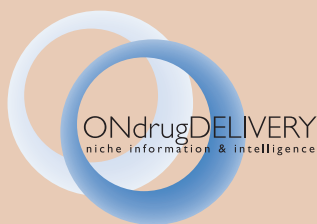


INJECTABLE DRUG DELIVERY 2013: FORMULATIONS FOCUS



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Sep: Drug Formulation & Delivery Solutions & Services 2013
Oct: Prefilled Syringes
Nov: Pulmonary & Nasal Drug Delivery
Dec: Delivering Biotherapeutics
Jan: Ophthalmic Drug Delivery
Feb: Prefilled Syringes
Mar: Transdermal Patches, Microneedles & Needle-Free Injectors
May: Injectable Drug Delivery: Devices Focus
June: Injectable Drug Delivery: Pharmaceuticals Focus
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ONdrugDelivery 2013/14 EDITORIAL CALENDAR

Publication Month	Issue Topic	Materials Deadline
September 2013	Drug Formulation & Delivery Solutions & Services 2013	August 5th
October 2013	Prefilled Syringes	September 2nd
November 2013	Pulmonary & Nasal Drug Delivery (OINDP)	October 7th
December 2013	Delivering Biotherapeutics	November 4th
January 2014	Ophthalmic Drug Delivery	December 9th
February 2014	Prefilled Syringes	January 13th
March 2014	Transdermal Patches, Microneedles & Needle-Free Injection	February 10th
April 2014	Pulmonary & Nasal Drug Delivery	March 10th
May 2014	Injectable Drug Delivery 2014: Devices Focus	April 14th
June 2014	Injectable Drug Delivery 2014: Pharmaceuticals Focus	May 12th
July 2014	Novel Oral Delivery Systems	June 9th



SMART FORMULATIONS OF LIQUID BIOTHERAPEUTICS

In this article, Jan Jezek, PhD, Chief Scientific Officer, Arecor Ltd, highlights the advantages of liquid formulations of biotherapeutics compared with lyophilised powder formulations. He describes the challenges of, and numerous factors affecting, liquid formulation stability, and introduces some of the innovative techniques and technologies that Arecor has developed to improve the stability of liquid formulations of vaccines and biotherapeutics.

STABILITY IS CRITICAL

The parenteral drug sector is experiencing a considerable growth, particularly due to the development of new biotherapeutics for a range of acute and chronic conditions. Although many products are still formulated as lyophilised powders requiring reconstitution prior to use, pharma companies can make considerably better use of liquid formulations, particularly in combination with

Stability of a biotherapeutic, a prerequisite for the development of a successful liquid product, is a function of numerous parameters, including the formulation, interactions with the container-closure system and stress conditions. Various stress conditions must be applied during the development of a robust biotherapeutic, such as: storage (a combined function of temperature and time); shaking; freeze-thaw stress; and exposure to light.

Protein instability can be divided into two

key categories: physical instability and chemical instability.¹ The main physical and chemical stability issues affecting biotherapeutics are shown in Figure 1.

Determining which stability issues are critical and which are non-critical is an important part of the product development process, together with setting speci-

fications for impurities resulting from the degradation processes. A suitable formulation must then be developed to meet the specifications.

FORMULATION IS CRITICAL FOR REQUIRED STABILITY

Formulation is a very powerful tool for controlling biotherapeutic stability and thus achieving the target product profile. Formulation is defined by the nature and quantity of specified excipients as well as other parameters such as pH. The excipients present in the formula-

"STABILITY OF A BIOTHERAPEUTIC, A PREREQUISITE FOR THE DEVELOPMENT OF A SUCCESSFUL LIQUID PRODUCT, IS A FUNCTION OF NUMEROUS PARAMETERS"

convenient, user-friendly and cost effective prefilled devices. The major benefits of the prefilled devices incorporating liquid formulations include:

- Minimisation of dosage errors due to the reduced number of steps involved
- Reduced risk of contamination
- Ease and speed of administration
- Potential for self-administration by the patient
- Improved patient compliance
- Sterility assurance
- Elimination of the need for overfill of costly biotherapeutics



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Physical instability	Chemical instability
Aggregation	Deamidation
Precipitation/particle formation	Aspartate isomerisation
Denaturation	Hydrolytic cleavage
Surface adsorption	Racemisation and β -elimination
Self association	Oxidation

Figure 1: Major degradation pathways of biotherapeutics.

Excipient type	Purpose
Buffer	Maintaining required pH
Tonicity modifier	Adjustment of osmolarity (tonicity)
Surfactant	Stability improvement, particularly with respect to agitation stress
Preservative	Prevention of microbial growth in multi-dose formulations
Antioxidant	Reduction in the rate of oxidation
Stabiliser	Stability improvement

Figure 2: Key excipient types in biopharmaceutical formulations.

tion define additional important parameters of the formulation such as osmolarity or ionic strength. Many of these parameters should be kept within optimal limits to minimise adverse side effects. For example, there is an optimum level established for formulation osmolarity to minimise the risk of injection pain.

Typically, each component serves a specific purpose in the formulation (Figure 2). It is strongly preferable to use excipients with a history of use in approved drug products because of their established safety profile, and ideally the excipients should be used within the approved concentration limit for the intended route of administration.

Traditionally, the stability of biologics is controlled by optimisation of basic formulation parameters, such as pH, ionic strength, and the nature of the key components. Determining the optimal pH and selecting an appropriate buffer is a critical step in formulation development. Near-neutral pH is preferable, but generally pH 5-8 is seen as safe. However, a number of successful products are formulated outside of this range, for example Lantus (insulin glargine, Sanofi Aventis), which is at pH 4.0, and Fuzeon (enfuvirtide, Roche) which has a pH of 9.0.² The tonicity modifier is typically the dominant component in a formulation, and its key purpose is to adjust osmolarity to a required level. Salts (sodium chloride, for example), sugars and sugar alcohols (such as trehalose and mannitol), are typically used as tonicity modifi-

ers. Surfactants, especially non-ionic surfactants (for example, polysorbate 20 and 80), are often added to protein formulations to prevent or minimise the surface interface induced damage, particularly in the presence of shaking or agitation stress. Antioxidants (e.g. methionine) can be used to prevent chemical changes due to oxidative stress. In the case of multi-dose formulations, preservatives (e.g. m-cresol or benzyl alcohol) must be included to prevent microbial growth.

Some excipients have been found to have a stabilising effect with respect to particular degradation pathways and maintaining structural

integrity of the biotherapeutic, so these can be added to the formulation as stabilisers. For example, metal cations can have a stabilising effect, particularly on proteins that contain a structural metal ion. Formulations of Factor VIII contain a small amount of calcium chloride,² as calcium ion binds within the structure of Factor VIII and improves its conformational stability.

Both anionic and cationic polymers have been reported to increase stability of some proteins.^{3,4} Cyclodextrins are another class of polymeric molecules reported and commercialised to improve stability and/or solubility of certain proteins.⁵ Amino acids, including histidine and arginine, have been described as having the ability to stabilise proteins by a variety of mechanisms, including preferential exclusion, direct protein binding and prevention of oxidation. Some proteins have also been used as excipients to improve stability, for example human serum albumin (HSA) and hydrolysed gelatin (predominately used in vaccine formulations).

