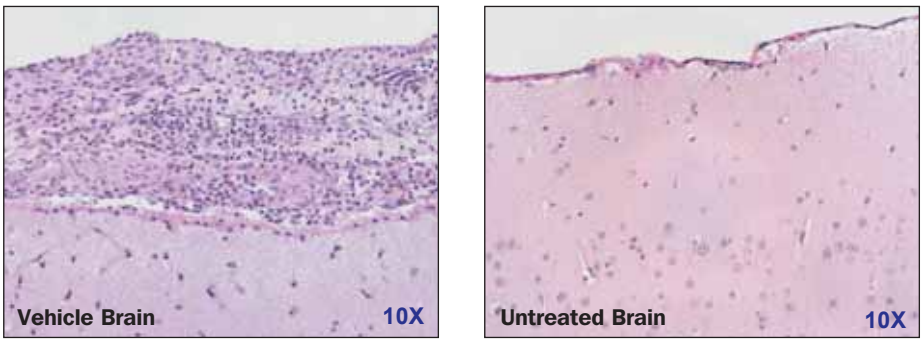

Figure 8: Histological Evaluation of Brain Sections Following Intrathecal Administration of a 20 mM Na Phosphate, pH 7.5, 135 mM NaCl Formulation, Showing Cellular Infiltrates into Meninges and Intrathecal Space for Vehicle Treated Group

of vehicle formulations of varying phosphate buffer concentrations and pH, as well as dose volumes (Figure 9).

In this screening study, four animals per arm were dosed four times on days 1, 5, 14, 19. The clinical observations noted in the animals receiving the initial vehicle (20 mM sodium phosphate, 130 mM NaCl, 0.005% polysorbate 20, pH 7.5) were reproduced with formulations containing a sodium phosphate concentration of ≥ 10 mM and a pH above 7.0.

Tolerability was improved by lowering the dose volume from 1.5 mL to 1.0 mL. Formulations with lower phosphate concentration pH 5.5-7.0 were well tolerated. Of the vehicles which were well tolerated, a vehicle comprising 5 mM sodium phosphate, 145 mM NaCl, 0.005% polysorbate 20 at pH 7.0 was found to be suitable for solubility and stability of the product. There were no adverse clinical signs from four IT administrations of the enzyme in this vehicle (14 mg enzyme in 1.0 mL dose volume) over three weeks. This low-pH, low-phosphate vehicle provided suitable enzyme stability for clinical development. These studies defined the formulation design space suitable for IT dosing (Figure 10).

In summary, CNS delivery of protein therapeutics is an underdeveloped area of research that requires a balancing act of identifying compositions which render adequate solubility, in vivo tolerability of the pharmaceutical composition, and adequate long-term stability to be able to commercialise the product (Figure 11).

Vehicles Tested	Clinical Reaction
20 mM Sodium Phosphate, 130 mM Sodium Chloride, +/- 0.005% Polysorbate 20, pH 5.5	NCS
10 mM Sodium Phosphate, 140 mM Sodium Chloride, +/- 0.005% Polysorbate 20, pH 5.5	NCS
10 mM Sodium Phosphate, 140 mM Sodium Chloride, +/- 0.005% Polysorbate 20, pH 7.5	CR
20 mM Na Phosphate, 130 mM NaCl, 0.005% Polysorbate 20, pH 6.0	NCS
20 mM Na Phosphate, 130 mM NaCl, 0.005% Polysorbate 20, pH 7.0	CR
5 mM Na Phosphate, 145 mM NaCl, 0.005% Polysorbate 20, pH 7.0	NCS
NCS – No Clinical Signs	
CR – Clinical Response, including tremors, hind leg tending, ataxia, displacement	

Figure 9: Summary of Clinical Response to Vehicles after Repeated Intrathecal Administration to Cynomolgus Monkeys

ACKNOWLEDGEMENTS

The pharmaceutical development work was conducted under Gaozhong Zhu's group at Shire HGT's Pharmaceutical and Analytical Development department, including Farah Natoli, Yuan Jiang, Rick Farhner, Brian Vernaglia, Jamie Tsung, Jennifer Terew, and Kris Lowe. The authors wish to acknowledge contributions by Nazila Miller and Kathy Taylor on the literature evaluation of CNS therapies, and animal safety studies by Julie Lieb, Teresa Wright, and Richard Pfeifer. We thank Jeannine Firestone from the Regulatory Department at Shire HGT for supporting the CNS programmes. Key collaborators on this endeavour include our clinical team, Drs Charles Richards, Ann Barbier, and Norman Barton.

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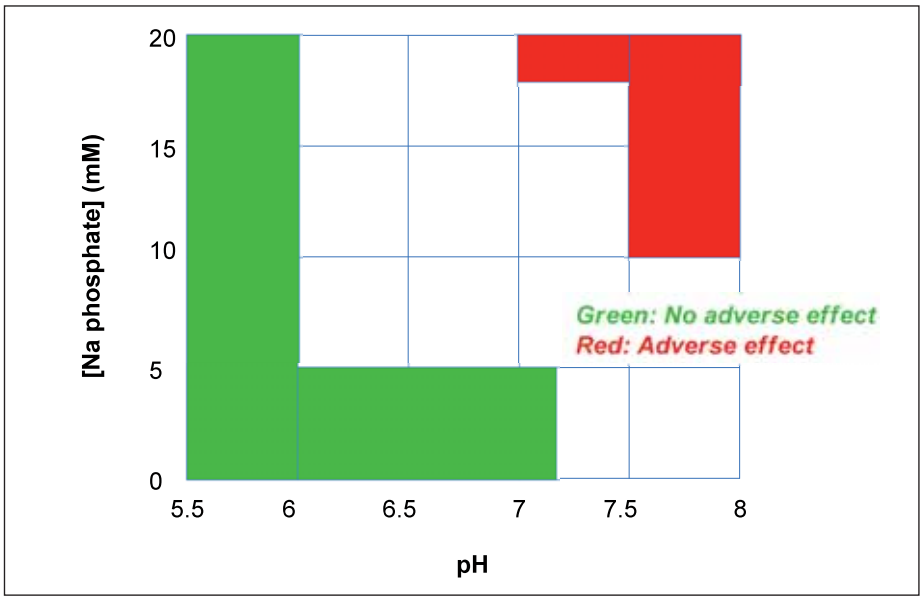

Figure 10: CNS-Tolerated Formulation Design Space

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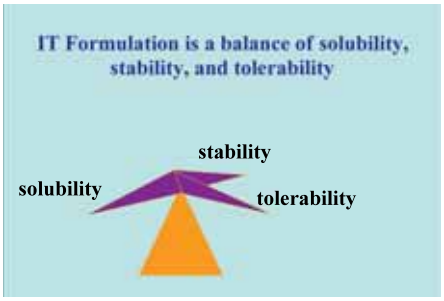

Figure 11: Fine Balance to Achieve Pharmaceutical Compositions Suitable for CNS Delivery

DENDRIMERS ENHANCE DELIVERY OF SMALL MOLECULE AND BIOLOGICAL DRUGS: CONTROL SOLUBILITY, HALF-LIFE, TOXICITY AND TARGETING

The use of macromolecule conjugates to enhance performance of drugs is currently an area of intense research for both small molecules and biological therapeutics. Starpharma's Dr David Owen, VP Research, and Dr Paul Barrett, VP Business Development, describe here the ways that Starpharma has been applying its dendrimer technology to address important challenges in drug delivery, an approach which is now gaining favour with professionals in the industry.

