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MIBio 2014

Stability of biopharmaceuticals
Getting the chemistry right

Tuesday 30th September 2014
Downing College, Cambridge

Stability of biopharmaceuticals

Getting the chemistry right

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An event jointly organised by the Formulation Science and Technology Group of the RSC and the BioMolecule focus group of the APS.

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The Formulation Science and Technology Group (FSTG)

The Formulation Science and Technology Group is a subject group of the Royal Society of Chemistry, London. It is the leading scientific organisation dedicated to product formulation. As a charitable organisation, it works for the benefits of its members and to further the awareness of formulation science. It fosters the advancement of formulation science across many scientific disciplines and industrial applications, including pharmaceuticals, cosmetics, foods and detergents. It is a point of focus for all industrialists and academics engaged in the practice of formulation science. The FSTG organises many events during the year for the benefit of its members, including conferences, training days, and networking events.

For more information visit: www.formulation.org.uk

The Academy of Pharmaceutical Sciences (APS)

The Academy of Pharmaceutical Sciences (APS) is the professional body for the Pharmaceutical Sciences in United Kingdom. A Pharmaceutical Scientist is an individual who contributes to bringing a new drug from concept through to a medicinal product. This includes, but is not exclusive to, individuals in academia, industry, regulation, clinical research and manufacturing. The mission of the Academy of Pharmaceutical Sciences is to champion innovation and opportunities in Pharmaceutical Sciences for the delivery of medicines. In order to achieve this mission, our aims are to:

- Promote the Pharmaceutical Sciences to stakeholders to facilitate understanding and achieve engagement
- Share learning, drive collaboration, partnership and innovation
- Deliver insights into industry and academic roles for aspiring scientists

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Joint Colloids Group (RSC/SCI)

Colloid science today is a very broad and dynamic subject spanning a wide range of areas from classical dispersions to novel nanoparticles for drug delivery. The Colloid Group aims to act as a focus for these interests for the UK colloid community (and wider). Whether you are a chemist, physicist, engineer, student, formulation scientist, nanotechnologist, pharmacist, biologist, polymer scientist, food scientist or any other related discipline interested in colloids, we invite you to join the Colloid Group. Today the group supports the UK colloid community by organising a range of meetings ranging from one day to multiday meetings. We also invite nominations for outstanding contributions to colloid science in the UK through our three awards, the McBain Medal, the Thomas Graham Lecture and the Rideal Award.

For more information visit: www.colloidsgroup.org.uk

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Foreword

MIBio - Molecular Interactions of Biopharmaceutical Formulation

It is a pleasure to welcome you to the 4th annual MIBio conference at Downing College, Cambridge. The aim of the MIBio series is to bring together the community of experts joint by their interest in various aspects of biopharmaceutical formulation.

The importance of formulation in the biopharmaceutical development process has been steadily increasing due to its ability to enable new stable products, compositions for convenient delivery formats or life-cycle management of existing products. It is one of our goals to communicate this importance more widely. We believe that in order to ensure future progress the industrial and academic community need to work together, and we are delighted to welcome a very healthy mix of academics and industrialists at our event. We are also delighted to have the support of the Biopharm formulation group of KTN as we recognise their critical role in bringing the biopharmaceutical community together.

Each year, the MIBio conference has a special theme. The main focus of MIBio 2014 is on understanding key chemical interactions that affect stability of biopharmaceutical products. Biopharmaceutical compositions are complex chemical systems involving various chemical interactions between active and inactive ingredients and the container holding the composition. These interactions can affect the product quality. Chemical stability aspects of a wide range of biopharmaceuticals and vaccines will be discussed during the day by international experts in the field.

Many thanks to all the authors who sent in abstracts and are contributing to the poster sessions. We are also grateful to all the sponsors and exhibitors who have made this meeting possible.

MIBio 2014 is a one-day event, but the discussion you start today may lead to new collaborations and discoveries that will help steer the development of the next generation formulation technologies.