EXPLORING A BROADER FORMULATION DESIGN SPACE

In order to develop better liquid formulations of biotherapeutics it is necessary to broaden the formulation design space, including:

- Screening of new excipients
- Understanding beneficial synergistic effects between excipients
- Understanding the synergies between the effect of excipients and other conditions such as ionic strength
- Understanding the destabilising effects of specific conditions and excipients

Whilst computational tools, such as Design of Experiment can be very helpful to map out the design space, the number of possible permutations of excipients and other param-

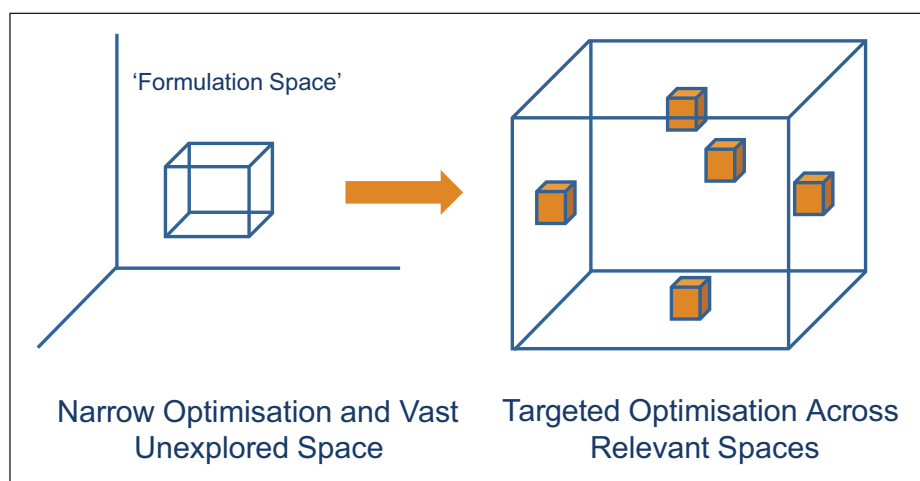


Figure 3: Identification of suitable subsets allows effective expansion of the design space over which a formulation can be optimised.

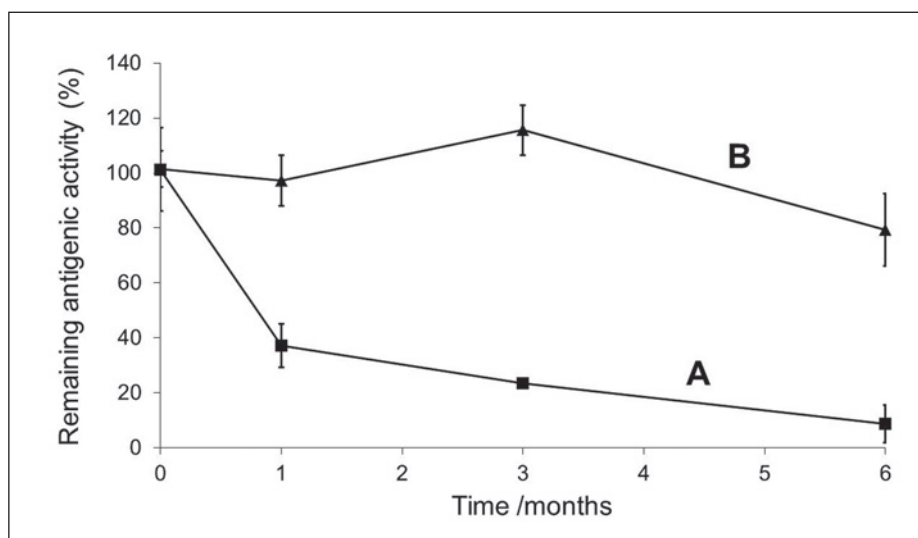


Figure 4: Recovery of antigenic activity of hepatitis B vaccine formulations at 45°C. The formulations were: (A) original Shanvac-B formulation; (B) 40 mM phosphate, 40 mM histidine, 100 mM NaCl, pH 5.2.

eters is extremely high, making evaluation difficult. It is therefore necessary to employ rational approaches to identify specific subsets of the broad design space to focus on (Figure 3) in order to develop formulations with superior stability.

Arecor Ltd, a biotechnology company based in Cambridge, UK, has developed a rational approach to identify these subsets. Arecor further develops subsets into robust formulation tools and uses these tools to develop improved liquid formulations of biotherapeutics.

The scope of this article does not allow a full explanation of the formulation technologies developed at Arecor, but a few brief examples are presented here.

As mentioned above, selection of appropriate buffer is an essential part of formulation development. The most common buffers used in parenteral formulations are phosphate, cit-

detrimental to certain aspects of protein stability, including aggregation. An alternative approach, based on using dual buffer systems with pKa values >1 unit from the pH of the composition has been demonstrated to improve stability.⁷

An example of the application of this technology has been reported⁸ using Hepatitis B vaccine in a joint project with colleagues from the Program for Appropriate Technology in Health (PATH; www.path.org) and the University of Colorado Denver, School of Pharmacy. The stability of the vaccine was particularly improved in the presence of excipients that did not comprise an ionisable group with pKa close to the pH of the formulation (i.e. conventional buffers), but comprised an ionisable group with pKa >1 unit away from the pH of the formulation.

The apparent importance of the pKa in the stabilising effect of the selected excipients suggests that exchange of hydrogen cations

phosphate at pH around 5.0 showed considerably improved stability at elevated temperatures compared with the currently marketed product, Shanvac-B (Shantha Biotechnics Pvt Ltd (Sanofi), Hyderabad, India) (see Figure 4). The stability of the product was also confirmed in an animal study.⁹

The new vaccine formulation has the potential to be used outside the cold chain for part of its shelf life. This is very likely to improve the immunisation coverage, simplify the logistics for outreach immunisation, and ensure the potency of the vaccine in areas where the cold chain is insufficient.

Another example of the application of two of Arecor's technologies is shown in Figure 5, using erythropoietin (EPO). The formulation of the existing marketed liquid product was compared with the new formulation based on Arecor's technology, using a combination of TRIS and benzoate anion (i.e. dual buffer system with pKa values >1 unit from the pH of the composition instead of phosphate (i.e. a conventional buffer). The formulation change resulted in a considerable improvement of stability with respect to aggregation (Figure 5).

Additional stability was achieved through using a second technology, the use of small amphiphilic species under specific salt conditions. It should be noted that the use of a small amphiphilic species (in this example, the benzoate anion) was found to be essential for achieving the required stability. It is believed that the synergistic effect of benzoate anion and salt conditions is due to non-covalent interactions with hydrophobic patches on the surface of the proteins.

CONCLUSION

Formulation is a very powerful tool to control the stability of biotherapeutics. Conventional approaches to formulation are well established and in many cases result in satisfactory stability to support suitable target product profiles. However, with increasing commercial pressures, it is essential to seek further improvements in stability to allow more convenient drug delivery options and, where possible, permit next-generation biotherapeutics. Innovation in formulation science is therefore very important.