Developing a new drug is a complex task in which many independent parameters must be optimised together, including potency, toxicities, bioavailability, biodistribution, clearance rates, formulability and defensible patent position.

There are a limited number of variables available to address all of these constraints in a single molecule. For this reason conjugating drugs to macromolecular "vehicles" is increasingly being considered as a means of breaking the problem into manageable pieces. For example, developers can choose to optimise potency in the structure of the drug itself, but leave control of biodistribution to a conjugated macromolecular vehicle. In this article we will describe how Starpharma's dendrimer-conjugate technology allows this division of labour between drug and vehicle to be achieved in practice.

Dendrimers certainly cannot be a panacea for all obstacles encountered when developing a drug. However, once a proper understanding of the strengths and limitations of the technology is achieved, its utility can be great indeed, whether for a new molecule, or for a marketed molecule entering development for a new application.

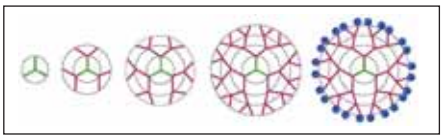

Figure 1: Dendrimers are macromolecules with well-defined, highly branched 3D structures, synthesised in spherical layers by adding monomers onto a core.

FOUR KEY APPLICATIONS

Starpharma has focused on four key drug delivery objectives:

- **Increase solubility** – by conjugating the drug to a dendrimer construct, very large increases in drug solubility have been achieved.
- **Control half-life** – the dendrimer can protect drugs from degradation and inhibit renal clearance. The half-life of drugs has thereby been substantially increased.
- **Control off-target toxicity** – different dendrimers can keep drug away from different tissues. This has been shown to reduce dose-limiting toxicities.
- **Target organ, tissue or receptor** – dendrimers have been used actively or passively to target a payload drug to particular destinations.

WELL-DEFINED, VERSATILE, SYNTHETIC MACROMOLECULES

Starpharma's dendrimers are highly-branched macromolecules with a well-defined structure. Starting with a core molecule, branching lysine units are repeatedly added in layers (or "generations"), until the desired structure is reached (Figure 1). The many surface points of attachment can then be functionalised with

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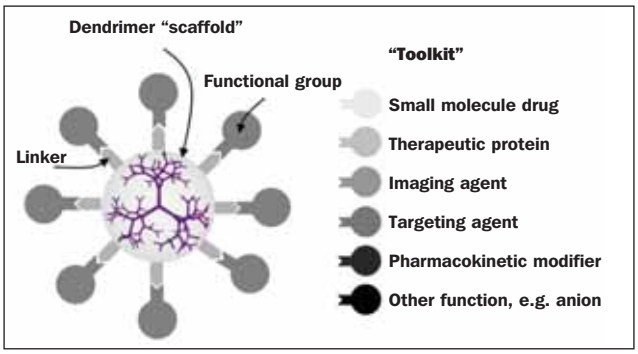


Figure 2: The surface of the dendrimer can be modified to achieve a wide range of functionality. Typically one or more functional groups are used on 32 to 64 attachment sites.

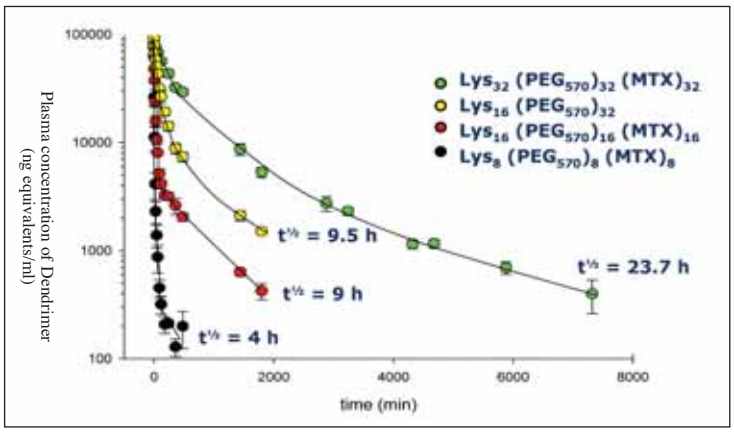
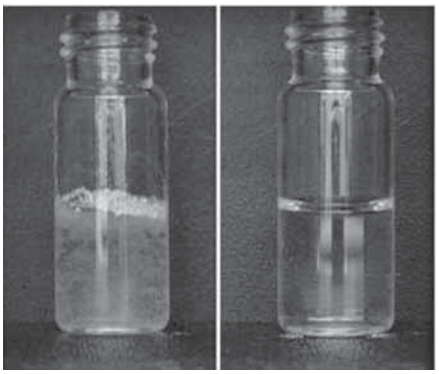


Figure 4: Attaching small-molecule drugs to dendrimer constructs allows control of half-life. In this study the half-life of methotrexate (MTX) was extended from 24 minutes for free DOX (data not shown), to 24 hours. For a different dendrimer-MTX construct, a 50-hour half-life was achieved.



Paclitaxel, no dendrimer solubility 0.8 μg/ml

Paclitaxel with dendrimer solubility >37 μg/ml

Figure 3: By conjugating paclitaxel to a dendrimer construct its aqueous solubility is enhanced 9000-fold.

one or more groups depending on the intended application (Figure 2).

Starpharma has taken its polylysine dendrimers into the clinic in the form of an antiviral vaginal gel, VivaGel®, currently in Phase II studies. VivaGel® is the subject of a licensing agreement with SSL, the owner of the Durex® condom brand as a microbicidal condom coating. VivaGel® is manufactured under cGMP and its structure is closely related to many of the dendrimers that Starpharma uses for drug delivery applications.

A growing number of pharmaceutical companies are now working with Starpharma: the company has announced collaborations with Lilly, GSK's Stiefel Laboratories, and Elanco. A number of additional collaborations with undisclosed partners are also under way.

Below we give some examples of well-known drugs that we have modified with dendrimers to achieve different objectives. It is hoped that the reader may consider the applicability of these techniques to his or her own molecules of interest.

INCREASE SOLUBILITY

Paclitaxel is a cancer drug well known for its poor aqueous solubility (<1μg/ml). When conjugated to a dendrimer construct, a 9000-fold increase in solublised paclitaxel is achieved (Figure 3). The structure is designed to release the API in the body so that it can achieve its intended pharmacological effects (see Boxed Text).

CONTROL HALF-LIFE

The half-life of a drug can be substantially enhanced by attaching it to a dendrimer construct. For example attaching methotrexate (MTX) to different members of a family of dendrimer constructs leads to a range of clearance rates in rats, ranging from 24 minutes (free MTX), to nearly 24 hours as shown in Figure 4, and 50 hours in a related construct.

A comparable result was achieved with doxorubicin (DOX): a $t_{1/2}$ of approximately 30 minutes for free DOX was extended to 34 hours for a DOX-dendrimer construct.

