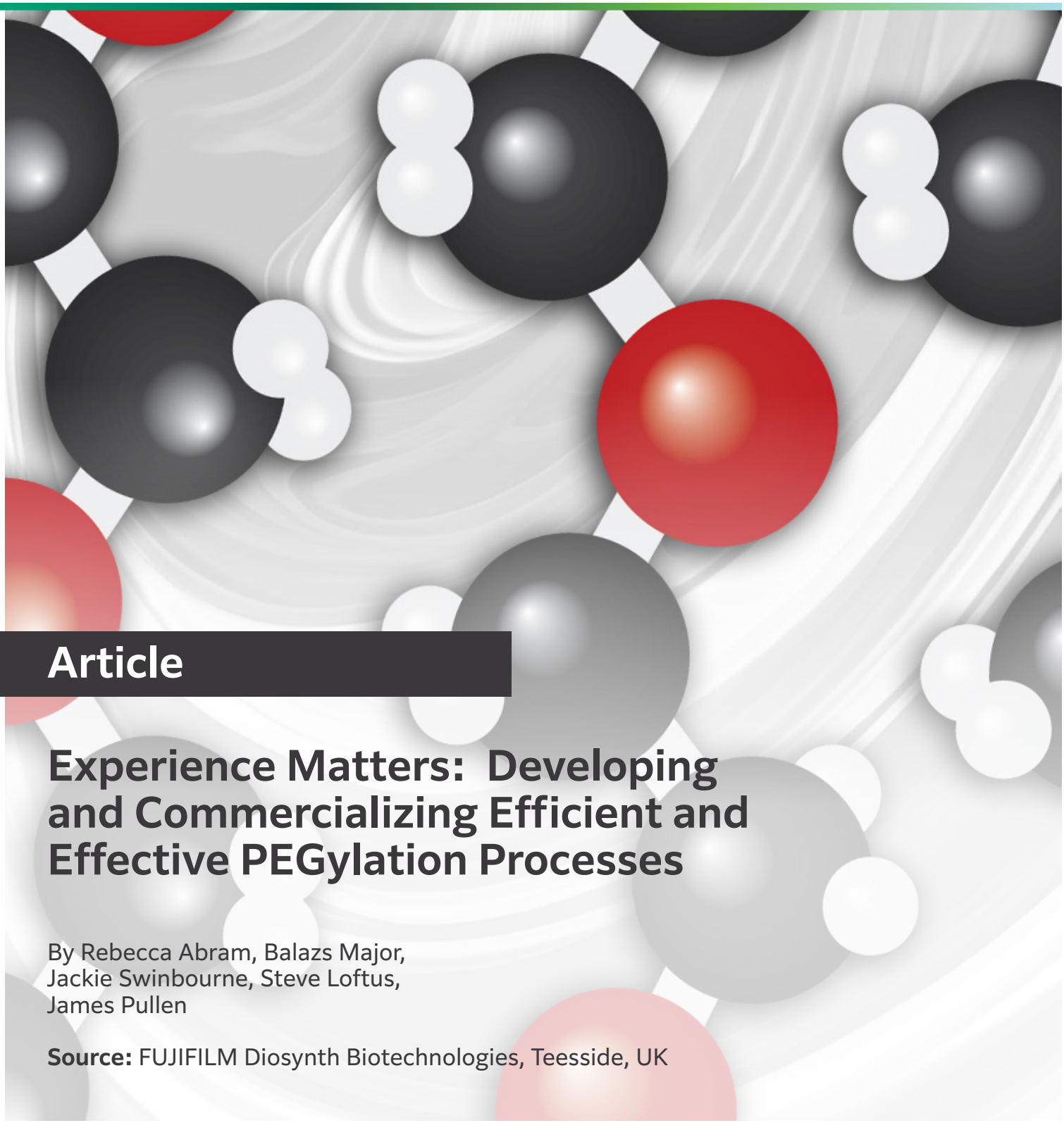
FUJIFILM

Diosynth biotechnologies



Article

Experience Matters: Developing and Commercializing Efficient and Effective PEGylation Processes

By Rebecca Abram, Balazs Major, Jackie Swinbourne, Steve Loftus, James Pullen

Source: FUJIFILM Diosynth Biotechnologies, Teesside, UK

PEGYLATED PROTEINS: SEAMLESS CLIENT SUPPORT FROM DEVELOPMENT TO COMMERCIALIZATION

Synthetic conjugation of proteins with polyethylene glycol (PEG) can enhance the protein's functionality, increase its half-life, change pharmacokinetic behaviour and/or enable specific application(s) [1]. Clinical successes of PEGylated therapeutics have fueled the development of new PEGylated proteins, as such at the time of writing there are 52 active clinical trials predominantly for PEGylated cytokines and enzymes [2]. These types of molecules are typically expressed in *E. coli.*

FUJIFILM Diosynth Biotechnologies (FDB) is a Contract Development Manufacturing Organization (CDMO) with a rich history and extensive experience in navigating our clients through development challenges for PEGylated microbial therapeutics. We support both liquid and solid conjugation chemistries that include reactions with amino acid side chains and backbone, as well as chemistries involving non-proteinogenic amino acids at fermentation volumes up to 3000 L. Our expanding capacity and end-to-end service has made us the partner of choice for clients on their journey to commercialization.