As shown in this article, rational formulation design can lead to novel formulations with considerably improved stability. Improvement can be achieved by eliminating excipients that are conventionally used in formulations as well as exploiting specific properties of excipients to achieve the required stability. The understanding of the physical and chemical degradation

"THE NUMBER OF POSSIBLE PERMUTATIONS OF EXCIPIENTS AND OTHER PARAMETERS IS EXTREMELY HIGH, MAKING EVALUATION DIFFICULT. IT IS THEREFORE NECESSARY TO EMPLOY RATIONAL APPROACHES TO IDENTIFY SPECIFIC SUBSETS OF THE BROAD DESIGN SPACE TO FOCUS ON"

rate and acetate,⁶ each being applicable within a specific pH range. Conventionally, buffers are selected based on their pKa constant which should be as close as possible to the pH of the composition.

However, Arecor found that the presence of the conventional buffers (i.e. those with the pKa very close to the pH of the formulation) can be

(H⁺) has a role to play in protein degradation, because excipients with different pKa have different abilities to exchange hydrogen cations with ionisable amino acid residues at the protein surface, resulting in different rates of charge fluctuation for each of the residues.

The optimal formulation of hepatitis B vaccine, based on 40 mM histidine and 40 mM

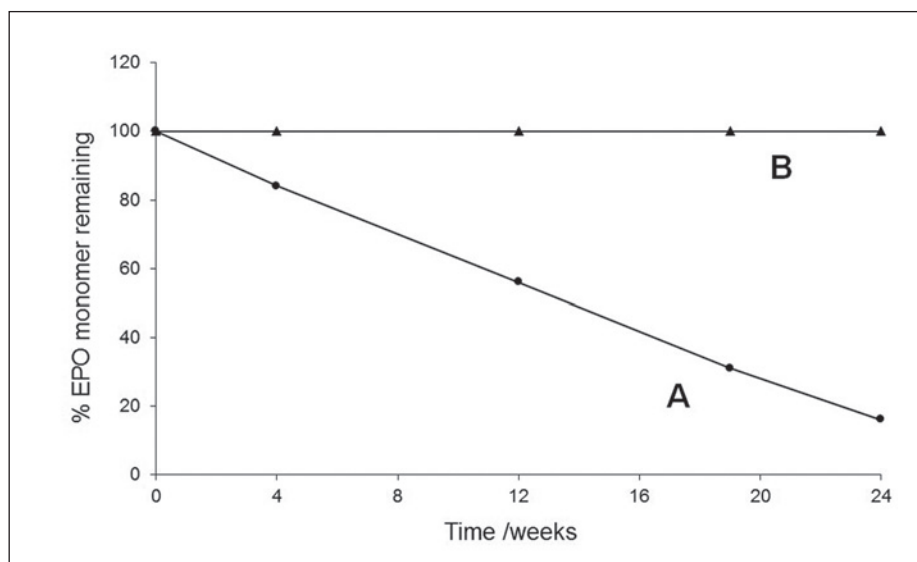


Figure 5: Retention of monomeric EPO in a marketed liquid formulation (A) and in a reformulated product based on a TRIS/benzoate anion dual buffering system (B).

pathways of proteins has enabled this rational design approach. The design and success of this formulation approach will be continually improved to tackle new demands from the biopharmaceutical industry and regulators, based on novel insights into protein stability through the development of new analytical technologies.

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USING HALF-LIFE EXTENSION TO MOVE A PIONEERING TREATMENT FOR AUTOIMMUNE DISEASES TOWARDS CLINICAL TRIALS

In this article, Mark Perkins, PhD, Customer Solution Manager at Novozymes Biopharma, and Anne S. De Groot, MD, Chief Executive Officer & Chief Scientific Officer at EpiVax, Inc, describe the partnership between the two companies, which uses Novozymes' Albufuse Flex half-life extension technology to improve the delivery of EpiVax's Type 1 diabetes treatment, Tregitopes, transforming this previously difficult-to-deliver protein into an extremely promising viable pharmaceutical product.

INTRODUCTION

Protein and peptide-based drugs, such as those used to treat autoimmune diseases, are widely known to display short plasma half-lives, meaning that they are rapidly cleared from the human body. Even some of today's best known drugs for the treatment of conditions such as diabetes, face challenges in bioavailability, resulting in the need for regular dosing of high levels of medication.

These limitations inevitably lead to reduced patient compliance and, consequently, increased healthcare costs. To illustrate one solution to this industry-wide challenge, Novozymes Biopharma and EpiVax, Inc, have developed a collaborative research agreement to help further the develop-

ment of epitopes), an immune-modulating therapy for the treatment of Type 1 diabetes, to Novozymes' Albufuse[®] half-life extension technology. The linkage with albumin will enhance Tregitope's pharmacokinetic and pharmacodynamic properties. As a result, it will be possible to modulate the half-life of the therapy to offer improved control, enhancing the overall efficiency of the treatment.

IMMUNOMODULATION TECHNOLOGY

Founded in 1998, EpiVax is an immunology company which has developed comprehensive analytical capabilities in the field of computational immunology. The company's main focus is the newly emerging discipline of immunoinformatics, by which scientists at EpiVax are able to harness the power of immune modulation for the development of improved vaccines and bio-therapeutics.

Using its proprietary computational tools that are widely used to screen for immunogenicity, EpiVax's expert team has identified a set of natural peptides

derived from Immunoglobulin G (IgG) that are T reg epitopes (Tregitopes). Tregitopes use the body's own natural mechanisms to induce tolerance to immunogenic proteins.



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"THE DISCOVERY OF THIS NOVEL TECHNOLOGY REPRESENTS A NEW PARADIGM FOR THE TREATMENT OF AUTOIMMUNE DISEASE AND HAS THE POTENTIAL TO REPRESENT A MAJOR STEP FORWARD IN PATIENT CARE"

ment of a potential 'paradigm-shifting' treatment for Type 1 diabetes and autoimmune diseases.

The research agreement involves linking EpiVax's proprietary Tregitopes (T regulatory

Over the past five years, EpiVax has been validating its hypotheses and demonstrating in a wide-range of models that the identified Tregitopes are immune modulators and can offer significant benefits when used in the treatment of autoimmune diseases.¹ EpiVax has confirmed that co-administration of Tregitopes with a range of proteins, such as Ovalbumin, allergens, insulin and Factor VIII, *in vitro* and *in vivo* leads to the suppression of inflammatory T cell responses and lowers antibody responses to the co-administered protein.

An increasing prevalence of Type 1 (juvenile) diabetes (T1D), an organ-specific autoimmune disease, which results from the destruction of insulin producing beta-cells, has led to the development of a number of novel biologics for this condition. Preliminary studies conducted in several different mouse models have shown that Tregitopes specifically induce naturally-occurring Tregs and, when co-administered with an antigen, lead to the expansion of antigen-specific regulatory T cells. Studies conducted in NOD mice, a strain that naturally develops a T1D-like condition, show that modulation of autoimmune responses to autologous epitopes by induction of antigen-specific tolerance may prevent on-going beta-cell destruction, maintaining the production of insulin.

Initial studies have indicated that Tregitopes reduce the incidence of disease when administered prior to the development of the condition, or during active disease, while also suppressing diabetes in therapeutic models that already displayed a high blood-sugar count prior to initiation of Tregitope treatment.² In each of these studies, Tregitope therapy was shown effectively to suppress inflammatory T cell responses *in vivo*. The discovery of this novel technology represents a new paradigm for the treatment of autoimmune disease and has the potential to represent a major step forward in patient care.