The approach is applicable both to small molecules and biological therapeutics. In Figure 5, the activity of insulin is prolonged so that glucose levels are suppressed longer in mice receiving insulin-dendrimer construct than for mice receiving insulin alone.

In the case of doxorubicin and methotrextate the API needed to be released from the construct to function. For insulin, release was not required (see Boxed Text).

LINKERS – CONTROLLING WHEN DRUG IS RELEASED

Some drugs continue to function whilst attached to dendrimers, whereas others need to be released before they can work. Starpharma uses different kinds of linkers between the dendrimer and the drug to control when and where the drug is released, for example:

"Permanent" Linker

If the drug is found to work satisfactorily when attached to the dendrimer, then there may be no need to release it. Insulin, for example, falls into this category.

Hydrolytically Unstable Linker

Sometimes, sustained release of the API may be all that is required, with no preferred release location. In this case a linker that breaks down with an appropriate half-life in aqueous solution may be the correct choice.

Acid-Labile Linker

Newly forming tumours are often hypoxic, and therefore have a low pH. By using a linker that is stable at neutral pH, but unstable at lower pH, it is possible preferentially to release drug at the tumour.

Enzymatically Cleaved Linker

It is possible preferentially to release a drug molecule at the location where a specific enzyme is expressed by connecting the drug to the dendrimer with an enzymatically cleavable linker. Such locations include tumours where certain enzymes are commonly over-expressed.

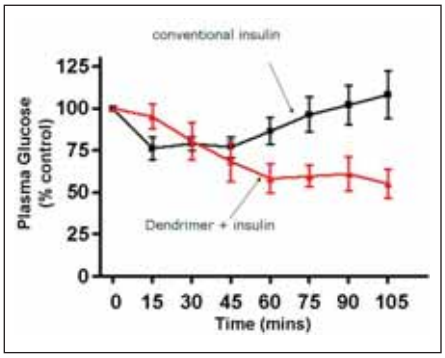


Figure 5: The half-life of biological molecules can be extended using dendrimers. In this case, in mice, the effective duration of activity of insulin is prolonged compared with insulin alone.

CONTROL TOXICITY

Damage to heart muscle can be a dose-limiting toxicity for cancer drug doxorubicin (Figure 6a). However, when doxorubicin is attached to a suitable dendrimer construct with preferential release of drug near a tumour (see Boxed Text), much less free drug reaches the heart, and cardiac damage is reduced (Figure 6c), whilst drug efficacy is maintained at the tumour site (Figure 6d).

TARGETING

There are two mechanisms that may be advantageously employed to control the destination of drugs when attached to a dendrimer.

Active Targeting – Adding a suitable targeting molecule to the construct, such as an antibody, allows the dendrimer to carry a payload to a target receptor. In Figure 7, this is achieved for a payload of gadolinium, allowing visualisation in an MRI scanner. The payload could equally have been a small-molecule API.

Passive Targeting – Even in the absence of any targeting group, tailoring the size and physico chemical properties of the dendrimer can achieve preferential accumulation in target tissues or organs (see Figure 8). Additionally, tumours can be passively targeted using the well known EPR effect.

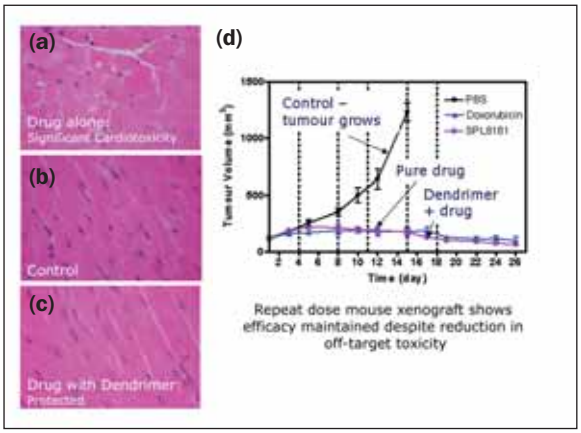


Figure 6: (a) Cardiac tissue damage can be a dose limiting toxicity for doxorubicin. (b) Image of undamaged cardiac muscle where negative control (PBS) is administered. (c) When DOX is conjugated to dendrimer, heart tissue no longer becomes damaged despite retention of efficacy against tumour as shown in xenograft study (d).

STRENGTHS AND LIMITATIONS

It is helpful to understand the strengths and limitations of the dendrimer-conjugate approach when assessing its use for specific applications:

Strengths

- The payload for small-molecule drugs is typically 20-40% w/w.
- A good level of purity / monodispersity can be achieved.
- Although some dendrimers naturally migrate

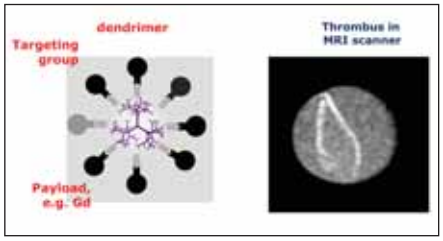


Figure 7: Left: By associating a dendrimer with a targeting group (e.g. an antibody), multiple "payload" molecules can be delivered to a molecular target. Right: Here the antibody fragment targets the dendrimer to a thrombus and the payload is successfully delivered. In a control study without the correct antibody, no such delivery was achieved, showing that the targeting in the image above was specific in nature. (Work conducted in collaboration with the Baker IDI Institute)

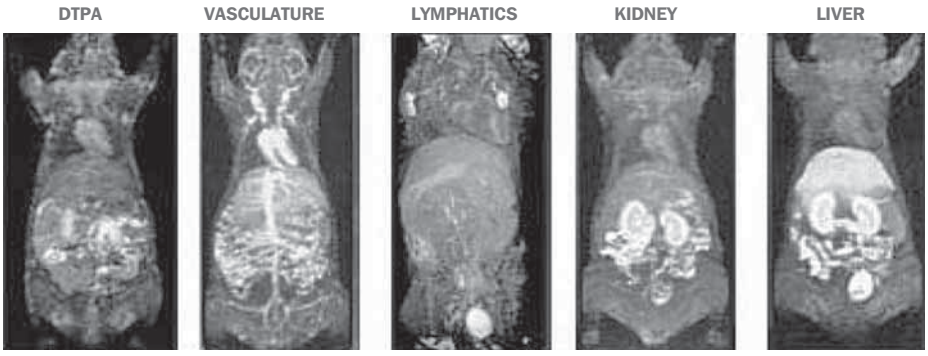


Figure 8: Passive targeting to tissues or organs can still occur in the absence of specific targeting groups. The left image shows the distribution of a non-dendrimer contrast agent control. The other four images are different dendrimer-gadolinium complexes, showing how different dendrimers target different tissues. (Image courtesy of M Brechbiel, NIH.)

towards the liver, others do not. This means that both the liver and other organs can be addressed using dendrimers.