We hope you enjoy the event,

Jan Jezek, Nick Darton, Tejash Shah and Stephen Harding
on behalf of MIBio 2014

MIBio 2014 Programme

08:00 Registration opens

09:00 Opening remarks

Morning session, chaired by Stephen Harding (University of Nottingham, UK)

09:15 **Stability of Biopharmaceuticals: Past, present and future**
Paul Varley (Medimmune, UK)

09:45 **Antibody glycoforms and stability**
Roy Jefferis (University of Birmingham, UK)

10:15 Speed Networking

11:00 **Primary packaging for biopharmaceuticals: challenges & options**
Horst Koller (Schott, Switzerland)

11:30 Exhibitors highlight

12:15 Lunch Break, Exhibition and Posters

Afternoon session, chaired by Ahmed Yassin (GSK, UK)

13:30 Introduction

13:45 **Developability evaluation during early pre-formulation: the meeting point between research and development**
Bernardo Perez-Ramirez (Genzyme, USA)

14:15 **Evaluation of chemical stability during developability assessment of therapeutic antibodies**
Daniel Heitmann (Novartis, Switzerland)

14:45 Coffee break, Exhibition and Posters

15:15 Discussion panel: Challenges of setting specifications for biopharmaceutical products

16:15 **Glycoconjugate vaccine stability and formulation**
Chris Jones (NIBSC, UK)

16:45 Concluding remarks

17:00 Conference ends

Stability of biopharmaceuticals

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Morning Session - Lecture Abstract

Stability of Biopharmaceuticals: Past, present and future

Paul Varley - MedImmune, UK

Having been involved in many aspects of biopharmaceutical product development for more than 20 years, Paul Varley will offer his personal perspective on the progress made in understanding and improving product stability in the broader context of the biopharmaceutical development process. He will also comment on the current and possible future trends in the industry.

Morning Session - Lecture Abstract

Antibody glycoforms and stability

Roy Jefferis - University of Birmingham, UK

Although exhibiting > 95 % sequence homology each of the four human IgG subclasses (isotypes) exhibit a unique profile of IgG-Fc mediated downstream effector activities. Immune complexes formed by IgG1 subclass antibodies have the potential to bind and activate one or more of ten endogenous ligands (Fc receptors, complement C1 etc.). The activation profile determines “Mechanism of Action” (MOA) of an antibody therapeutic. Glycosylation of the IgG1-Fc is essential for binding and activation of these endogenous ligands and the glycoform profile of an antibody therapeutic is a “Critical Quality Attribute” (CQA). Protein and glycosylation engineering has been employed to generate variants of both glycosylated and aglycosylated IgG molecules exhibiting enhanced and/or selective effector profiles. However, the dramatic impact on functional efficacy achieved is not reflected in gross structural changes, as determined by x-ray crystallography and NMR. The dynamic nature of the antibody molecule provides a “test bed” for structure/function studies and the generation of novel molecules with therapeutic potential.

Jefferis R. (2012) Isotype and glycoform selection for antibody therapeutics. *Arch. Biochem. Biophys.* 526: 159-166.

Jefferis R. (2011) Aggregation, immune complexes and immunogenicity. *mAb* 3: 503-4

Mimura, Y., Jefferis, R. (2007) Contrasting glycosylation profiles between Fab and Fc of a human IgG protein studied by electrospray ionization mass spectrometry. *J Immunol Methods.* 326:116-26.

Stability of biopharmaceuticals

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Morning Session - Lecture Abstract

Primary packaging for biopharmaceuticals: challenges & options

Horst Koller (Schott, Switzerland)

Biopharmaceutical therapies have grown in importance and an increasing number of such therapies have received regulatory approval. For many years now, the majority of delivery systems and devices are made out of glass / plastic / rubber combinations. But heightened regulatory requirements as well as increased focus on drug container interactions pose a daunting challenge on the design, development and manufacturing of such systems. All end-users like health care professionals and patients take it for granted that the packaging protects the drug product from the external environment without compromising the product potency and efficacy. This task is not only the responsibility of the drug product manufacturer but also of the container, component and device manufacturer. One of the hot regulatory topics is the migration of possible extractable compounds into the drug product, the so called leachables. This leachable behavior can be minimized but not completely avoided by, for example, coating of the container surface or rubber surface which is in direct product contact. This talk will focus on extractables and therefore possible leachables from different container materials and also show options for an improved leachable behavior and delivery systems to ensure drug product stability, especially for sensitive biopharmaceuticals.