DEVELOPING A PIONEERING THERAPY

After seeing the results of its pioneering research in animal models, EpiVax began to make steps to bring the therapy into the clinic. However, a key barrier in developing the technology has been finding an effective and safe method of delivering the Tregitopes into the human body. Peptides are notoriously difficult drugs to deliver in humans as they are limited to an average half-life of approximately 20 minutes, with many being undetectable in the serum after this period of time. Therefore, means of stabilising the Tregitope peptides and delivering them to the target cells efficiently are essential. During the initial research and development stages, EpiVax

had been using saline, liposomes and various lab-level solutions as delivery vehicles for the therapy that could not be translated into the clinic due to safety and purity concerns.

To help move the therapy closer to clinical trials, the company required a technology that would not only provide a carrier, but that would

greatly enhancing the overall efficacy of the treatment.

The two companies are currently working together to fuse the identified Tregitopes to human serum albumin. The joint partnership will see Novozymes' team using its unique expertise in genetic manipulation of albumin and commercial

A KEY BARRIER IN DEVELOPING THE TECHNOLOGY HAS BEEN FINDING AN EFFECTIVE AND SAFE METHOD OF DELIVERING THE TREGITOPES INTO THE HUMAN BODY. PEPTIDES ARE NOTORIOUSLY DIFFICULT DRUGS TO DELIVER IN HUMANS AS THEY ARE LIMITED BY AN AVERAGE HALF-LIFE OF APPROXIMATELY 20 MINUTES, WITH MANY BEING UNDETECTABLE IN THE SERUM AFTER THIS PERIOD OF TIME"

also facilitate greater control of the therapeutic half-life of the treatment.

Novozymes' Albufuse® Flex half-life extension technology represented an ideal solution for the company with its widely documented profile as a well-tolerated carrier. In comparison to alternative solutions it offered a strong safety track record for intravenous administration, with a well understood half-life and reduced side-effects, offering both improved tolerance and efficacy. By genetically fusing proteins or peptides with albumin, their bioavailability can be enhanced, allowing effective therapeutic levels to be maintained for longer.

The technology represents a series of engineered human albumins with modified binding affinity to the human FcRn receptor. FcRn is a dual-binding receptor that binds both IgG and albumin, at distinct sites, and protects them from intracellular degradation. Since this receptor interaction is key in modulating the pharmacokinetics of albumin *in vivo*, the result is a series of albumin molecules with an enhanced half-life compared to wild-type albumin.

By combining the engineered albumin with their drug candidate, EpiVax has enabled the delivery of Tregitopes directly to the cells that the immune modulation technology needs to target, and prolonged the therapeutic half-life in a manner previously unachievable with native human albumin and other half-life extension technologies. In particular, albumin with extended half-life offers a highly-effective delivery vehicle and opens the door towards dosing bi-monthly or monthly. In addition, attaching the therapy to albumin improves the solubility of the peptides,

albumin production to undertake the necessary protein engineering, while EpiVax's scientists are now investigating the effectiveness of a range of Tregitope delivery vehicles in test models.

NOVEL HALF-LIFE EXTENSION TECHNOLOGY

The utilisation of the Albufuse platform for peptides and proteins is already well established in the field of diabetes, as well as haemophilia and neutropenia, with the first products incorporating the technology expected to receive marketing approval during 2014. As a natural alternative to PEGylation, Albumin has a long serum half-life of 19 days due to the aforementioned FcRn interaction plus its large hydrodynamic radius, which protects the 67 kDa molecule from renal clearance.

The fusion of numerous therapeutically relevant proteins and peptides to albumin using the Albufuse half-life extension technology can extend a therapy's half-life significantly and its pharmacokinetic properties can be considerably improved. The multiple benefits of the platform have been validated in both clinical and preclinical trials showing its capability to reduce frequency and level of dosing, while also conferring benefits of batch-to-batch consistency and reduced toxicity, leading to decreased side effects. As a result, patient experiences of treatment regimes can be significantly improved and compliance can be enhanced.

In the development of the technology, Novozymes recognised that the pressure to reduce production and processing times is ever increasing

in the biopharmaceutical industry for manufacturers such as EpiVax. The broadly applicable platform helps to streamline processes and has an established regulatory pathway enabling products to reach the market more quickly and cost effectively. These advances help provide for flexibility and efficiency for manufacturers developing novel therapies.

CONCLUSION

The partnership between EpiVax and Novozymes will contribute significant advances in the treatment of a wide-range of autoimmune diseases. By taking the pioneering immune modulation technology and combining it with a clinically-proven and versatile half-life extension platform, considerable progress will be made in moving the treatment closer to clinical trials. The result will be a safe and effective platform for the application of Tregitopes to multiple autoimmune diseases, as well as transplantation and allergy conditions. Offering improved circulatory half-life for reduced frequency and level of dosing, as well as diminished side-effects and improved tolerance and efficacy, the Tregitope-albumin fusion will make significant steps in improving patient quality of life.

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ABOUT EPIVAX:

EpiVax, Inc. is a Providence, Rhode Island biotechnology company focused on the development of vaccines and immunotherapeutics. EpiVax is one of the world's leading innovators in the field of immunogenicity screening. Through the application and utilisation of these computational tools, EpiVax is helping to engineer safe, more effective therapeutic proteins and to rapidly design protective and efficacious new vaccines.

Led by Dr Anne S. De Groot, MD, immune-informatics and vaccine design thought leader, EpiVax has enjoyed success in the fields of immunology and bioinformatics, and has developed proprietary immune-informatics tools for the development and improvement of biotherapeutic drug candidates.

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- ✓ Disrupting the future of healthcare with drug delivery
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SELF-INTERACTION CHROMATOGRAPHY LENDING INSIGHT INTO FORMULATION STUDIES

In this article, Michelle Amaral, PhD, Science Writer and PR Consultant, Soluble Therapeutics, Inc, introduces self-interaction chromatography (SIC), and its advantages over traditional methods such as light-scattering techniques, as a means to determine the optimum formulation for developing a protein formulation into a biotherapeutic product.

A new day dawned on the pharmaceutical industry when recombinant technologies enabled the use of proteins as therapeutics. A wide range of approaches opened up, from replacing aberrant proteins in a disease state or introducing novel proteins to enhancing delivery of small-molecule pharmaceuticals with monoclonal antibodies (MAbs). Underlying each of these tactics, however, is the requirement that a protein be highly concentrated, stable, and active in solution when delivered. Ensuring that these qualities are met is not a simple feat. Self-interaction chromatography (SIC) is now being

more manageable volume of drug can be administered. However, at such high concentrations proteins have a tendency to precipitate out of solution, aggregate, or form a highly viscous phase – none of which are amenable to storage and delivery of the drug, since the protein's activity becomes compromised. Care must be taken to formulate a buffer solution with conditions that are optimal for proper folding and stability of the highly concentrated protein.

Unfortunately, standard recipes for these formulations do not exist, and the conditions for each protein can vary greatly. Each component

must be determined empirically, varying such characteristics as pH, additives, and salt concentration. But with such an array of components from which to choose, finding optimal formulations can prove to be a monumental task requiring upwards of one year to complete.

Traditionally, light-scattering techniques have been used to obtain measurements of a protein's size and, therefore, its

stability in various formulations. This method is quite laborious though, requiring a high level of skill for operating the instrument and a large amount of time just to take the measurements. Moreover, certain additives such as detergents and surfactants interfere with light scattering, making it impossible to obtain protein solubility information under these conditions.