- Dendrimers are synthesised using standard chemical processes.
- A long shelf-life has been observed in ambient and accelerated stability studies of dendrimer product VivaGel® (>2 years ambient).
- Dendrimer constructs often yield a readily soluble powder when freeze-dried.
- Even when PEG has a large molecular weight, its "spaghetti-like" form means that it can still be cleared through the kidney quicker than may be intended ("reptation"). The branching, 3D structure of a dendrimer can be used to obstruct this clearance mechanism. On the other hand it is possible to make smaller dendrimers that are excreted reasonably quickly through the kidney, if required.
- Starpharma's dendrimers are metabolised to natural lysine which is readily processed by the body.
- Dendrimers have a covalent structure. This contrasts with liposomes which are often considered metastable so that they can eventually rearrange to form planar bi-layers.

Limitations

- An intact dendrimer-drug construct does not generally cross the gut wall and so the approach may not be a good choice for certain oral administration applications. Obviously this limitation does not apply where the drug is solublised with dendrimer and then released in the gut for absorption.
- The drug-dendrimer construct will generally be considered to be a New Chemical Entity (NCE), meaning that clinical testing of the new construct may be required. This is normally not seen as an issue for preclinical drugs, which will require full clinical testing anyway, but the approach may require review before application to a candidate already in the clinic. Helpfully, the construct may be considered to be a prodrug allowing bridging data from previous studies to be used.

BEYOND PHARMACEUTICALS

Starpharma is pursuing applications of its dendrimer technology beyond pharmaceuticals, for example, in cosmetics, coatings and agrochemicals. Starpharma would be pleased to discuss the application of its dendrimer technology to any sector, pharmaceutical or otherwise.

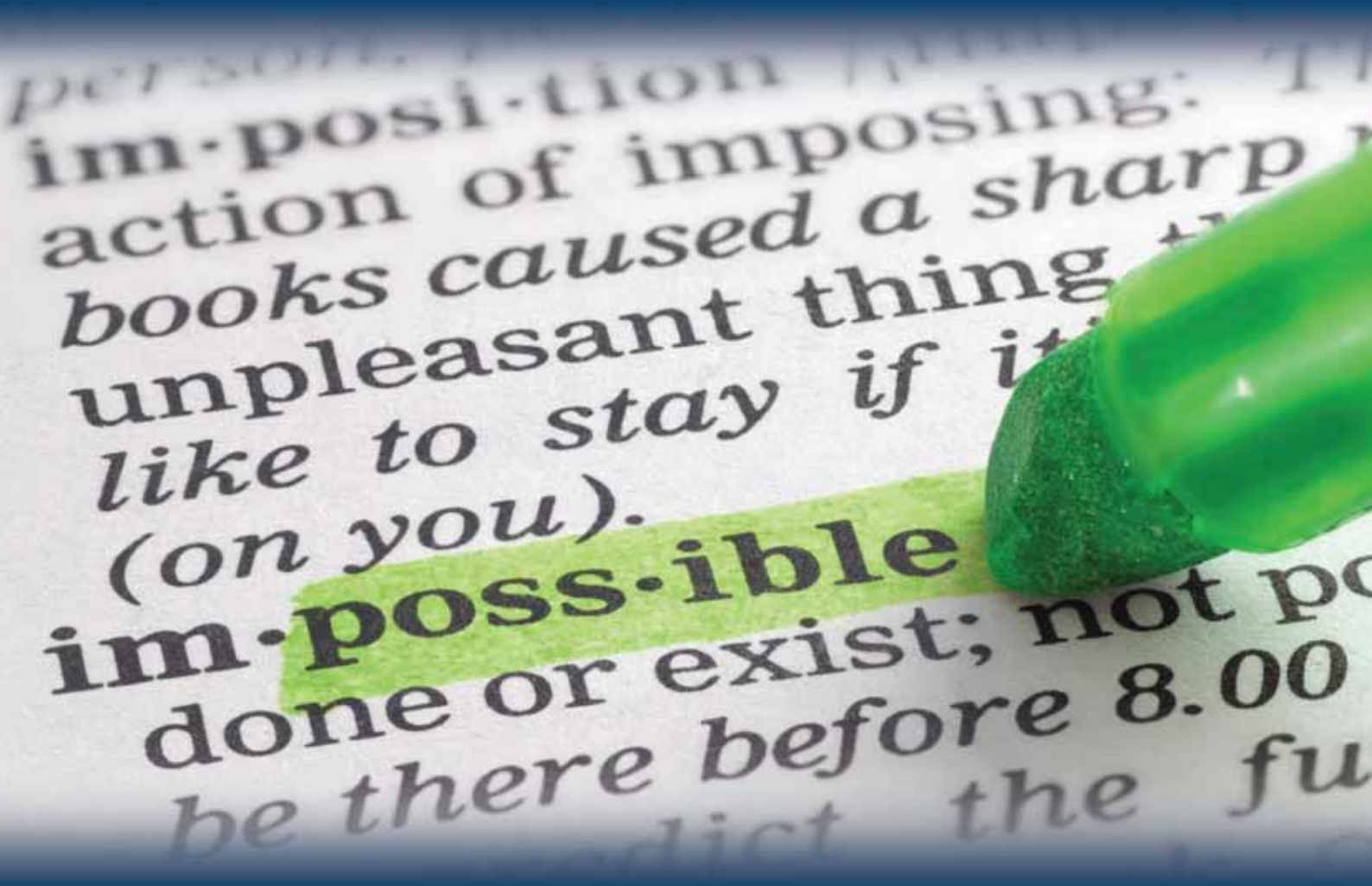
ABOUT STARPHARMA

Starpharma Holdings Limited (ASX:SPL, OTCQX:SPHRY) is a world leader in the development of dendrimer technology for pharmaceutical, life-science and other applications. SPL has two operating companies, Starpharma Pty Ltd in Melbourne, Australia, and DNT, Inc in the USA. Products based on Starpharma's dendrimer technology are on the market in the form of diagnostic elements and laboratory reagents through licence arrangements with partners including Siemens and Merck KgA.

The company's lead pharmaceutical development product is VivaGel® (SPL7013 Gel), a vaginal microbicide designed to prevent the transmission of sexually transmitted infections, including HIV and genital herpes. In September 2008, Starpharma signed a full licence agreement with SSL International plc (LSE:SSL) to develop a VivaGel®-coated condom. SSL manufactures and sells Durex® condoms, the market-leading condom brand worldwide.

Starpharma also has agreements with Lilly, Elanco, Stiefel Laboratories (a GSK Company), and Unilever as well as many research collaborations with some of the world's other leading organisations.

More companies are now working with Starpharma's dendrimer technology to find new ways of delivering drugs



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CONTRASOL™: NOVEL NANOTECHNOLOGY FOR THE ENHANCED DEVELOPMENT OF INTRAVENOUS FORMULATIONS

Pharmaceutical pipelines are becoming increasingly populated with poorly soluble drug candidates presenting a significant challenge to the industry in terms of the development of intravenous formulations. Here, Alison Foster, PhD, Laboratory Director at IOTA NanoSolutions Limited, introduces the ContraSol™ technology, a novel non-attrition approach to nanoparticles of poorly soluble API with the potential to address common formulation issues associated with intravenous delivery.

