ENHANCING PROTEIN PRESERVATION DURING BIOLOGICAL DRUG PREPARATION AND DELIVERY

In this article, Adrien Bouillet, R&D Mechanical & Plastics Manager, EVEON, Loïc Girois, PhD Student, Laboratoire des Matériaux et du Génie Physique (LMGP), and Marianne Weidenhaupt, PhD, Associate Professor, LMGP, discuss the results of work carried out in joint efforts by EVEON and LMGP in the field of protein adsorption and aggregation, and how the results of this work have led to improvements in the design of EVEON's proprietary micropump.

Therapeutic proteins are injectable biological drugs with a high specificity, which are used for the treatment of many diseases, including diabetes, autoimmune diseases and cancer. They are regularly marketed as lyophilised powders that need to be reconstituted, either manually or automatically, prior to injection.

It is well established that exposure to certain material and air interfaces, to which proteins adsorb, can have a strong impact on their stability.[1] While adsorbing at interfaces, proteins change their conformation, which can lead to the formation of protein aggregates. This can entail a loss of function or lead to the development of immunogenic responses and, consequently, adverse reactions in patients. Moreover, these aggregates can also obstruct the fluid flow in reconstitution and injection devices, having a negative impact on their performance. Improving the stability of these drugs is generally achieved by formulation excipients, such as surfactants, but can also be maximised by a precise design of device components and fluidic protocols.

In order to enhance protein preservation during drug preparation and delivery, EVEON and LMGP have worked together through the LabCom programme to improve EVEON's technology platform. Three different versions of EVEON's proprietary micropump were studied for their mechanical performance, analysing the stability of an unformulated drug solution during pump cycles at different speeds (20, 50 and 80 rotations per minute).

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Adrien Bouillet
R&D Mechanical & Plastics Manager
T: +33 4 76448417
E: abo@eveon.eu

EVEON SAS
305, rue Aristide Bergès
38330 Montbonnot-Saint-Martin
France

www.eveon.eu

Loïc Girois
PhD student
T: +33 4 56529309
E: loic.girois@grenoble-inp.fr

Dr Marianne Weidenhaupt
Associate Professor
T: +33 4 56529335
E: marianne.weidenhaupt@grenoble-inp.fr

Laboratoire des Matériaux et du Génie Physique
Minatec
3 parvis Louis Néel
CS 50257-38016
Grenoble cedex 1
France

www.lmgp.grenoble-inp.fr

The drug chosen was an unformulated human insulin solution at 0.7 mg/mL in a 25 mM tris buffer of pH 7.4 with 125 mM NaCl. Unformulated insulin is well known for its high tendency to adsorb and aggregate at interfaces,[2] and can therefore be considered a suitable test case for a worst-case scenario with respect to drug aggregation. The unformulated protein solution was transferred through the pumps over 500 suction/discharge cycles. The operation specifications define 300 pumping cycles as a target limit. Typically, the torque values increase with pump cycles at all speeds.

Figure 1 shows the correlation between torque increase and speed for EVEON's original micropump and comparative results for the three pumps are shown at 80 rotations per minute in Figure 2. The micropump evolution (siliconised) showed the lowest and most stable torque values.

Insulin stability was monitored by Thioflavin T fluorescence (480 nm), a conformation-sensitive dye indicative of amyloid aggregates. The appearance of Thioflavin T-positive insulin aggregates in unformulated solutions was recorded for all pumps. The extent of fluorescence depended on insulin concentration, pumping speed and the number of cycles through the pumps. A thorough comparative analysis was completed on the micropump evolution (silicone free) and the micropump evolution (siliconised), the two pumps that showed the lowest torque values. At 80 rotations per minute and 500 cycles, aggregating was detected in one out of nine experimental runs for the micropump evolution (siliconised), whereas it was detected in four out of nine for the micropump evolution silicone free (Figures 3 and 4).

These results show that reduced torque values correlate positively with a lower aggregation potential when tested with unformulated insulin solutions in the pumps. All EVEON's micropumps showed no Thioflavin T-positive insulin aggregates when tested with formulated insulin solutions. The results led to a better understanding of the original version of the micropump, which allowed for a drastic reduction of the aggregation potential with the design of the new micropump evolution and its siliconised version.