**"SELF-INTERACTION CHROMATOGRAPHY
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PROTEIN FORMULATIONS – AND IN JUST
A MATTER OF ABOUT TWO MONTHS"**

used as a fast and effective method for determining the optimal formulation of a protein solution so that the product can be used successfully in downstream development applications.

In order to reach efficacious levels, protein pharmaceuticals must be delivered to a patient at high concentrations. An added benefit of high concentration protein delivery is that a smaller,



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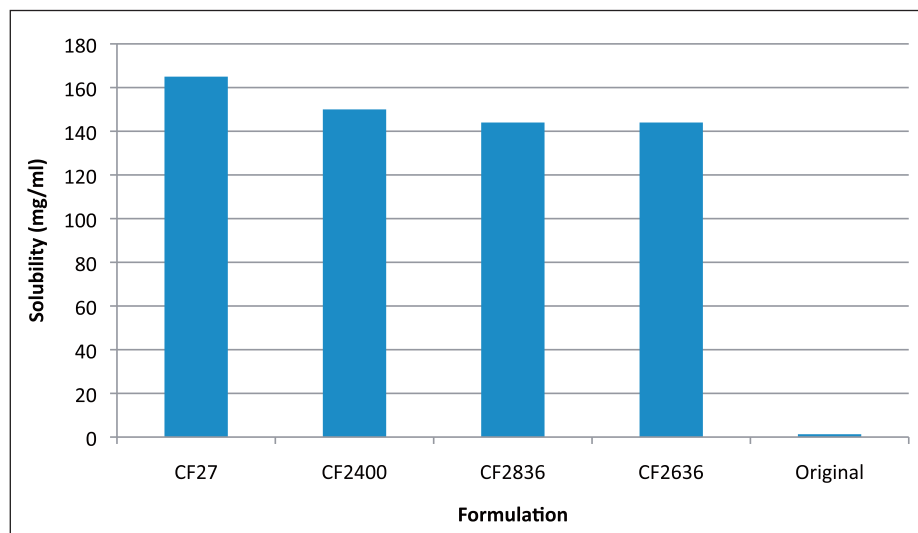


Figure 1: Formulations for Improving the Solubility of an MAB

SIC has been recently introduced as a more powerful method for developing highly concentrated protein formulations – and in just a matter of about two months. SIC is a technique that measures the extent of a protein's interaction with itself, and its applicability is generally unaffected by the vast array of additives available to the typical formulation professional. Other methods of measuring protein-protein interactions are sometimes limited because of the light scattering effects of some additives. SIC requires a small amount of protein that is covalently attached to beads and packed into a microcapillary HPLC column. The mobile phase of the SIC experiments consists of the formulation being tested, along with a 1 μ l bolus injection of the protein of interest. The elution of the protein injected in the mobile phase is measured via UV detection and the retention time is used to evaluate whether or not the formulation or additive of interest is causing attraction or repulsion between the protein molecules.

Data collected via SIC enables the calculation of a parameter that quantitatively describes the protein's interaction with itself; this is the second virial coefficient, or B value. The B value is the sum of all potential forces between two proteins including ionic, dipole, hydrophobic, and van der Waals forces; it is a measure of protein-protein interactions in all orientations and distances. In general, positive B values indicate a net repulsion between two protein molecules while negative B values indicate a net attraction. When additives are introduced into solutions, the B value is altered such that the protein molecules display mild attraction to each other, which is conducive to crystallization, or enhanced repulsion, which increases the protein's physical stability and solubility.

Experimentally determined B values can be used to predict the B value of a protein in over 12,000 other formulations using an artificial neural network. This is a wealth of information for groups that are developing a new biopharmaceutical. Differential scanning calorimetry and other biophysical techniques are performed to confirm a complete characterisation profile on the final solutions.

CASE STUDY: CONCENTRATING A MONOCLONAL ANTIBODY

SIC methodology was valuable in concentrating a monoclonal antibody for an industry collaborator. Despite their repeated attempts to increase the solubility of the antibody using “standard” additives and excipients, the highest concentration they could achieve was 1.3 mg/ml in PBS before aggregation would occur. Screens were performed and, using this data, the artificial neural network predicted and ranked

highly concentrated, stable, and active in solution without aggregation or other phase changes that are detrimental to the drug delivery process and the patient. An optimal formulation for the protein of interest is crucial, then, for its performance. High-throughput methods that screen components of the solution save time and money for a company developing a promising drug.

ABOUT SOLUBLE THERAPEUTICS INC

Soluble Therapeutics, Inc. was founded in 2008 to commercialise the HSC™ Technology. The company's services enhance the drug development process, by rapidly optimising protein solubility and stability. Soluble Therapeutics brings transformational technology to the world of protein-based pharmaceuticals, vaccines, and therapeutics.

Led by Chief Executive Officer Dr Joseph N. Garner, Soluble Therapeutics' management team consists of science industry professionals

“THE B VALUE IS THE SUM OF ALL POTENTIAL FORCES BETWEEN TWO PROTEINS INCLUDING IONIC, DIPOLE, HYDROPHOBIC, AND VAN DER WAALS FORCES; IT IS A MEASURE OF PROTEIN-PROTEIN INTERACTIONS IN ALL ORIENTATIONS AND DISTANCES”

over 12,000 possible formulations. The top four candidates, outlined in Figure 1, were tested for maximum solubility and yielded concentrations 110-127 times higher. The formulations were subsequently used with animals in clinical trials.

The use of proteins as therapeutics has revolutionised the field of drug discovery. However, care must be taken to ensure that a protein is

bringing more than 60 years combined experience from organisations such as NASA, the University of Alabama at Birmingham, and Mississippi State University.

Soluble Therapeutics, Inc. is the first company to introduce protein formulation solutions that deliver the price and performance advantages of the HSC™ Technology.

SYRINGE SILICONISATION: TRENDS, METHODS, ANALYSIS PROCEDURES

In this paper, Bruno Reuter, Director of Product Development, and Claudia Petersen, Director, Business Development, both of Gerresheimer Bünde, provide a detailed description of silicone oil and its applications in syringe siliconisation, highlighting the advantages and challenges siliconisation brings to the drug formulation and recent developments in the field.

Ready-to-fill, i.e. sterile, prefilled glass syringes, are washed, siliconised, sterilised and packaged by the primary packaging manufacturer. They can then be filled by pharmaceutical companies without any further processing. These days the majority of prefilled syringes are made of glass and the trend looks set to continue. The siliconisation of the syringe barrel is an extremely important aspect of the production of sterile, prefilled glass syringes because the functional interaction of the glass barrel siliconisation and the plunger stopper siliconisation is crucial to the efficiency of the entire system. Both inadequate and excessive siliconisation can cause problems in this connection. The use of modern technology can achieve an extremely uniform distribution of silicone oil in glass syringes with reduced quantities of silicone oil. Another option for minimising the amount of free silicone oil in a syringe is the thermal fixation of the silicone oil on the glass surface in a process called baked-on siliconisation. Plastic-based silicone oil-free, and low-silicone oil, prefilled syringe systems are relatively new developments. Silicone oil-free lubricant coatings for syringes are also currently in the development phase.