One of the first documented records of parenteral drug administration was that of Alexander Wood who, in the mid 19th Century, described an injection of morphine for the treatment of neuralgia given because the patient was not able to take opium orally.[1,2] Generally, injection – and specifically the intravenous (i.v.) route – is the fastest method to deliver medication throughout the body and is often used to deliver drugs that show poor bioavailability when given orally or via other routes of administration, allowing drugs to bypass all absorption barriers and gain direct entry into the general circulation. Other advantages and some disadvantages of i.v. administration are summarised in Figure 1.

An i.v. formulation is always required in preclinical development for toxicological evaluation, especially if this is the intended end-use. Typically, an i.v. study is also conducted to determine absolute bioavailability of a drug administered via other routes.[3] In addition, the delivery of multiple therapeutic agents to a patient through a central i.v. infusion line is common practice in hospitals and thus it is desirable to develop critical care products that can be admixed with an i.v. fluid for infusion.[3]

Nonetheless, it is becoming increasingly difficult to develop i.v. formulations for drugs emerging from pharmaceutical pipelines because a vast number of these are poorly soluble. These new chemical entities (NCEs) would normally benefit from i.v. administration as they often show poor bioavailability when administered via other routes. However, an aqueous formulation is required for administration into the blood (a predominantly aqueous entity, 90% of blood plasma being water [4]), so developing i.v. formulations of poorly soluble compounds presents a huge problem for the pharmaceutical industry.

Nearly 40% of NCEs are never brought to market because of biopharmaceutical issues such as low solubility, low dissolution rate and low permeability.[5] Indeed a significant proportion of all new drugs that are entering the market are hydrophobic, falling into Class II or IV of the Biopharmaceutics Classification System. Permeability is not a concern for Class II compounds and thus solubility or the dissolution rate of the drug becomes the limiting factor. Several approaches have been used in attempts to solve this problem (see Figure 2).[6]

Advanced drug delivery systems have been developed to overcome many of the pitfalls associated with traditional formulation techniques. Over the past 10 years, nanoparticle engineering processes have been developed and are quickly becoming a popular method for the formulation of poorly soluble drugs as the increased surface curvature and surface area of the drug particles enhances solubility and dissolution rate for these materials (Noyes-Whitney and Kelvin equation)[8-9]. Thus, methods for producing small particles of a poorly soluble drug are quickly becoming the key driver for formulation scientists.

For intravenous administration, it is vitally important that these particles are below a cer-

Advantages	Disadvantages
• Fast delivery of API: 15-30 sec for i.v. • 100% bioavailability of drug • Suitable for drugs not absorbed by the gut or those that are too irritant • API can be formulated in a controlled release medication • Preferred method to administer a drug at high dose as the medication can be delivered continuously (infusion) • Continuous infusion maintains a constant level of drug with short biological $t_{1/2}$	• Patients cannot always self-administer • Many people have a fear of needles and injection • i.v. bypasses most of the body's natural defenses, exposing the user to possible issues (hepatitis, abscesses, infections and undissolved particles or additives/ contaminants) • Potentially fatal air boluses can occur if not administered by a professional • Formulations need to be sterile

Figure 1: Advantages and disadvantages of i.v. administration

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tain size to ensure that they are not taken up by macrophages of the liver (potentially targeting toxicity in the liver). On the other hand, they should not be so small that they migrate into the spleen and bone marrow.[10,11] Ideally nano-formulations should be developed to mimic an injected solution whilst aiming to minimise side effects of current i.v. formulations due to poorly tolerated excipients.

Numerous methods to form nanoparticles for injectable products have been developed and are classed as top-down or bottom-up processes. Many of the methods to produce organic nanoparticles are the subject of a comprehensive review by Horn and Rieger.[12] However, not all of these methods can be adopted for injectable drugs due to the strict limitations on permissable excipients. Some of the more common methods are summarised in Figure 3 on the next page.

IOTA NANOSOLUTIONS LIMITED

IOTA NanoSolutions Limited is a specialist formulation company that has developed a generic approach, ContraSol™ , that overcomes some of the limitations of other nanotechnologies by forming stable dry-powder formats that can be re-constituted at the point of use.

ContraSol™ involves the blending of oppositely soluble materials, typically a blend of a poorly soluble drug within a water-soluble matrix. This blend, on addition to water, rapidly liberates nanoparticles of the drug, stabilised by the soluble matrix material.

Method	Advantages	Disadvantages
pH Adjustment	• Relatively inexpensive • Useful for drugs that are less stable at physiological pH • Easy to manufacture	• Extreme pH could limit the infusion rate • Could cause tissue irritation • Drug precipitation possible during infusion
Salt Formation/ Prodrugs	• Highly soluble	• New chemical entity • May be less pharmacologically active • May be slower to act as needs to undergo conversion to parent drug • Development of new water-soluble derivatives is costly
Co-solvents	• Good for medications where small amounts of active required • Readily available safety data for a variety of cosolvents	• Systemic toxicity • Drug precipitation upon infusion • Local irritation that could cause pain at injection site • Cosolvent must hydrogen bond with water for water miscibility and this limits the capacity for the cosolvent to favourably interact with the drug
Surfactant Systems (micelles)	• Useful for drugs that can be dissolved in surfactant (low dose drugs) • Easy to process • Polymeric micelles can be tailored to control size and morphology of micelles[7] • Polymeric micelles have high loading capacity and better control of drug release	• Systemic toxicity • Formulation irritation potential & some surfactants are poorly tolerated • High amounts of surfactants may be required for high dosages • Solubilised drug may precipitate out as results of micelle dissociation on dilution with blood
Complex Formation	• Solubilises and stabilizes the active • Good physical stability • May reduce local tissue reaction upon infusion • Precipitation upon infusion unlikely	• Potential irritants • Only possible with certain drugs • Inherent toxicity of complexing agents • Viscosity of resulting complex may be a limiting factor • Equilibrium process – may not reach completion • High concentrations required
Emulsions	• Improve chemical stability • Can reduce adverse reactions	• Thermodynamically unstable • Processing can be difficult
Liposomes	• Reduces systemic toxicity • Targeted drug delivery	• Difficult to formulate • Stability problems • Potential manufacturing problem with regard to the raw material • Cost • Final drug concentration in product is load even though drug loading high in terms of lipid:drug ratio

Figure 2: Traditional Methods Used to Formulate Poorly Soluble Drugs

ContraSol™ offers significant benefits over existing nanoparticle technologies including:

- Processing of a wide range of chemicals (no chemical modification required)
  - Liquids and low-melting-point drugs (including waxes)
  - Amorphous and crystalline drugs
  - Thermally sensitive drugs
- High loadings of API (up to 85% w/w)
- Excipients selected from those used in FDA-approved i.v. products
- Rapid single-step processing
- Scalability through existing cGMP/aseptic facilities
- Narrow particle size distributions

IOTA NanoSolutions™ has various platforms underpinning the ContraSol™ approach that can be selected depending on the properties of the drug and end application (Figure 4).

EM-POSS™: EMULSION-TEMPLATING OF POROUS SOLUBLE SOLIDS

EM-PoSS™ uses technology synonymous with the formation of polyHIPEs (that is, the use of high internal phase emulsions (HIPEs) as templates to create highly porous materials (polyHIPEs).[15] However, polyHIPEs are insoluble in water, using cross-linked polymers to maintain their porous structure, which can swell to absorb liquids.

