Exploring Amino Acid-Based and Stable Spray-Dried Vaccinations

With researchers endeavouring to develop thermostable and needle-free vaccines, addressing complications associated with liquid preparations has become more important than ever

Dr Jens Altrichter, Dr Kristina Kemter, and Rebekka Ströver at LEUKOCARE and Professor Dr Martin Scholz, Biotechnology Consultant

The development of therapeutic biologics (including vaccines) requires significant efforts to avoid severe issues due to molecular instability. In a recently published survey, several key findings were discussed, including formulation issues, project failures, and solutions to overcome such hurdles (1). One key finding was that formulation issues cause significant drug development delays and even complete project failures due to formulation issues, as experienced by more than 60% and 6% of survey participants, respectively. Consequently, deploying a commercially viable formulation early enough in the drug product development process could help to prevent failure of such projects.

Furthermore, competitive advantages and reduced time to market are key decision criteria in formulation development, and aspects on reduced manufacturing costs, longer shelf life, and facilitated transport, as well as logistics, were considered as important aspects of formulation (1). Within the vaccine market, the need for cold storage and the impossibility of terminal sterilisation are major drawbacks in vaccine manufacturing and distribution, particularly in the case of spray-dried vaccines. Hence, facilitated transportation at room temperature and terminal sterilisation of vaccines would result in clear competitive advantages.

Molecular Damage During Manufacturing of Vaccines

Today, major challenges in the field of vaccination are to develop thermostable vaccines and to establish needle-free vaccination strategies to fight viral diseases, even in developing countries, which is also one strategic aim of the WHO Global Immunization Vision and Strategy (2-3). Furthermore, to be better prepared for pandemic outbreaks, stockpiles of stable vaccine formulations are required (4).

Liquid preparations face a high risk of instability during storage due to high molecular mobility and increased likelihood of chemical reactions and physical instability (5). For this reason, many liquid vaccine preparations need to be stored and transported under refrigeration and often only feature a short shelf life. For liquid vaccine preparations, stability is often enhanced by selection of pH buffering salts and the use of amino acids for stabilisation (6-7).

The stability of such preparations, especially their thermal stability, can be greatly increased by drying the antigens, for instance, by freeze drying, thereby minimising molecular mobility and the risk of intermolecular reactions. This approach requires the antigen to be stabilised via, for example, exchange of water with other hydrophilic molecules or by formation of a sugar glass matrix during both the step of freezing (cryoprotection) and the subsequent step of drying (lyoprotection) (8-9).

Spray drying is often the method of choice, as it avoids the freezing step and requires lower energy costs as compared to lyophilisation. The products obtained can be designed to have good dispersion characteristics, as well as low agglomeration and adhesive tendencies to allow powder handling, packaging, and efficient re-dispersion upon contact with water or buffer. This is especially true for spray drying which, accordingly, is particularly suitable for bulk production of large amounts of vaccines (10).

Another hurdle in vaccine production is the sterilisation of vaccine preparations without significant loss of material. Standard sterile filtrations of biomolecules leave the risk of contamination during aseptic fill and finish and may hence lead to significant patient risks or economic loss (11). Therefore, the need for technologies that enable terminal sterilisation of biomolecules increases (12-14).

The main bottlenecks in this development are to:

- Obtain sufficient amounts of vaccine powder
- Sterilise the vaccine powder

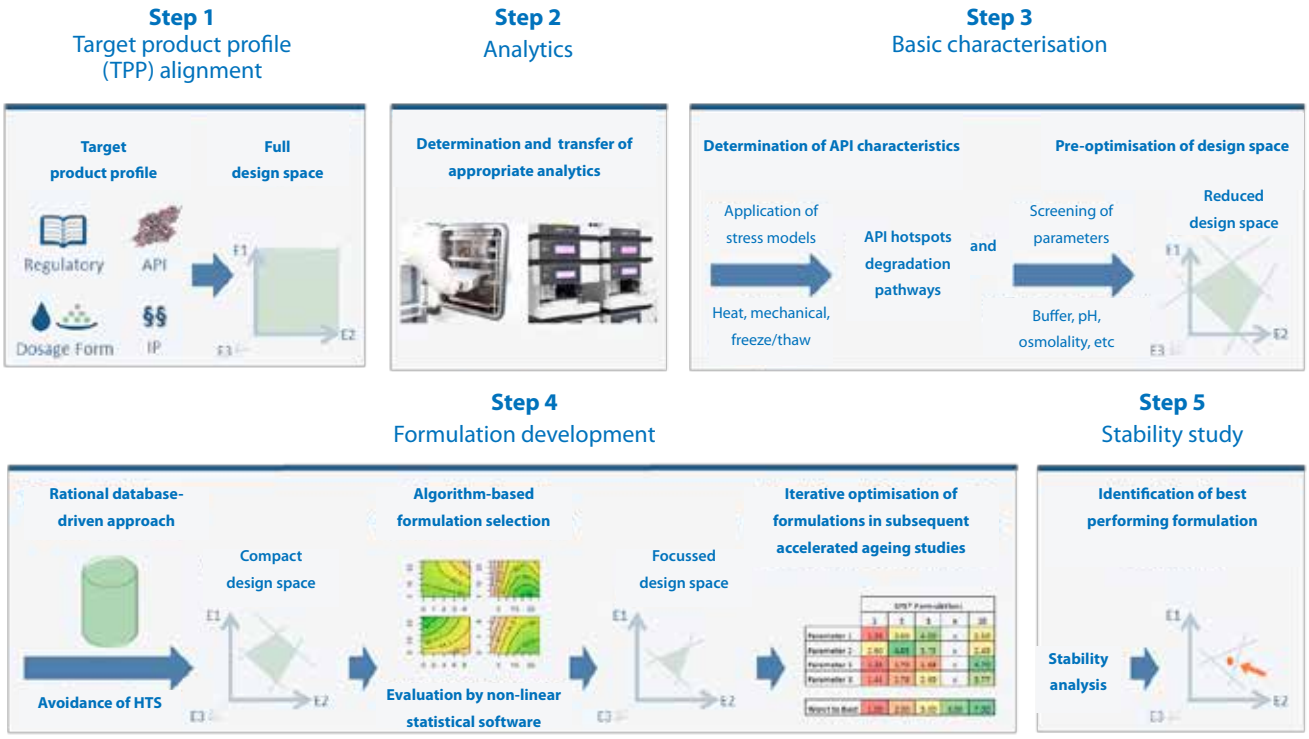


Figure 1: Rational and iterative excipient selection process to obtain tailored amino acid-based formulations with maximum stabilisation efficacy

	DNA viruses*	RNA viruses*
Liquid	Poxviridae, herpesviridae Adenovirus	Coronaviridae, Arteriviridae, other
Lyophilised	Modified vaccinia Ankara (MVA), Adenovirus, Parvovirus	Paramyxovirus strains, influenza strains, arteriviridae, MVA as vector, Picornavirus
Spray-dried	MVA Adenovirus	H1N1 influenza strain, Picornavirus
Viral vectors	MVA Adenovirus	Paramyxovirus strains, MVA as vector for flaviviridae

*Enveloped; Non-enveloped

Table 1: Tailored amino acid-based formulations stabilised a broad range of viruses/vaccines

- Avoid unappreciated antigen modifications
- Avoid loss of specific immunogenicity and, thus, efficacy

Spray Drying and Molecular Integrity

Spray drying involves the atomisation of a liquid feed into very small droplets within a hot drying gas, leading to flash drying of the droplets into solid particles. Increased mechanical shear stress, high temperatures, and high energy input can result in loss of molecular integrity and functionality by means of oxidation, deamidation, isomerisation of amino acid residues, and hydrolysis of the peptide bonds or by physical loss of secondary and tertiary structure, unfolding and denaturation, or aggregation. A multitude of spray drying process parameters influence the powder morphology, amorphous or crystalline particle size distribution, particle shape, particle density, residual moisture content, and the glass transition temperature (T_g), which have a strong impact on the application route and storage stability of both the amorphous powder and the spray-dried therapeutical molecule. Specifically adapted spray drying process steps, combined with tailored formulations, contribute to molecular and functional integrity, physical characteristics of the resulting powder particles, and to storage stability.