INTRODUCTION

Primary packaging for injectables almost exclusively comprises a glass container (cartridge, syringe, vial) and an elastomer closure. Ampoules are an exception. Elastomers are by nature slightly sticky, so all elastomer closures (plunger stoppers for syringes and cartridges, serum or lyophilisation stoppers) are siliconised.

Siliconisation prevents the rubber closures from sticking together and simplifies processing of the articles on the filling lines. For example, it minimises mechanical forces when the stop-

pers are inserted. Siliconisation is therefore essential to process capability.

Although syringes and cartridges are always siliconised, this applies to a lesser extent to vials and ampoules. On the container the siliconisation provides a barrier coating between the glass and drug formulation. It also prevents the adsorption of formulation components on the glass surface. The hydrophobic deactivation of the surface also improves the containers' drainability. In prefilled syringes and cartridges, siliconisation also performs another function. It lubricates the syringe barrel or cartridge body enabling the plunger to glide through it. Siliconisation of the plunger stopper alone would not provide adequate lubrication.

Silicone oils are ideal as lubricants because they are largely inert, hydrophobic and viscoelastic. Chemical and physical requirements for lubricants are set out in the relevant monographs of the US Pharmacopeia (USP) and the European Pharmacopoeia (Ph Eur).^{1,2} Section 3.1.8 of the Ph Eur also defines a kinematic viscosity of 1,000-30,000 mm²/s for silicone oils used as lubricants.³ In contrast, the monograph for polydimethylsiloxane (PDMS) in the USP 2 permits the use of silicone oils with a viscosity of 20-30,000 centistokes (cSt).

However, increasingly stringent quality requirements and new bioengineered drugs are now taking siliconisation technology to its limits. Non-homogenous siliconisation, which can occur when simple coating techniques are used on longer syringe barrels, can in some cases lead to mechanical problems. These include the incomplete drainage of the syringe in an auto-injector or high gliding forces. Silicone oil droplets are always observed in filled syringes.

The number of silicone oil droplets increases in line with the quantity of silicone oil used. Droplets which are visible to the naked eye



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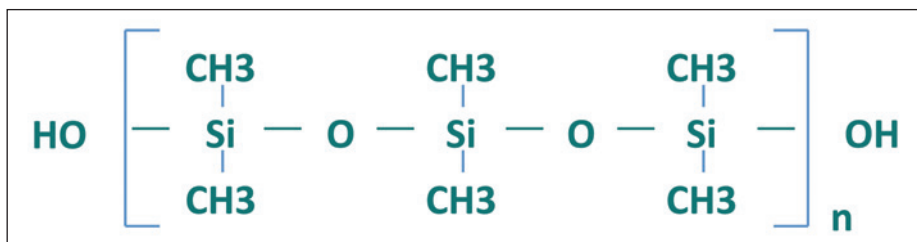


Figure 1: Polydimethylsiloxane.

could be viewed as a cosmetic defect. At sub-visual level, the issue of whether silicone oil particles could induce protein aggregation is currently under discussion.⁴

In light of this development, there is an obvious trend towards optimised or alternative coating techniques. Attempts are being made to achieve the most uniform possible coating with a reduced quantity of silicone oil and to minimise the amount of free silicone oil by way of baked-on siliconisation. In this context, reliable analysis technologies that can be used to make qualitative and quantitative checks on the coating are absolutely essential. Alternative coating techniques are also being developed.

SILICONE OILS & THEIR PROPERTIES

Silicone oils have been used for half a century in numerous pharmaceutical applications. For example, they are used as lubricants in pharmaceuticals production and as inert pharmaceutical base materials (e.g. soft capsule walls).⁵ Trimethylsiloxy end-blocked polydimethylsiloxane (PDMS, dimethicone) in various viscosities is generally used for siliconisation (see Figure 1).

The most frequently used silicone oil for the siliconisation of primary packaging components is DOW CORNING® 360 Medical Fluid. PDMS is produced by reducing quartz sand to elemental silicon. In the next step, the silicon reacts directly with methyl chloride in a process called Müller-Rochow synthesis to create methyl chlorosilanes. In this process, a mixture of different silanes is produced, the majority of which (75%–90%) are dimethyldichlorosilane $(\text{CH}_3)_2\text{SiCl}_2$. After distillative separation, the dimethyldichlorosilane is converted by hydrolysis or methanolysis into silanols which condense into low-molecular-weight chains and cycles.

In an acidic (cationic) or alkaline (anionic) catalysed polymerisation, polydimethylsiloxanes with hydroxyl functions are generated. After the addition of trimethylchlorosilane they are furnished with trimethylsiloxy end groups. The short-chain molecules are removed from the resulting polydisperse polymers by way of vaporisation, leaving deployable PDMS.

The characteristic aspect of the PDMS molecule is the Si-O bond. With a bond energy of

108 kcal/mol, it is considerably more stable than the C-O bond (83 kcal/mol) or the C-C bond (85 kcal/mol). PDMS is accordingly less sensitive to thermal loads, UV radiation or oxidation agents.

Reactions such as oxidation, polymerisation or depolymerisation do not occur until temperatures exceeding 130°C. The molecule also typically has a flat bond angle ($\angle \text{Si-O-Si} = 151^\circ \pm 12^\circ$) which has low rotation energy and is especially flexible (Figure 2). A high bond length (1.63 Å Si-O as

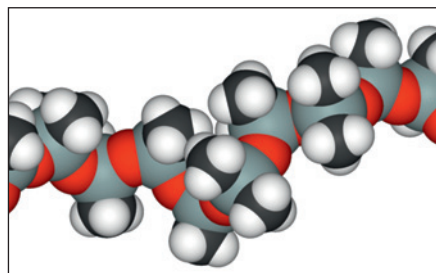


Figure 2: 3D-structure of polydimethylsiloxane.

compared with 1.43 Å for C-O) makes the molecule comparatively gas-permeable.⁶

The spiral shaped (and therefore easily compressible) molecule is surrounded by CH_3 groups which are responsible for the chemical and mechanical properties of PDMS. The molecule's methyl groups only interact to a very limited extent. This ensures low viscosity, even with high molecular weights, which simplifies the distribution of PDMS on surfaces and makes it a very effective lubricant. PDMS is also largely inert and reactions with glass, metals, plastics or human tissues are minimal. The CH_3 groups make PDMS extremely hydrophobic. It is insoluble in water, but soluble in non-polar solvents.⁶

SILICONISED SYRINGES

As already explained the syringe system only works if the glass barrel and plunger stopper siliconisation are homogenous and optimally harmonised. For needle syringes, siliconisation of the needle is also essential to prevent it sticking to the skin, thereby minimising injection pain. For the so-called oily siliconisation of the syringe glass barrel DOW CORNING® 360 with a viscosity of 1,000 cSt is used. The DOW CORNING® 365 siliconisation emulsion

is often used in the baked-on siliconisation process (describe later). The needle is siliconised using a wipe technique during ready-to-fill processing. DOW CORNING® 360 with a viscosity of 12,500 cSt is used. Another option is the thermal fixation of silicon oil on the needle during the needle mounting process.

The goal of syringe barrel siliconisation is to obtain the most even anti-friction coating possible along the entire length of the syringe in order to minimise break loose and gliding forces when the plunger stopper is deployed (Figure 3).