In contrast, IOTA NanoSolutions™ uses the same emulsion-templating principle and dissolves water-soluble excipients in the aqueous continuous phase of an emulsion whilst dissolving the drug into the discontinuous oil phase. This emulsion is frozen in a cryogenic liquid to 'lock' the emulsion structure and then freeze-dried to remove the water and volatile oil phase leaving a highly porous matrix containing the poorly soluble drug dispersed within (Figure 5). This highly porous structure rapidly dissolves in water to release nanoparticles of the drug. The early research supporting this platform is the subject of a recent paper.[16]

The EM-PoSS™ platform requires very small amounts of API (see EM-PLATE™) and is particularly relevant to injectables as aseptic lyophilisation is well established.

EM-POWER™: EMULSION – PARTICLE ENGINEERING FROM OIL & WATER EMULSION ROUTES

EM-POWER™ utilises the same basic emulsion formation techniques as EM-PoSS™ except with EM-POWER™ the drying step uses spray-drying rather than freeze-drying.

Method	Description	Advantages	Disadvantages
Wet milling	Attrition process where micron-sized crystals are wet-milled in the presence of grinding media and a surface modifier[13]	• Crystallinity is maintained • Commonly used process	• Time-intensive (>4 days to mill <400nm) • Potential contamination from grinding media • Batch process (batch-to-batch variation) • Risk of microbiological issues if milling over a few days at 30 °C[14] • Difficult to mill low melting point actives
Nano-suspension	Produced by high pressure homogenization of drug suspensions (typically in a surfactant solution)	• Relatively simple process	• High pressures can cause changes in crystal structure • Batch process (batch-to-batch variation) • Stability of nanosuspensions may be an issue
Controlled Precipitation	A solution of the drug is added to a miscible anti-solvent which cause the drug to precipitate (typically stabilizers are added to stabilize the precipitated drug)	• Amorphous material produced has improved dissolution kinetics compared to the crystalline form	• Amorphous material may be unstable
Nanoemulsion	Emulsions with a droplet size of <1micron with the drug dissolved in the oil phase	• Biocompatible	• High energy required to reduce emulsion droplet size • Destabilised by Ostwald ripening leading to creaming
Solvent Evaporation	A drug is dissolved into the volatile oil phase of an emulsion. The oil is then evaporated leaving an aqueous dispersion of the drug	• Simple to produce	• High energy required to reduce emulsion droplet size • Difficult to remove all solvent

Figure 3: Traditional Nanoparticle Formation Methods

Unlike EM-PoSS™ , the structure of the emulsion is not 'locked' during the drying step and the droplets that are sprayed through the spray-drier nozzle can form a variety of shapes (either ruptured, expanded, collapsed or fragmented) depending on the spray-drying conditions (Figure 6).[17]

IN-PRESS™: PARTICLE ENGINEERING FROM SINGLE PHASE SOLUTIONS

IN-PrESS™ uses a similar principle to the other IOTA NanoSolutions™ platforms by dissolving all excipients and the drug prior to the drying step. However, in this case an emulsion is not formed as the mixture of solvents (or one solvent in some cases) form a single phase. The solvents are miscible. The excipients are selected to be soluble in the application medium and are also soluble in the initial solvent solution. The removal of the volatile liquids can be carried out either by freeze-drying or by spray-drying as above. As emulsion-templating is not carried out in this case, the freeze-drying process does not lead to such highly porous structures (Figure 7).

Figure 4: IOTA NanoSolutions™ Platform Technologies

IN-GRANE™: GRANULATION TECHNIQUES

In the platforms above, the drying step has involved freeze-drying or spray drying. Spray-granulation is an alternative method for drying that can be used. Both emulsion and single solution routes can be used and the drying technique can form large particles (up to 10 mm) with different morphologies depending on the process conditions. Dense particles can be produced by layering solids onto the rapidly growing nuclei produced in the process in one method (Figure 8).[18] This layered, 'onion-like' structure offers the potential to coat the growing nucleus with specific materials, such as enteric polymers.

Alternatively a 'raspberry-type' structure can be produced by agglomerating smaller particles produced by spraying more of the feedstock, which forms a binder between the particles (Figure 9).[19] These are less dense than the 'onion-like' structure, are easily compressible with adjustable porosity.

EM-PLATE™: WELL-PLATE SCREENING

EM-PLATE™ uses the EM-PoSS™ platform on a very small scale (typically 1 mg of active per sample) to allow screening of multiple formulations when only small amounts of a NCE are

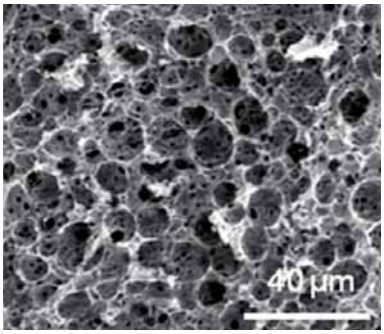

Figure 5: Emulsion-Templated Structure formed through EM-PoSS™

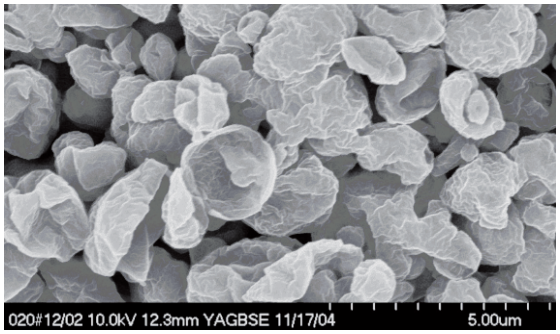

Figure 6: Spray Dried Sample Produced by EM-POWER™

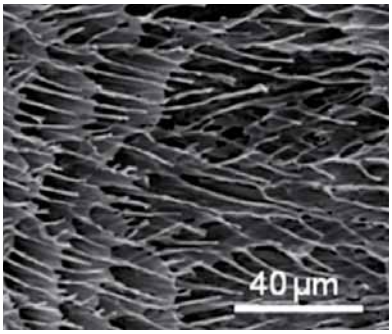

Figure 7: Freeze-Dried Sample Produced by IN-PrESS™

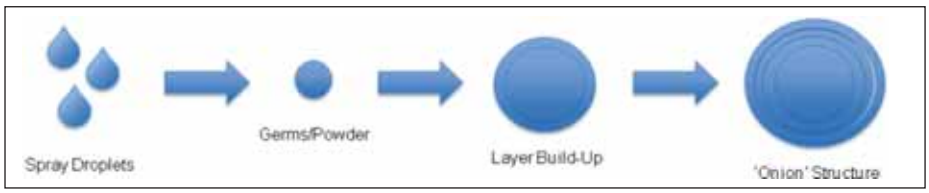

Figure 8: Spray Granulation to form 'Onion' Structures (Reproduced from Glatt Group)[18]