Afternoon session – Lecture Abstract

Developability evaluation during early pre-formulation: the meeting point between research and development

Bernardo Perez-Ramirez (Genzyme)

Development of robust formulations for therapeutic proteins requires a synergistic approach between different departments to select protein constructs that could endure the stresses that usually happen during manufacturing, storage and clinical administration. Some of the process stresses can be mimicked in the laboratory and used as probes to select the proper constructs to move into formulation development. Among those stresses are viral inactivation, freeze-thaw and shear stress. The expected outcome of those studies should be no change in the physical and chemical properties of the proteins analyzed. In addition, stresses at high temperature (45 °C) as function of low and high pH could serve to differentiate the candidate proteins and rank them. The use of key process stresses in combination with selective analytical assays to evaluate the robustness of four IgG constructs that recognize the same biological target will be presented in this talk. The approach discussed assists in de-risking protein constructs based on their physical, chemical and biological properties before moving them into formal development.

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Afternoon session – Lecture Abstract

Evaluation of chemical stability during developability assessment of therapeutic antibodies

Daniel Heitmann - Novartis, Switzerland

The pharmaceutical industry faces two major changes that will transform the practice how it operates. Biopharmaceuticals have enabled the treatment of diseases that could not be addressed by traditional small molecule approaches so far. Controlling the costs for the development of such innovative medicines is a prerequisite to deliver affordable drugs to patients. Consequently, the research and development process has to be optimized in a way that the overall spending per project can be reduced and project failures are minimized. The Integrated Biologics Profiling Unit (IBP) was founded within Novartis Pharmaceuticals to de-risk the technical development process. Among many other characteristics, the stability of the biological compounds is tested early on to avoid problems (increased efforts) in late phase technical development. This presentation will highlight in three case studies the assessment, detection and potential risks arising from deamidation. Deamidation is one of the major concerns for chemical stability arising from the primary amino acid sequence and liable sequence motifs are ideally deselected early on. As occurrence, kinetics, impact on product quality and shelf life by deamidation are hard to predict solely from the primary sequence data or since in some cases liable motifs cannot be deselected, formulation stress testing, biophysical characterization and bioactivity assays play a major role in assessing the potential risk for the overall technical development process.

Afternoon session – Lecture Abstract

Glycoconjugate vaccine stability and formulation.

Chris Jones - NIBSC, UK

Glycoconjugate vaccines, in which the cell surface polysaccharide, or a fragment from it, is covalently coupled to a suitable carrier protein are widely used to protect against a range of pathogenic bacteria, including Haemophilus influenzae, Neisseria meningitidis, Streptococcus pneumoniae and Salmonella Typhi which currently kill more than a million under-5s per year. These vaccines are key components of World Health Organisation (WHO)-sponsored mass immunisation programmes in developing countries. Instability most commonly arises from the depolymerisation of the polysaccharide chain. In this talk I will discuss the structure of these vaccines, and how stability relates to the different structures of the repeat units. Spectroscopic and chromatographic methods are used to assess product stability and define the pathways for degradation. This may require the development of lyophilised products, mixed lyophilised/liquid formulations for multivalent vaccines or controlled interaction with aluminium-based adjuvants in combination vaccines. To support delivery of these vaccines to isolated communities, WHO are developing criteria for enhanced stability after the vaccine final fills leave the cold chain.

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Exhibitors contact details -

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Oval Medical Technologies

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Poster Abstract

A stability-indicating HPLC-DAD method for determination of STP: development, validation, kinetics, structure elucidation and application to commercial dosage form

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Siripentol (STP) is a newly approved orphan antiepileptic drug (AED), marketed under the name Diacomit® (Biocodex Inc.). In the current study, a simple, sensitive and accurate stability-indicating HPLC-DAD method has been developed and validated for the determination of STP and its degradation product in bulk and capsule form. Chromatographic separation was achieved on a Symmetry® C18 column, using a mobile phase consisting of acetonitrile and 50 mM potassium dihydrogen phosphate buffer (apparent pH ~ 4.1 ± 0.1) (60:40, v/v) at a flow rate of 1.0 mL.min⁻¹, quantification was achieved using photodiode array detector (DAD) at 262.5 nm. The method was validated in accordance with the International Conference on Harmonization (ICH) guidelines showing specificity, linearity ($r^2 = 0.9996$, range of 1-25 µg/mL), precision (relative standard deviation lower than 2%), accuracy (mean recovery 100.08±1.73), limits of detection and quantification (LOD= 0.024 and LOQ = 0.081 µg/mL) and robustness. STP, was subjected to various stress conditions. STP has shown marked stability under alkaline hydrolytic stress conditions, thermal, oxidative and photolytic conditions. It degraded only under acidic conditions, forming a single degradation product which was well resolved from the pure drug with significantly different retention time values. This degradation product was identified by ¹H-NMR and ¹³C-NMR spectroscopy as well as ion trap mass spectrometry. This degradation process revealed a first order kinetics with a half life ($t_{1/2}$) of 1.42 hours. The results demonstrated that the method would have a great value when applied in quality control and stability studies for STP.