Inadequate siliconisation of the syringe barrel, particularly the existence of unsiliconised areas, can cause slip-stick effects that impair the syringe's function. The forces in the injection process can then be too high or the entire system can fail. Since inadequate siliconisation and gaps in the coating are often found on the lower end of the syringe (luer tip/needle end), it is possible that the syringe will not be completely emptied. Such defects can remain undiscovered, particularly in auto-injectors since these are closed systems. The result could be that an inadequate dosage of the medication is administered.

The obvious solution is to increase the amount of silicone oil used to achieve a homogenous coating. However, as already mentioned, increasing the amount of silicone oil used is also associated with higher quantities of silicone particles in the solution.

With protein-based drugs in particular, undesirable interactions with silicone oil particles cannot be ruled out. Sub-visual silicone oil particles are thought to promote protein aggregation which can increase the severity of immune responses and reduce the drug's tolerability. However, the underlying mechanism is not yet fully understood. There is a discussion as to whether protein aggregation is influenced by additional motion, e.g. shaking the syringe.⁷

Experiments have also shown that when silicone oil in excess of 1 mg/syringe is used the additional silicone oil does not further reduce gliding forces.

The interior siliconisation of glass syringe barrels has another advantage. It prevents the drug solution from interacting with the glass surface and minimises related problems such as the loss of active ingredients through adsorption or pH value changes due to alkali leaching.

Prefillable glass syringes are only manufactured from high-quality type 1 borosilicate glass. However, sodium ions can still leach out of the glass surface if the syringe contains an aqueous solution and is stored for a long period of time. This leads to higher pH values which could be problematic in unbuffered systems.

Acidic environments foster this process:

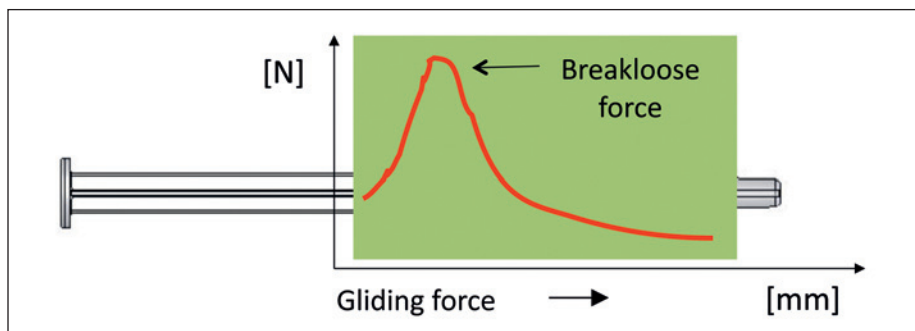


Figure 3: Extrusion force profile of a prefillable syringe.

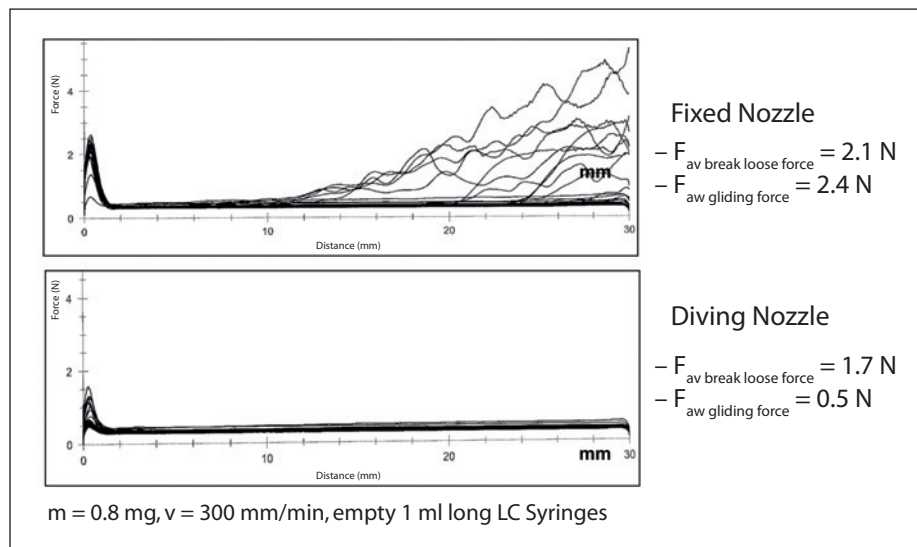


Figure 4: Comparison of extrusion force profiles – fixed nozzle (top) versus diving nozzle (bottom).

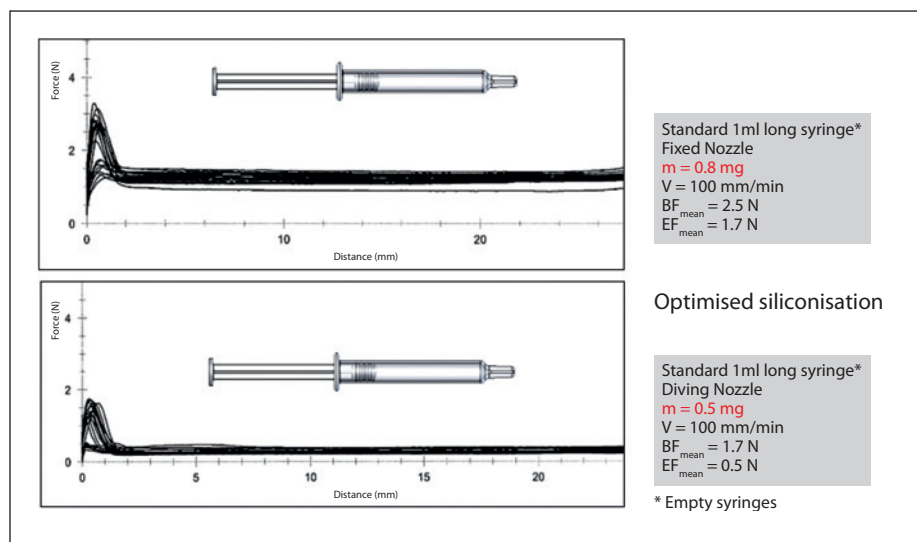
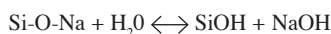
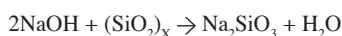


Figure 5: Baked-on siliconisation.



In alkaline environments, on the other hand, an etching process is observed:



Aqueous solutions with a high pH value

cannot therefore be stored for long periods in unsiliconised borosilicate glass containers. They have to be lyophilised and reconstituted before use. In extreme cases, the etching of the glass surface can cause delamination. Hydrophobic deactivation of the container by siliconisation effectively protects the glass surface.

OPTIMISED SILICONISATION

For the abovementioned reasons, the main objective in siliconisation is to achieve the most homogenous possible coating with the minimum possible quantity of silicone oil. Initially it is necessary to establish the minimum quantity of silicone oil which will reliably satisfy the quality requirements of the application. In the production of ready-to-fill syringes, siliconisation generally takes place after washing and drying. Fixed nozzles positioned at finger flange level under the syringe barrel spray the silicone oil onto the inside surface. In long syringes, the silicone oil is sometimes unevenly distributed and the concentration of the silicone oil is lower at one end of the syringe (luer tip/needle end). The use of diving nozzles can considerably improve the evenness of the coating across the entire length of the syringe body. In this process, the nozzles are inserted into the syringe to apply the silicone oil (finely atomised) in motion. The result is practically linear as is shown by the closely bundled gliding forces in the force path diagram (Figure 4).