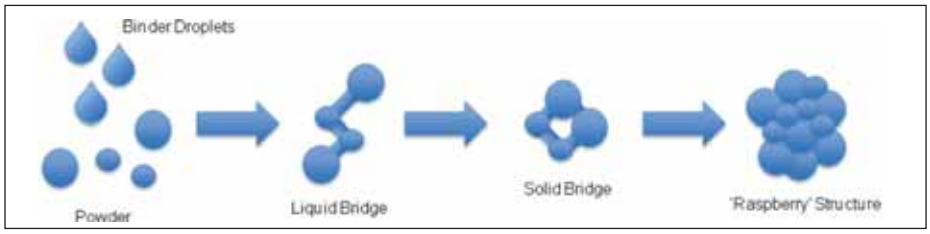

Figure 9: Spray Granulation to form 'Raspberry' Structures (Reproduced from Glatt Group)[19]

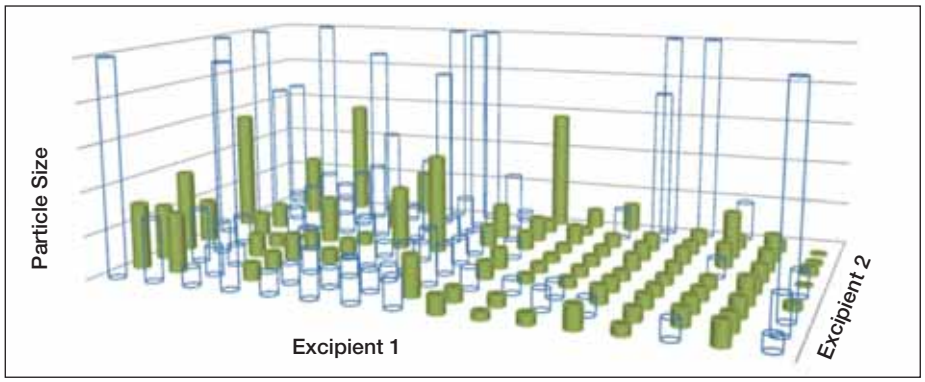

Figure 10: EM-PLATE™ Screening Showing 'Hits' in Green (160mg of API used to generate the 160 sample library)

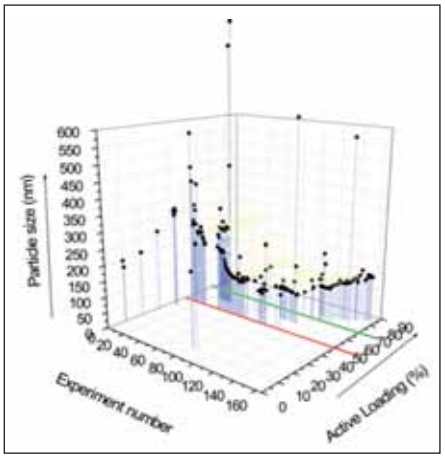

Figure 11: IOTA NanoSolutions™ Formulation Discovery Programme (i.v. product)

available and the 'solubilised' active is required to test for efficacy or toxicity, for example. Ranges of excipients are investigated and samples are selected on the particle size distributions obtained (both in terms of polydispersity and average size). Figure 10 shows a typical output of an EM-PLATE™ screen where the formulations that meet the target criteria are shown in green.

All IOTA NanoSolutions™ platforms use excipients from FDA-approved injectable ingredients. No new molecules are used or formed during the processing of the actives, ensuring that regulatory clearance is kept as simple as possible.

The platforms have a number of other advantages, especially those involving emulsion techniques, as a number of variables in the process can be varied depending on the properties of the end formulation required. Parameters that can be changed include the oil/water phase ratio and the concentration of API within the oil phase. Both can dramatically change the loading of API within the formulation and the particle size/zeta potential obtained. In addition it is possible to co-formulate more than one poorly soluble active or, alternatively, include a water-soluble active as part of the water-soluble matrix.

A number of i.v. formulations have been developed by IOTA NanoSolutions™ for partner programmes and all have shown improvement over conventional formulation techniques.

In one particular case study, IOTA NanoSolutions™ developed an i.v. formulation of a new anti-infective that was required as a nanoparticulate to try to overcome its solubility issues. The formulation was developed using the EM-POWER™ platform with an iterative approach to identify a formulation that met the target criteria. Numerous formulations were generated during the study (>200 formulations) and Figure 11 summarises some of the data obtained. During this iterative process a number of different i.v. acceptable excipients were evaluated and Figure 11 shows the loading of active for each formulation and its effect on particle size.

The maximum loading of API achieved for this formulation was 83% w/w. However, many of the formulations performed well in terms of pharmacokinetics and efficacy. The particle-size distribution data (obtained by dynamic light scattering) from a selection of the formulations are summarised in Figures 12-14.

In a study of the *in vivo* efficacy and pharmacokinetics of this poorly-soluble i.v. anti-infective, the formulations generated by EM-POWER™ produced results comparable with those obtained from the solvent-based formulation (Cremophor/DMSO).

Figure 15 compares two IOTA NanoSolutions™ formulations with the solvent-based formulation, and both IOTA NanoSolutions™ formulations give comparable AUC and C_{max}. Figure 16 shows pharmacokinetic data (equal dose administered to mice) presented in summary and compared with a milled formulation of the same API. The improvement in AUC and C_{max} of the EM-POWER™ formulations over the milled equivalent is clearly visible.

The lead formulation was tested for efficacy and compared against a current commercial treat-

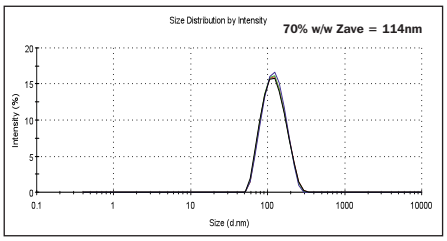

Figure 12: Particle Size Distribution of 70%w/w API Formulation (i.v. product)

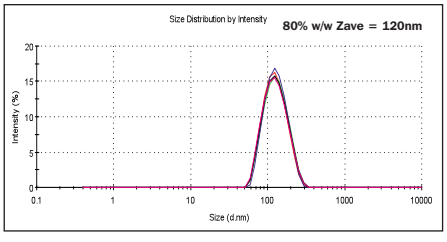

Figure 13: Particle Size Distribution of 80%w/w API Formulation (i.v. product)

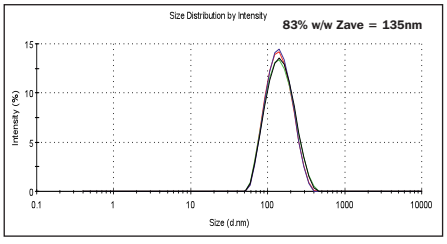

Figure 14: Particle Size Distribution of 83%w/w API Formulation (i.v. product)

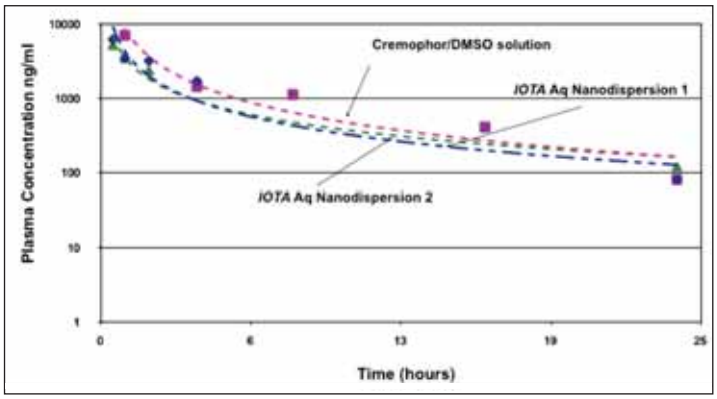

Figure 15: Pharmacokinetic Data showing Two IOTA NanoSolutions™ Formulations Compared to a Solvent Solution of the Same Active (i.v. administration, 20mg/kg dose into mice)