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Poster Abstract

Taylor Dispersion Analysis: An emerging technique for characterising the stability of proteins

Rachel Bott, Matthew Brown, Oksana Leszczyszyn

Malvern Instruments Limited, Malvern, Worcestershire, UK

Stability of biopharmaceuticals is fundamental to their efficacy as a biotherapeutic, but understanding how different factors influence their stability can often be challenging; complicated by complex backgrounds, limited sample volumes, as well as high and low protein concentrations. Combining Taylor Dispersion Analysis (TDA) with UV area imaging, we show how this emerging technique can address all of the above and give complementary information about sample stability compared to more established techniques such as DLS and SEC.

The principals of TDA allow the behaviour and stability of analytes to be monitored through changes in hydrodynamic size, and can be applied to a variety of different analytes; ranging from proteins and peptides down to small molecules. Here we demonstrate how TDA can be employed to characterise the stability of Insulin and other proteins through several key examples: We show how aggregation induced in forced degradation studies, using for example thermal or chemical stress, can be assessed. We also show how TDA can be used to monitor changes in the oligomeric state of Insulin after the addition of different excipients, to determine transitions between active and inactive oligomeric states.

Poster Abstract

Dextran-Based Nanoparticulate Carriers for Peptide Delivery to the Brain

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The blood-brain barrier (BBB) forms a very significant challenge for drug delivery to the central nervous system (CNS). Particularly for recently developed macromolecular therapeutic agents. One such agent is octapeptide OP, an endozepine-like peptide, which shows potential in the treatment of various neurological and endocrine diseases [1]. Previous research in our group has shown that modified dextran nanoparticles are able to cross the BBB. This work focuses on the development of these modified dextran nanoparticles as delivery vehicles for OP into the brain. This technology could be extended for the treatment of various disorders of the CNS. As a model peptide, Angiotensin 2 (ANG2) was entrapped with an efficiency of 34% and 43% using the double emulsion (WOW) and the solid in oil in water dispersion (SOW) methods respectively. Similar results were achieved with both BSA and OP. The size of the resulting nanoparticles was measured using dynamic light scattering (DLS) and nanoparticle tracking analysis (NTA) and was found to be around 250 nm. The zeta potential of the resulting nanoformulations (ca. -30 mV to -40 mV) was indicative of their good stability. Release studies using nanoparticles loaded with ANG2 have shown a 50% burst release, followed by an extra 30% of the total amount of peptide being released gradually over a period of one week. The activity of the released ANG2 was found to be comparable to that of free ANG2 using a bioactivity assay that employs HUVEC cells. The ANG2 loaded nanoparticles were also shown to be non-toxic to human endothelial cells. Finally, the ability of these peptide-loaded nanoparticles to cross the blood brain barrier will be investigated using a brain endothelial cell based BBB permeability model.

References: J. Leprince, P. Gandolfo, J.L. Thoumas, C. Patte, J.L. Fauchere, H. Vaudry, M.C. Tonon, *Journal of Medicinal Chemistry* 1998, 41, 4433-38.

Stability of biopharmaceuticals

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Poster Abstract

Temperature denaturation and aggregation of a multi-domain protein (IgG1) investigated with an array of complementary biophysical methods

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The challenge of biopharmaceutical manufacturing that is often seen as greater than that for small molecule drugs is largely associated with protein stability. Differential scanning calorimetry, used to optimise formulation conditions by monitoring thermal denaturation midpoints, may not apply in cases of irreversible aggregation or independent transitions of constituent domains.