In conclusion, the results confirmed that the new micropump evolution, even in its silicone-free version, may be considered

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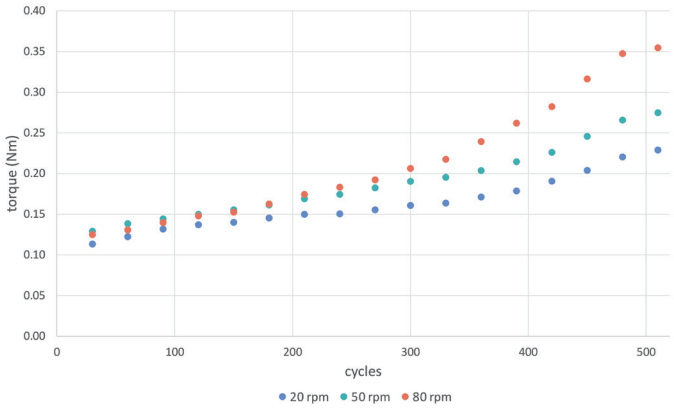

Figure 1: Torque as a function of number of cycles at three different speeds for EVEON's original micropump.

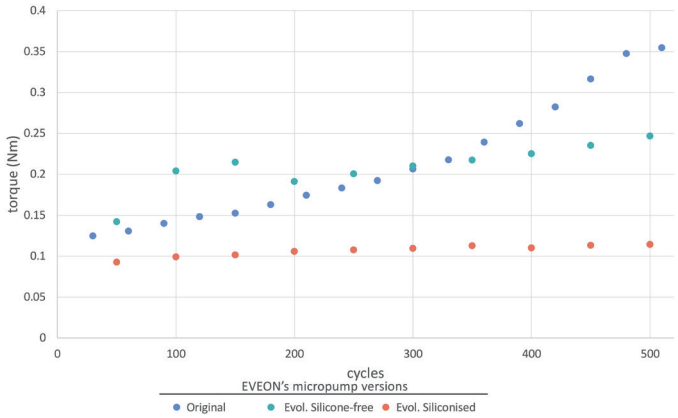

Figure 2: Torque as a function of number of cycles at 80 rotations per minute for three different versions of EVEON's proprietary micropump: original, evolution (silicone-free) and evolution (siliconised).

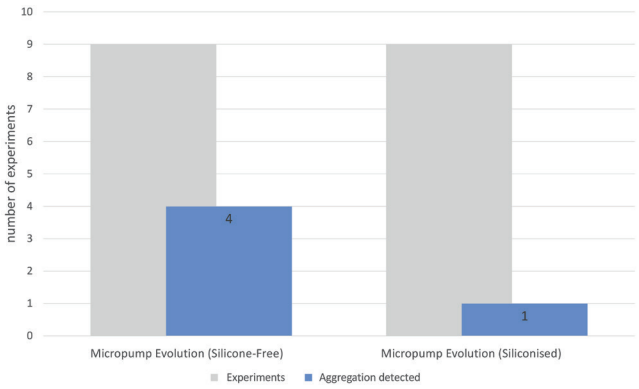

Figure 3: Insulin aggregation after 500 cycles at 80 rotations per minute for the two evolution versions of the pump. The number of experiments with Thioflavin T-positive insulin aggregation is shown with blue bars, and the total number of experiments (nine) is shown with grey bars.

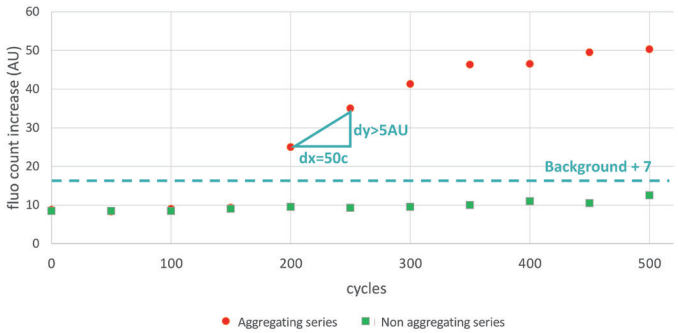

Figure 4: An experiment is considered ThioflavinT-positive when the fluorescence increment after 50 pump cycles is greater than five, and the fluorescence signal at 500 cycles is greater than seven times the baseline fluorescence.