Studies on 1 ml long syringes have revealed considerable potential for reducing the amount of silicone oil required. In one experiment, the quantity of silicone oil per syringe could be reduced by 40% without any impairment of the system's functional properties (see Figure 5). In practice the calculation of the optimum quantity of silicone oil has to take syringe volume, plunger stopper type (coated/ uncoated), plunger stopper placement method (seating tube/vacuum) and application requirements (injection systems) into account. Plunger stoppers from different suppliers not only differ in terms of the type of rubber used and their design, they are also coated with silicone oils of different viscosities. The siliconisation methods also differ considerably. These variables can have a bigger impact on the syringe system's functional properties than the syringe siliconisation of different suppliers, as shown by Eu *et al.*⁸

BAKED-ON SILICONISATION

Another key advancement in siliconisation technology is the baked-on siliconisation technology. It involves the application of silicone oil as an emulsion which is then baked on to the glass surface in a special kiln at a specific temperature and for a specific length of time.

In the baked-on process, both hydrogen and covalent bonds form between the glass surface and the poly-dimethylsiloxane chains. The bonds are so strong that part of the silicone oil cannot be removed with solvent and a permanent hydrophobic layer is created (Figure 6).

In addition the average molecule weight increases as a result of polymerisation and the vaporisation of short chain polymers. The resulting extremely thin layer of silicone, in conjunction with the low quantity of silicone oil used in the emulsion, minimises free silicone in the syringe and ensures that the required quality of finish is achieved. The layer thickness measures 15-50 nm. By comparison, the average layer thickness with oily siliconisation is 500-1,000 nm.

Baked-on siliconisation reduces the measurable quantity of free silicone oil to approximately 10 % of the normal value. As a result, there are fewer sub-visual and visual silicone oil particles in the solution. This siliconisation process is therefore recommended for use with sensitive protein formulations. It is also advantageous for ophthalmological preparations which are associated with very stringent requirements as regards particle contamination.

Another benefit is the stability of the mechanical properties of the filled syringe throughout its shelf life. The ribs of a plunger stopper press into the silicone layer when a syringe with oily siliconisation is stored for long periods of time and the glass comes into direct contact with the rubber. Since elastomers are always slightly sticky, the break-loose forces increase over the storage period.

With baked-on siliconisation, however, this phenomenon is not observed to the same extent (Figure 7). The break-loose force remains practically constant over the entire storage period.

OUTLOOK

There is a trend towards reduced-silicone systems or baked-on siliconisation in glass syringe finishing. Improved analysis techniques and a better understanding of the phenomena involved support optimised use of silicone oil.

New issues are arising as a result of the use of innovative materials or coatings. In light of the increasing complexity of devices and the more widespread incidence of biopharmaceuticals with specific requirements, new alternative materials for primary packaging products are becoming increasingly interesting. For example, the inside surfaces of vials and syringes can be coated with pure SiO_2 in a plasma process to minimise their interaction with drugs. Plastic systems based on cyclic olefins (COP/COC) are also gaining in significance for prefilled syringes and vials. COP syringes such as the ClearJect™ TasPack™ by from Kako Co Ltd (Osaka, Japan) have glass-like transparency. Additionally, they have a higher break resistance, their pH stability range is larger and there is no metal ion leaching.

Excellent dosage precision is also very

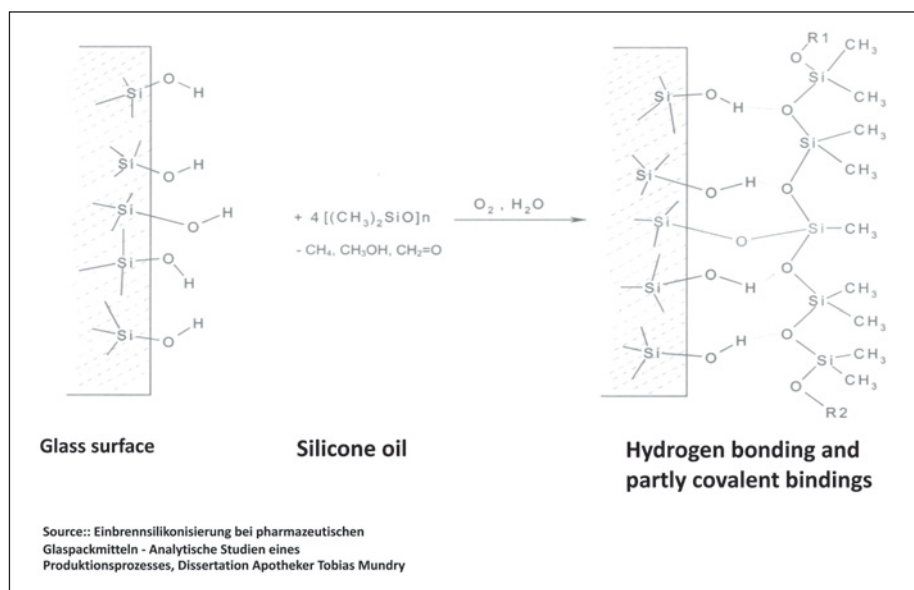


Figure 6: Extrusion force profile after optimised siliconisation.

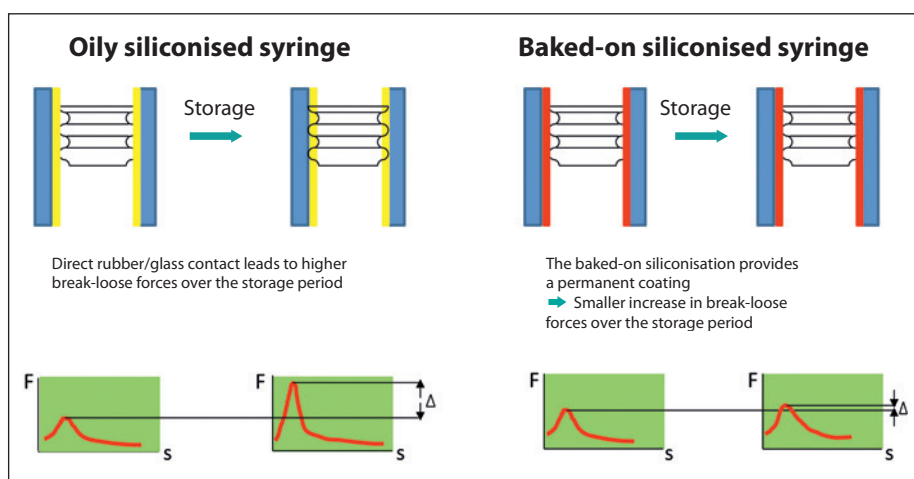


Figure 7: Comparison of syringes with oily and baked-on siliconisation.


important in packaging for bio-pharmaceuticals. In most cases siliconisation is also essential in COP syringes. Silicone oil-free systems are a brand new approach. The gliding properties of the fluoropolymer coating on specially developed plunger stoppers eliminate the need to siliconise plastic syringes. There are as many innovative ideas for the development of primary packaging products as there are innovative drugs and syringe systems.

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