An approach capable of tackling several aspects of structural integrity may be necessary in these and other cases where different structural transitions are likely to lead to degradation. Using as a monoclonal antibody, we provide an example of how complementary biophysical techniques can support a rationale for improving and stabilising active conformation. In comparison, our data demonstrates how optimisation of storage conditions, which is based only on thermal stability studies, could lead to the stabilisation of an undesired product.

Poster Abstract

Reformulation of protein therapeutics to improve their stability

Nicholas Darton, David Gerring, Jayne Curry, Leon Zakrzewski and Jan Jezek

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The stability of biopharmaceutical molecules is key to their successful therapeutic or diagnostic application. A number of processes, both reversible and irreversible, affect the rate of the degradation processes and therefore the stability of biological molecules. The ability to control the rates of these processes by selection of the right formulation represents a powerful tool for stabilization of biological molecules. The rates can be controlled successfully by optimizing a number of conventional formulation parameters, such as pH or selection of a surfactant. However, various additional formulation features can be implemented to further improve the stability. Presented here are three case studies demonstrating considerable improvement in stability of existing protein therapeutics by specific changes in their formulation. The nature of these formulation changes is explained, and a possible mechanism of action of the stabilizing excipients is suggested. The stability conferred by the reformulations of these products potentially enables cold chain free shipment providing better vaccine and medicine coverage.

Stability of biopharmaceuticals

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Poster Abstract

Formulating a stable natural product: pumpkin seed oil bodies

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In order to develop a compound for pharmaceutical interest from natural sources maintenance of the stability is a key issue. Numerous environmental factors such as temperature, humidity and pH can influence the stability of the product. Therefore, characterisation of physical properties of the active substances in the solution is crucial for therapeutic industry to obtain a stable biopharmaceutical product. Pumpkin seeds encompass a treasure for nutraceutical compounds. The health benefits associated with pumpkin seed and seed oil lists prevention against a number of diseases. In current research we have isolated and characterized pumpkin seed oil bodies (PSO) with respect to their stability as a colloid. The purpose behind extracting oil bodies from pumpkin seed is to provide an alternative source for lipid droplet that could be used in its natural form (protected by its proteins and phospholipid layer) for multiple industrial applications mainly in the biopharmaceutical capacity. This could involve their role for hydrophobic drugs, adjuvants for vaccines, encapsulation for prebiotics and purification for recombinant proteins. The colloidal solution of oil bodies (oil bodies in the specific buffer with variable salt concentration) itself present a naturally stabilized molecule. Characterisation of this colloid by changing pH and temperature provides us an array of environmental conditions. Knowledge of zeta potential is also the key point for characterisation of electrochemical surface properties. Alteration in any of these parameters of the solvent altogether changes the behaviour of PSO. We have previously extracted and isolated PSOs using an aqueous extraction method. Colloidal solutions of PSOs were characterized for their stability under increasing temperature and change in ionic strength (Adams, Imran et al. 2012). In the present work stability of PSOs were analysed at increasing ionic strength (0-250mM NaCl) and temperature. The size distribution analysis and zeta potential measurements were carried out using dynamic light scattering. Thus extracted PSOs can be used alone (in natural intact form) or in combination with other polymers for biomedical applications. In general, stability of PSOs is a critical factor for their effective applications. For commercial purposes, enhancement of oil-body stability tends to reduce processing cost by attenuating the degradation of oil bodies and may also promote the development of novel applications. These results are crucial to deeply recognize the structure and behaviour of oil bodies from pumpkin seeds in conditions closely related to natural physiological environment in order to explore the potential of these substances (protein and oils) as colloids in biopharmaceutical industry.

Reference: Adams, G. G., S. Imran, et al. (2012). "Extraction, isolation and characterisation of oil bodies from pumpkin seeds for therapeutic use." *Food Chemistry* 134: 1919-1925.