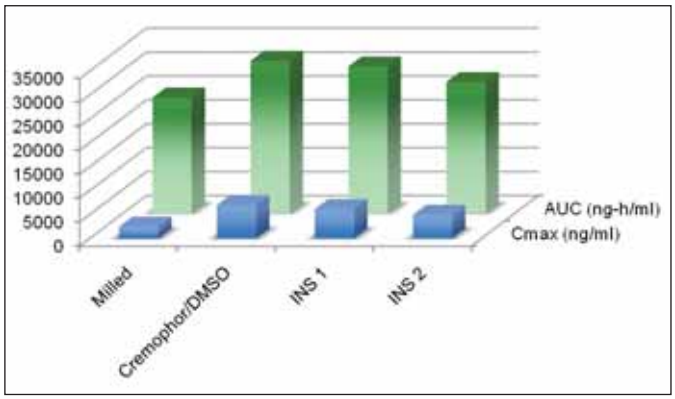

Figure 16: Pharmacokinetic Data of Two IOTA NanoSolutions™ Formulations (INS 1 & INS 2) vs. a Milled and Solvent Solution of the Same Active (i.v. product)

ment and a solvent solution for a specific indication (equal dose; mouse model). The commercial product has significant drug-drug interactions leading to patient complications such that its use in the clinic is decreasing. The IOTA NanoSolutions™ formulation showed better efficacy in this indication compared with both the commercial treatment and the solvent solution (Figure 17).

The dispersion stability of the IOTA NanoSolutions™ formulation was tested by re-constituting the formulation into a saline solution. Samples were taken periodically and the particle size analysed to ensure no particle aggregation over the period (8 hr). The formulations proved to be stable for at least 48 hr (Figure 18) at the dispersion concentration of 2%wt active which satisfied the target criteria for this potential product. Clinical trials are expected to begin in 2010.

Many of IOTA NanoSolutions' partnered projects are progressing through the early stages of drug discovery into the next stages of development. This includes its i.v. products as well as those developed for other routes of administration. The company has consistently demonstrated highly efficacious formulations of poorly soluble materials that are at least as effective as the API dissolved in a solvent solution.

Using the expertise and know-how of the highly qualified IOTA NanoSolutions™ scientists, each formulation is tailor-made to the active ingredient in question, with no two formulations being identical. This leads to extensive opportunities in patent protection and lifecycle management both of NCEs and generic medicines.

The data provided herein is for information only. IOTA NanoSolutions Limited does not warrant its completeness or suitability for any purpose.

IOTA NanoSolutions is the trading name of IOTA NanoSolutions Limited. IOTA NanoSolutions™ is the registered trademark of IOTA NanoSolutions Limited, Number 004660452.

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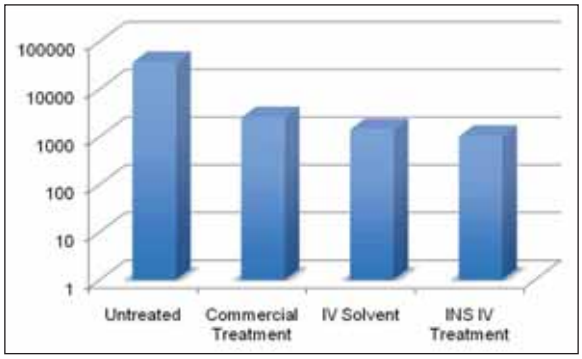

Figure 17: Efficacy Data of IOTA NanoSolutions™ i.v. Formulation (INS IV) vs. a Commercial Equivalent and a Solvent Solution Plotted on a Log Scale

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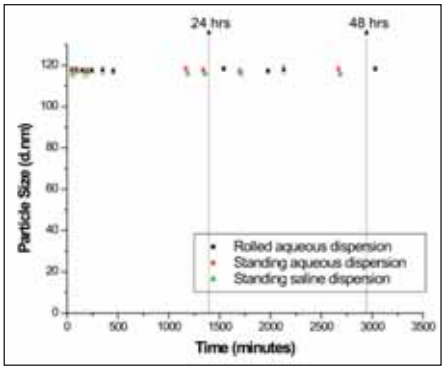

Figure 18: Stability Data of IOTA NanoSolutions™ i.v. Formulations in Dispersion

IOTA NanoSolutions Limited specialising in non attrition routes to nanoparticles of poorly soluble and insoluble APIs

IOTA NanoSolutions™ specialises in the enhanced formulation of poorly soluble and insoluble APIs through its proprietary platform technology ContraSol™. The ContraSol™ approach produces dry, solid blends e.g. powders or tablets, of poorly soluble API (with no chemical modification) within a soluble matrix. When added to liquids, the matrix dissolves to liberate the poorly soluble API as a nanoparticulate dispersion (Z-average typically 100-300nm). IOTA NanoSolutions' formulations can be used to effectively incorporate poorly soluble and insoluble APIs into tablet, gel, powder and liquid formats for parenteral, inhalable, ocular, oral and topical administration.

Powders and dispersions generated by the application of ContraSol™ have demonstrated:

- Improved solubility kinetics
- Improved bioavailability/bioactivity
- Reduction in or elimination of the use of organic solvent
- Improved clarity of solution
- Ability to generate "fastest dissolving" claims
- Potentially valuable extensions to intellectual property

ContraSol™ offers significant benefits over existing nanoparticle formation technologies including:

- Processing of a wide range of materials
  - * amorphous and crystalline APIs
  - * liquids and low melting point APIs (including waxes)
  - * thermally sensitive APIs
- High powder loadings of API (up to 85 w/w%)
- Narrow particle size distribution
- Rapid single-step processing (does not involve high energy, time consuming nanoparticle formation steps)
- Scale-ability through existing cGMP facilities

IOTA NanoSolutions™ is a spin-out company from Unilever R&D and is headquartered at the MerseyBIO Incubator in Liverpool, UK. Our best results are achieved through collaboration, understanding your formulation needs and optimising the performance of your poorly soluble / insoluble API. For further information concerning IOTA NanoSolutions™ and the ContraSol™ technology please contact:

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ContraSol™ platform technologies:

- EM-PoSS™ • EM-POWER™ • IN-PrESS™
- IN-Grane™ • EM-PLATE™

ContraSol™
Dispersions are the Solution

www.iotanano.com

Shire

A shared pursuit of innovative treatments

- We are committed to collaborating with academic researchers, healthcare providers, and patient associations around the world
- Our shared goal is a deeper understanding of life-threatening genetic disorders
- Our work together includes the investigation and development of therapeutic approaches in mucopolysaccharidoses (MPS) and other rare genetic disorders

To learn more about MPS and other rare genetic disorders, visit www.bravecommunity.com or www.hunterpatients.com.

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