Sterilisation of Biologics

Aseptic processing of biologics is not expected to result in a 10^{-6} sterility assurance level, leaving the risk of contamination during fill and finish and potentially leading to significant economic loss (12-13, 15). Pharmaceutical guidelines recommend terminal sterilisation methods so that the need for technologies and procedures that enable terminal sterilisation of biomolecules (including proteins) increases with cost pressure and safety concerns (14, 16-17). On the other hand, irradiation is currently not considered a standard and valid sterilisation protocol for biologics and other proteins since it is associated with a high-energy input and increased risk of chemical and physical modifications, misfolding, formation of

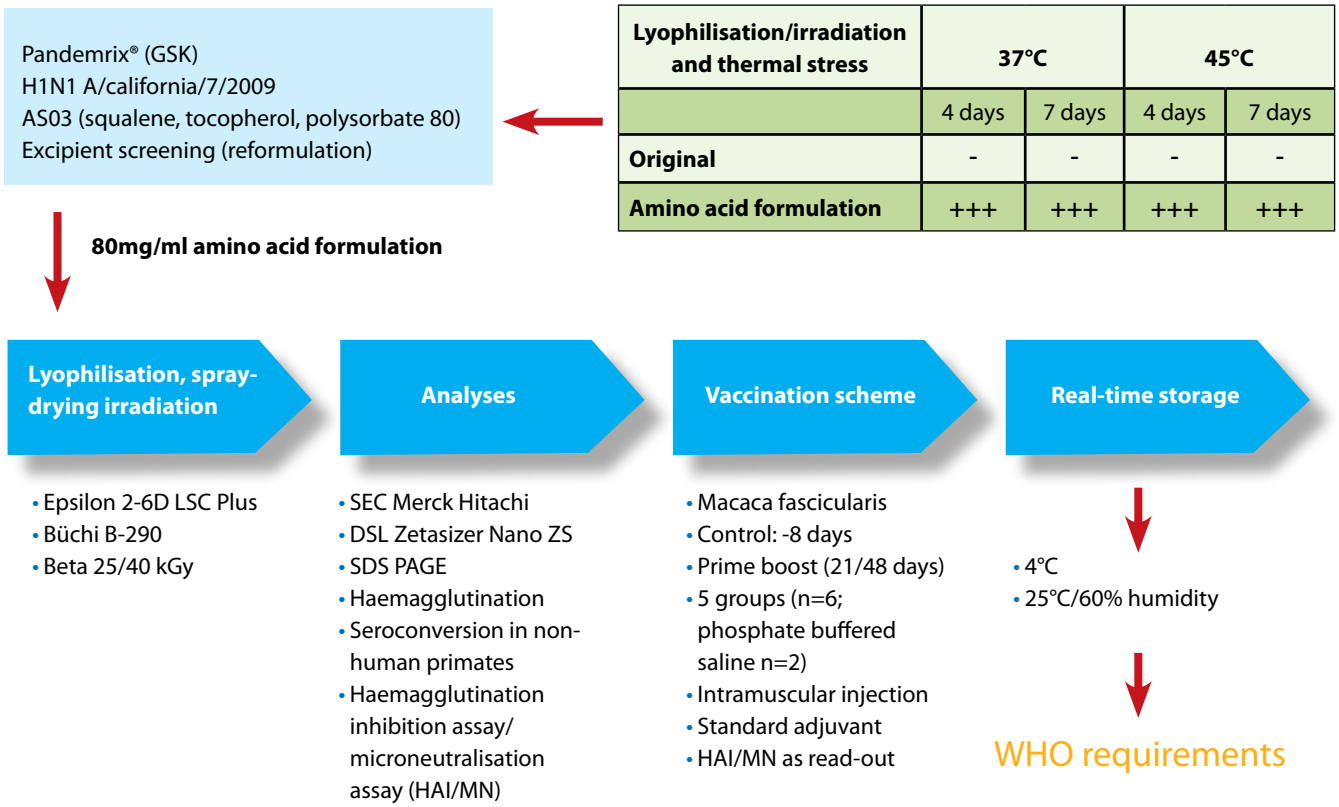


Figure 2: Selection process of stable and efficient formulations for the validation of stress-exposed Pandemrix in a non-human primate model

aggregates, and fragmentation (17). In particular, aggregates may lead to modified immunogenicity of therapeutically applied biologics (18).

Tailored Amino Acid-Based Vaccine Formulations

Specifically tailored amino acid-based formulations have been shown to stabilise complex therapeutic biomolecules (19). Moreover, different viruses and vaccines, including enveloped and non-enveloped DNA and RNA viruses that underwent different manufacturing relevant stress conditions, were efficiently stabilised (see Table 1, page 30). Due to the individual complexity of viruses and vaccines, the amino acid-based formulations have to be specifically tailored to enable maximum stability during manufacturing (downstream processing) and subsequent delivery and storage. Usually, the tailoring process of the formulation underlies a strict and rational algorithm-based excipient selection procedure along with defined stress simulation models and monitoring of molecular integrity, which will be tackled in more detail later in the article.

Excipient Selection Process Principle