Figure 5: EVEON's patented micropump.

a preferable solution when looking to mitigate the risk of protein aggregates in biopharmaceuticals during drug preparation and delivery.

EVEON's proprietary micropump (Figure 5) is a modular platform design that may offer a reliable solution to pharmaceutical and biotech companies aiming to develop an automated drug preparation device for at-home patient care.

ABOUT THE ORGANISATIONS

EVEON is an ISO 13485 certified company that designs and produces automatic, secure and connected medical devices for the preparation and delivery of therapeutic treatments to improve patient quality of life. EVEON places the needs of patients and healthcare practitioners at the heart of its developments, designing simple and intuitive devices to improve therapeutic performance, compliance and homecare conditions. Its expertise has just been recognised by Forbes magazine, which ranks EVEON as the third most inventive company in France in the medical technologies category. As an end-to-end innovation partner, from concept to CE marking, with strong know-how and capabilities in fluidics, mechanics and electronics, EVEON is recognised as a key partner for innovative companies offering a global service from feasibility to manufacturing.

Laboratoire des Matériaux et du Génie Physique (LMGP) is a joint research unit of the Grenoble Institute of Technology and the French National Centre for Scientific Research (le centre national de la recherche scientifique, CNRS), developing research in materials for science and materials for biomedical engineering. It is active in the fields of functional thin films, surface nano-engineering and interactions between materials and biological matter. In the latter field, LMGP has long-standing expertise in the analysis of therapeutic protein stability at interfaces using biophysical surface-sensitive techniques and biochemical assays. LGMP and EVEON operate a common laboratory supported by the IDEX funding programme, with the aim of optimising the stability of therapeutic proteins in their automated preparation and injection devices. This academic-industrial alliance allows for the improvement of EVEON's products, while simultaneously gaining fundamental knowledge about protein stability at interfaces.

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REFERENCES

1. *Pinholt C et al, "The importance of interfaces in protein drug delivery- why is protein adsorption of interest in pharmaceutical formulations?". Expert Opin Drug Deliv, 2011, Vol 8(7), pp 949–964.*
2. *Sluzky V et al, "Kinetics of insulin aggregation in aqueous solutions upon agitation in the presence of hydrophobic surfaces". Proc Natl Acad Sci USA, 1991, Vol 88(21), pp 9377–9381.*

ABOUT THE AUTHORS

Adrien Bouillet holds a Masters degree in Mechanical Engineering and Integrated Design. He went abroad for the first years of his career, and worked as a Mechanical Design Engineer in Scotland, New-Zealand and Australia; upon moving back to France, Mr Bouillet joined EVEON in 2015. After five years, he took on the position of R&D Mechanical & Plastics Manager, leading a team of four Mechanical Design Engineers, two Plastics Engineers and one Mechanical CAD Technician. Placed at the heart of EVEON's development, the R&D Mechanical & Plastics team works very closely with both the Digital and Fluidics teams, to design ergonomic, smart and reliable products.

Loïc Girois, holds a Masters degree from Université Grenoble Alpes (France) in Nanobiosciences and is currently working on a collaborative project for EVEON and the LMGP on the stability of therapeutic proteins in automated drug preparation and administration systems.

Marianne Weidenhaupt, PhD, is Associate Professor at the Grenoble Institute of Technology (France) and a researcher at LMGP, where she leads the team researching the interface between materials and biological matter. Dr Weidenhaupt received her PhD from ETH Zurich (Switzerland) in 1986 and worked as a postdoctoral fellow at the French Alternative Energies and Atomic Energy Commission (Paris, France) and the European Molecular Biology Laboratory's site in Grenoble. She is a trained molecular biologist and biochemist and is currently studying protein adsorption and aggregation at interfaces in the context of therapeutic protein stability. She develops molecular tools and techniques to monitor and quantify these processes. Dr Weidenhaupt is the author of 33 peer-reviewed papers and three patents.

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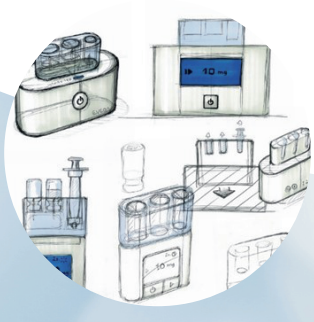

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