SUPPORTING OUR PARTNERS EVERY STEP OF THE WAY MEANS

Efficiencies of Scale: From the very beginning, FDB starts by focusing on the long-term cost of goods to ensure commercial viability. For early phase clients, process development starts with a Design of Experiments (DoE) to optimize the conjugation step with the aim of reducing the conjugate/protein molar ratio, increase process efficiency, optimize reaction time and improve reaction specificity. Readiness for scale up is assessed by evaluating factors including PEG preparation, handling, mixing mechanism and speed of addition, material compatibility, hold times and large-scale safety aspects.

Integrated Heterogeneity Assessments: Complex multi-step and side chemical reactions increase the likelihood of heterogeneity and increase the risk of product degradation, resulting in a mixture of different species with varying degrees of PEGylation, presenting both separation and purification challenges. During the process development phase, DoEs are performed to optimize the PEGylation reaction conditions, with the aim of generating confidence in consistent lab-scale performance. Maintaining a line of sight to manufacture FDB utilizes process development data to select compatible pre- and post-conjugation unit operations and de-risk conjugation steps to ensure minimal losses of reagents whilst balancing the need for increased yield versus increased heterogeneity. FDB's analytical teams efficiently integrate analytical methodologies to monitor real-time reactions during development and design and validate appropriate control and release methodologies.

Characterization and Stability Services: The characterization of conjugated proteins can be challenging due to the complexity of the whole molecule, masking of some critical quality attributes and presenting challenges in terms of identifying conjugation sites. Conjugation may affect the stability/degradation profile of the protein and may potentially alter conformation and biological activity, which can impact its efficacy. FDB provides comprehensive analytical and stability services to identify and characterize the degree of PEGylation and the stability of the final product, including bioinformatics, biophysical assessment, cell-based potency assay development, mass spectrometry and cGMP physicochemical testing.

Creation of Control Strategies that Last the Entire Life-Cyle of Your Molecule: Our experts are housed in laboratories that are purposefully designed and dedicated to the systematic investigation of operating conditions and parameters that are important in ensuring product quality and process consistency in manufacturing. We perform studies to examine the re-usability of processing raw materials such as chromatography resins and assess the stability of processing solutions including buffers and process intermediates. The result is an optimized, robust and well-understood manufacturing process that consistently produces drug substance within pre-determined specifications. Read more in our process characterization blog

In the past 10 years, across our US and UK sites, our experts have navigated an increasing number of clients through diverse and complex challenges on their journey from process development to create commercially viable manufacturing processes for multiple conjugation modalities and PEGylation mechanisms. We offer bespoke services from development to drug substance and finished goods that include regulatory life-cycle management.

PEGYLATION: FUTURE PERSPECTIVES

The future of conjugation and PEGylation in biologics is promising, with advancements focused on enhancing drug efficacy and reducing immunogenicity. Many emerging strategies involve site-specific attachment of therapeutic molecules to biologics, minimizing off-target effects. Other novel PEGylation approaches aim to optimize drug delivery, including alternatives to traditional polyethylene glycol modifications, such as multi-arm PEGylation and controlled-release systems. Outside the realm of direct conjugation to proteins post expression, there continues to be an increase in biologics that are fusion proteins or bio-conjugations such as albumin fusions, PASylation® and XTENylation™. Here the host cell produces the protein-based conjugate along with the target biologic, removing the need for conjugation reactions downstream.

Purification of PEGylated biologics in the future will require advanced chromatographic techniques by leveraging mixed-mode chromatography, customized resins and adsorbents, plus the utilization of automation and high-throughput systems to enhance chromatographic efficiency. With the rise of continuous processing, strategies will also need to be developed that allow PEGylation/conjugations reactions to slot seamlessly into connected processes, minimizing hold times. For this, a thorough understanding of the kinetics and chemistry of the reactions is essential, thus necessitating the advancement of analytical techniques to support development. Computational fluid dynamics (CFD) could be used to model mixing at scale with a view to maximize the reaction efficiency and accelerate program timelines.

To learn more about how FDB can support PEGylation development and commercialization and the benefits that our partnership mindset provides, contact us.

[1] Francesco M Veronese and Anna Mero. The impact of PEGylation on biological therapies. BioDrugs; 2008;22(5):315-29

[2] Yongsheng Gao, Maithili Joshi, Zongmin Zhao, Samir Mitragotri. PEGylated therapeutics in the clinic. Bioengineering and Translational Medicine; 22 September 2023

About the Author

Dr Rebecca Abram holds the position of Director, Strategic Technical Marketing at FUJIFILM Diosynth Biotechnologies. She has global responsibility for leading technical marketing activities for all service offerings. Rebecca joined FUJIFILM Diosynth Biotechnologies at the Billingham (UK) site in the Analytical Development department in 2019 where she led the analytical component for over 70 early and late phase customer programs. She received her BSc in Chemistry and subsequently completed her doctoral research into anti-inflammatory peptides before joining FDB.

About FDB

FDB is a global CDMO operating in Europe and North America with integrated platforms to meet client demands and deliver medicines to patients faster. FDB has capabilities not only with drug substance manufacturing but also with finished goods manufacturing on both sides of the Atlantic, allowing a customer to work with a single provider for their end-to-end manufacturing needs. To learn more about their CDMO offerings, contact the FDB team.