The principle procedure of the excipient selection process can be divided into five major steps (see Figure 1, page 30). The first step is the alignment of the target product profile considering different aspects such as regulatory requirements, intellectual property issues, drug dosage limitations, and the known molecular characteristics of the API. This information allows for the definition of the full design space as a basis for subsequent rational selection experiments. After selection of appropriate analytics (step 2), the molecule is challenged under defined stress conditions to identify sensible hot spots and degradation pathways (step 3). After systematic screening of various parameters and reduction of the design space, a combined data-driven and algorithm-based approach results in the definition of a focussed design space. This formulation development approach does not require high-throughput screening (HTS) (step 4). Iterative optimisation of formulations in API challenging models results in the identification of the most suitable formulations which then will be used in stability assays (step 5: eg, real-time storage).

Amino Acid-Based Formulation for Influenza Vaccine

In a recent publication, it was studied whether amino acid-based formulations are able to stabilise the influenza A vaccine Pandemrix (see Figure 2) even during extreme stress conditions, heat stress, spray drying, irradiation (>25 kGy beta irradiation), and storage (see Figure 3a, page 34) (20). Preselection of formulations was done by means of functional haemagglutination assays and molecular integrity analytics, such as size exclusion chromatography and dynamic light scattering. After identification of the best formulation (according to steps 3-5 of the selection process scheme),

Standard formulation with mannitol
Antigen concentration [ng]

+B = bulking agent; SD = spray drying

Selected amino acid formulation
Antigen concentration [ng]

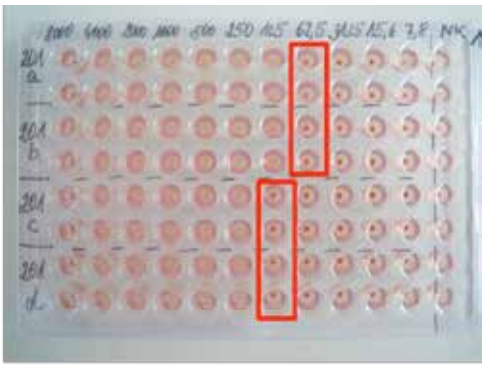
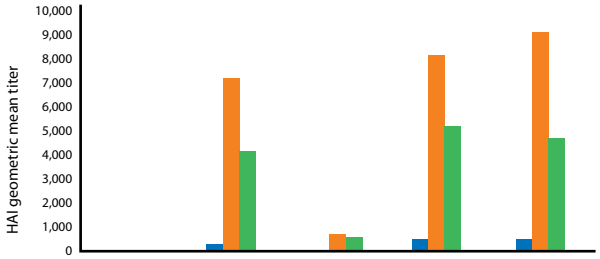