Poster Abstract

A novel in vitro method for estimating formulation performance of subcutaneously administered biopharmaceuticals

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Subcutaneously administered biopharmaceuticals typically provide a shelf-life of two years but often low or largely variable bioavailability outcomes. It has been recently suggested that the chemical, physical and physiological environment of the subcutaneous tissue may play a role in determining the extent of biotherapeutic absorption from the injection site following a subcutaneous injection [1]. A novel in vitro method for predicting how the characteristics of the subcutaneous tissue may influence the post-injection stability and diffusional properties of subcutaneously administered biopharmaceuticals has been developed. This Subcutaneous Injection Site Simulator (SciSSor) enables identification of any unwanted interactions that may hinder the biotherapeutic absorption from the sc injection site in a tractable manner, thus allowing for rational formulation design that in addition to a sufficient shelf-life will also provide optimum absorption from the injection site.

[1] Kinnunen, H. M. and R. J. Mrsny (2014). "Improving the outcomes of biopharmaceutical delivery via the subcutaneous route by understanding the chemical, physical and physiological properties of the subcutaneous injection site." *Journal of Controlled Release* 182: 22-32.

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Poster Abstract

Taylor Dispersion: a tool for mass-weighted, UV selective analysis of hydrodynamic size in complex formulations

[Oksana I. Leszczyszyn](#), Karl Hampton, Rachel Bott

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Taylor Dispersion is a theoretical model that describes the behaviour of a sample plug traversing a cylindrical pipe under Poiseuille flow [1]. The advective and diffusive properties of the molecules contained within the sample plug give rise to characteristic flow profiles – known as Taylorgrams - from which the molecular diffusion coefficient and, in turn, hydrodynamic radius can be determined. Using data acquired with the Viscosizer 200 system (Malvern Instruments, Worcestershire UK) - which couples a microcapillary with UV detection to visualise the flow profiles of nanolitre sample plugs – we show, with particular examples, the characteristic Taylorgrams of small molecules, proteins and samples with multi-component species. Principally, the analysis of such Taylorgrams can be used to selectively obtain the mass-weighted hydrodynamic size of UV active species within samples; however, we further extend the discussion to the potential applications for this technique in characterising the behaviour, conformation and stability of proteins and other target molecules within complex media e.g. excipient-laden bioformulations, aggregated solutions and other mixtures.

[1] Taylor G., Proc. Royal Soc. London, Series A, Math. Phys. Sci., 1953, 219:(1137)186-203

Poster Abstract

Aggregation stability of an IgG4 antibody during formulation studies

Mariam M. Nuhu and Robin Curtis

School of Chemical Engineering and Analytical Science, University of Manchester

One of the largest and fastest growing classes of biopharmaceutical products on the market includes antibodies and antibody-derived products. Like other protein therapeutics, antibodies have the tendency to aggregate under a range of formulation conditions. Thus, it is important to generate optimal solution conditions which ultimately control the formulation of the protein drugs. The main aim of this work is to study the aggregation behaviour and the stability characteristics of a monoclonal antibody (IgG4) under a range of operating conditions in the presence of a novel cationic excipient. Conformational and colloidal stability of the antibody was determined from temperature controlled light scattering and intrinsic fluorescence studies. Dynamic and static light scattering were used to measure protein-protein and protein-excipient interactions at room temperature. Experiments reveal that the nature of the solvent environment affects the conformational and aggregation stability of the antibody where a decrease in the conformational stability of the protein increases the tendency for oligomerization and vice versa. Measurements are made as a function of pH and ionic strength in order to change the charge properties of the antibody and understand the intermolecular forces controlling the formulation stability.

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Poster Abstract

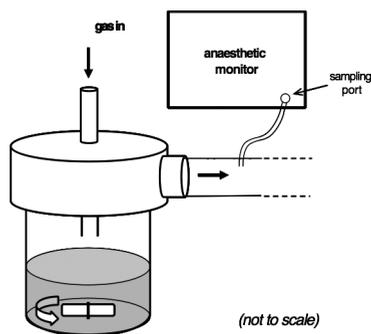
Volatile fluorinated nanoemulsions: a chemical route to controlled delivery of inhalation anaesthesia

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Novel dispersions of the volatile inhalation anaesthetic sevoflurane have been formulated that can provide controlled, sustainable release of anaesthetic over clinically useful timescales. The emulsions can be simply formed with manual shaking, reproducibly yielding droplets of the order of 250 nm diameter, i.e. within the nanoemulsion range. Using a custom flow-rig, release of anaesthetic gas from the emulsion has been evaluated and clinically useful levels achieved through appropriate stirring of the formulation. Stirring can also be used to temporarily increase or decrease the amount of anaesthetic released. Once some essential factors are taken into account relating to the unusual nature of the fluorinated systems and the highly perturbed environment of their evaluation, the observed behaviour regarding sevoflurane evaporation can be reasonably well explained by existing theoretical models of evaporation from emulsions and links between release and emulsion structure defined, providing the basis for future development.