Figure 3a: Results with amino acid-based formulations for stress-exposed H1N1 influenza A vaccine (A) haemagglutination read-out. Selected amino acid-based formulations prevented loss of antigenic function after spray drying and βirradiation

a preclinical non-human primate (*Macaca fascicularis*) safety and efficacy study was conducted. Animals receiving original Pandemrix exhibited expected levels of seroconversion after 21 days (prime) and 48 days (boost), as assessed by haemagglutination inhibition and microneutralisation assays. Animals vaccinated with spray-dried and irradiated Pandemrix failed to exhibit seroconversion after 21 days, whereas spray-dried and irradiated amino acid-based formulated vaccines elicited similar seroconversion levels to those vaccinated with original Pandemrix (see Figure 3b). In conclusion, rational and

Amino acid-based formulated H1N1 influenza A vaccine demonstrates highly efficient vaccination even after irradiation



	Phosphate buffered saline	Pandemrix	Pandemrix-mannitol spray dried/β-irradiated	Protected spray dried	Protected spray dried/β-irradiated
Day 21	5	180	10	360	160
Day 34	5	7241	508	8128	9123
Day 48	10	4064	359	5120	4561

Figure 3b: Highly efficient vaccination was achieved even after βirradiation as demonstrated in a non-human primate model. Seroconversion was determined by haemagglutination inhibition assay

systematic approaches to design specifically tailored amino acid-based formulations in conjunction with appropriate stress models and analytics have a strong potential to identify highly stabilising formulations. The stability of the complex split virus Pandemrix vaccine after an extreme stress challenge could be even validated in non-human primates.

Formulations in the Future

Advanced and tailored amino acid-based formulations enable spray drying and terminal sterilisation of vaccines by avoiding stress-mediated molecular loss of molecular integrity, increasing stability and safety of the end product. The rational design of the formulation by support of design of experiments approaches ought to consider stress-mediated molecular challenges during each step of downstream processing (see Figure 3a and 3b) along with accurate monitoring of the molecular integrity. Successful stabilisation of vaccines by amino acid-based formulations during manufacturing might be essential for spray drying vaccines in the future.

“Advanced and tailored amino acid-based formulations enable spray drying and terminal sterilisation of vaccines by avoiding stress-mediated molecular loss of molecular integrity, increasing stability and safety of the end product”

> **Successful stabilisation of vaccines by amino acid-based formulations during manufacturing might be essential for spray drying vaccines in the future**

References

1. Visit: www.rentschler-biopharma.com/fileadmin/user_upload/News/Downloads/20181106_Full_report_for_release.pdf
2. Scherliess R, Delivery of antigens used for vaccination: Recent advances and challenges, *Ther Deliv* 2(10): pp1,351-68, 2011
3. WHO, GIVS: Global Immunization Vision and Strategy. Geneva, 2005
4. Amorij JP *et al*, Development of stable influenza vaccine powder formulations: Challenges and possibilities, *Pharm Res* 25(6): pp1,256-73, 2008
5. Hasija M *et al*, Forced degradation studies: An essential tool for the formulation development of vaccines, *Vaccine: Development and Therapy*: pp11-33, 2013
6. Brandau DT *et al*, Thermal stability of vaccines, *J Pharm Sci* 92(2): pp218-31, 2003
7. Wang S *et al*, Viscosity-lowering effect of amino acids and salts on highly concentrated solutions of two IgG1 monoclonal antibodies, *Mol Pharm* 12(12): pp4,478-87, 2015
8. Wang W, Lyophilization and development of solid protein pharmaceuticals, *Int J Pharm* 203(1-2): pp1-60, 2000
9. Amorij JP *et al*, Rational design of an influenza subunit vaccine powder with sugar glass technology: Preventing conformational changes of haemagglutinin during freezing and freeze-drying, *Vaccine* 25(35): pp6,447-57, 2007
10. Lal M, Technologies for the Development of Thermostable Vaccines: *PATH*; 2013
11. Son YJ and McConville JT, Development of a standardized dissolution test for inhalable formulations, *Int J Pharm* 382(1-2): pp15-22, 2009
12. Rathore N and Rajan RS, Current perspectives on stability of drug products during formulation, fill and finish operations, *Biotechnol Prog* 24(3): pp504-14, 2008
13. Shire SJ, Formulation and manufacturability of biologics, *Curr Opin Biotechnol* 20(6): pp708-14, 2009
14. Bhamra K *et al*, Aseptic vial filling, *Methods Mol Med* 40: pp313-7, 2000
15. Visit: www.fda.gov/ICECI/EnforcementActions/WarningLetters/default.htm
16. Visit: www.edqm.eu/en/european-pharmacopoeia-ph-eur-9th-edition
17. Garrison WM *et al*, Radiation-induced oxidation of protein in aqueous solution, *Radiat Res* 16: pp483-502, 1962
18. De Groot AS and Scott DW, Immunogenicity of protein therapeutics, *Trends Immunol* 28(11): pp482-90, 2007
19. Kemter K *et al*, Amino acid-based advanced liquid formulation development for highly concentrated therapeutic antibodies balances physical and chemical stability and low viscosity, *Biotechnol J* 13(7): e1700523, 2018
20. Scherliess R *et al*, Induction of protective immunity against H1N1 influenza A(H1N1)pdm09 with spray-dried and electron-beam sterilised vaccines in non-human primates, *Vaccine* 32(19): pp2,231-40, 2014

About the authors

Dr Jens Altrichter is a Medical Doctor and Biochemist. Researcher periods took him to various US competence centres. In 1998, Jens left his research and teaching position at the University of Rostock, Germany, and founded biotechnology company CellTech, where he became CEO. In 2006, Jens joined LEUKOCARE as Managing Director, and, in 2012, he became Chief Operating Officer, adopting responsibilities for R&D, production management, and regulatory affairs. In 2017, he co-founded ARTCLINE and became CEO at the company.
Email: jens.altrichter@leukocare.com

Dr Kristina Kemter works as a Project Leader in R&D at LEUKOCARE. In her current position, Kristina is responsible for continuous development of LEUKOCARE's proprietary Stabilizing and Protecting Solutions (SPS®) formulation platform. Between 2005-2010, Kristina worked in a postdoctoral fellowship and as Project Leader at the Department of Molecular Pediatrics, Dr von Hauner Children's Hospital, Ludwigs Maximilians University, Germany. Kristina also worked in a postdoctoral fellowship and studied chemistry with a focus on biochemistry at the Technical University Munich, Germany, at the Chair of Biochemistry.
Email: kristina.kemter@leukocare.com

Rebekka Ströver studied translation studies at the University of Heidelberg, Germany, specialising in Spanish and English language, and medical translations. Rebekka held various positions in the field of medical translations in pharma and biotech. She joined LEUKOCARE in 2016 and is now part of the marketing and sales team serving as communication and marketing specialist.
Email: rebekka.stroever@leukocare.com

Professor Dr Martin Scholz studied biology in Frankfurt am Main, Germany, and specialised in experimental medicine, immunology, and virology. Martin is associated with the Heinrich Heine University Hospital in Düsseldorf, Germany, as an APL Professor, where he was Head of Research in the Department of Traumatology between 2005-2010. Martin founded LEUKOCARE in 2001, where he worked as Chief Scientific Officer until the end of 2017. He wrote a large number of scientific publications and patent applications in the field of stabilisation of biologics. As a consultant, Martin currently supports LEUKOCARE and biotech start-up companies.
Email: martin.scholz@starts-and-ups.de