Poster Abstract

Dissolution-permeability apparatus with integrated in situ concentration monitoring of both donor and receiver compartments.

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Introduction: Development strategies for insoluble compounds require not only measurement of solubility enhancements achieved through formulation but also assessment of the effect a formulation has on permeability. A new dissolution-permeability measurement platform (μ FLUX™) allows simultaneous monitoring of both effects thereby providing an invitro path to early in vivo predictive biorelevant absorption testing.

Methods: The μ FLUX™ device is an add-on option to the μ DISS Profiler™ instrument (Pion Inc.) consisting of three pairs of temperature controlled side-by-side permeability chambers mounted on top of the stirring platform. Each pair (Figure 2) consists of a donor and a receiver compartment separated by a filter-supported GIT-optimised artificial membrane (Double-Sink™ PAMPA). The donor compartment is filled with 10 - 13 mL of the media of interest. For this study the receiver compartment contained Acceptor Sink Buffer at pH 7.4 (ASB-7.4, Pion Inc). The integrated fiber-optic UV probes were positioned in the donor and receiver compartments allowing real time concentration monitoring in all chambers.

Conclusions: It is interesting to note, that although the total concentration of Danazol in the donor compartment was up to 40 times higher in the case of FeSSIF versus FeSSIFblank media, the flux increased only 5.4 fold. This indicates that solubility enhancement due to micelle formation does not lead to the same magnitude of absorption increase. In many cases, dissolution experiments alone cannot correctly predict the in vivo response to formulations due to the peculiar interplay of solubility and permeability in complex media. The μ FLUX device extends the utility of in situ concentration monitoring to improved assessment of formulation on absorption potential and more realistic IVIVC modeling.

[1] A. Avdeef, et al. Absorption-Excipient-pH classification gradient maps: Sparingly-soluble drugs and pH partitioning hypothesis. *Eur. J. Pharm. Sci.* 2008, 33 (1), 29-41.

[2] A. Avdeef, O. Tsinman. PAMPA—A drug absorption in vitro model. 13. Chemical selectivity due to membrane hydrogen bonding: In combo comparisons of HDM-, DOPC-, and DS-PAMPA models. *Eur. J. Pharm. Sci.* 2006, 28 (1), 43-59.

[3] J. H. Fagerberg, et al. Dissolution Rate and Apparent Solubility of Poorly Soluble Drugs in Biorelevant Dissolution Media. *Mol. Pharm.* 2010, 7 (5), 1419-1430.

Stability of biopharmaceuticals

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Poster Abstract

Nanoparticulate drug delivery systems for the local delivery of growth factors for fracture healing

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Aquasomes are nanoparticulate carrier systems which comprise ceramic nanocores coated with a polyhydroxyl oligomer to which drug/antigen/peptide can be adsorbed. The rigid nanocore (hydroxyapatite) retains the conformational integrity of adsorbed bio-pharmaceuticals by conferring structural rigidity to the system and its polyhydroxyl coating (trehalose) protects from dehydration and degradation usually associated with the delivery of biochemically active molecules. Bone morphogenetic protein (BMP) is a growth factor that stimulates ectopic bone formation. They have been exploited in orthopaedics to aid in bone regeneration. Hydroxyapatite nanocores were coated with trehalose, the polyhydroxyl oligomer of choice. BMP was then adsorbed onto the surface of the coated cores and lyophilised to fabricate the desired aquasomes. In vitro drug release studies of BMP from BMP-loaded aquasomes to osteoblasts (SAOS-2) and pre-osteoblasts (MG-63) were performed. Alkaline phosphatase (ALP) production was used to analyse the differentiation effect of BMP released from aquasomes on pre-osteoblasts. Results showed that ALP secreted is relatively constant which can be compared with BMP release. The use of aquasomes for sustained delivery of growth factors can be implicated in fracture healing of which both cases require an increase in bone cell proliferation.

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