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Review

Liquid phase separation methods for N-glycosylation analysis of glycoproteins of biomedical and biopharmaceutical interest. A critical review

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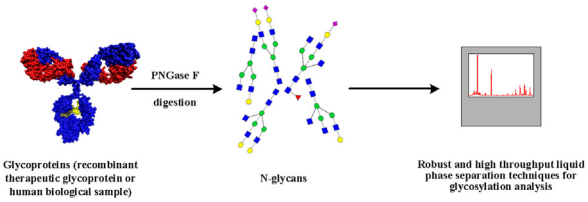
GRAPHICAL ABSTRACT



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ABSTRACT

Comprehensive carbohydrate analysis of glycoproteins from human biological samples and biotherapeutics are important from diagnostic and therapeutic points of view. This review summarizes the current state-of-the-art liquid phase separation techniques used in N-glycosylation analysis. The different liquid chromatographic techniques and capillary electrophoresis methods are critically discussed in detail. Miniaturization of these methods is also important to increase throughput and decrease analysis time. The sample preparation and labeling methods for asparagine linked oligosaccharides are also addressed.

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Abbrevations

OKT3	Ortho Kung T3
IgG	immunoglobulin G
FDA	Food and Drug Administration
EMA	European Medicines Agency
QbD	Quality by Design
ADCC	antibody-dependent-cell mediated cytotoxicity
CDC	complement-dependent cytotoxicity
Gal	galactose
GlcNAc	N-acetylglucosamine
Man	mannose
Fuc	fucose
Endo H	Endoglycosidase H
PNGase F	peptide-N-glycosidases F
PNGase A	peptide-N-glycosidases A
2-AB	2-aminobenzamide
2-AA	2-aminobenzoic acid
PA	2-aminopyridine
ANTS	8-aminonaphthalene-1,3,6-trisulfonic acid
APTS	8-aminopyrene-1,3,6-trisulfonic acid
ABEE	2-aminobenzoic acid ethyl ester
PSC	1-pyrenesulfonyl chloride
HPAEC-PAD	high pH anion exchange chromatography with pulsed amperometric detection
NPLC	normal phase liquid chromatography
HILIC	Hydrophilic Interaction Liquid Chromatography
RP	reverse phase
PGC	porous graphitized carbon
WAX	weak anion exchange
UPLC	ultra-performance liquid chromatography
MALDI-TOF	matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
CE	capillary electrophoresis
LIF	laser induced fluorescence
CGE	capillary gel electrophoresis
COPD	Chronic Obstructive Pulmonary Disease
ME	microchip electrophoresis
LOD	limit of detection

1. Introduction

Development of biopharmaceuticals is currently stimulated by the emergence of recombinant protein-based drugs (e.g., monoclonal antibodies), which is the fastest growing sector in the pharmaceutical industry nowadays. Glycosylation, as one of the most common post-translational modifications on the recombinant protein therapeutics [1], is the result of complex enzymatic processes that covalently attach oligosaccharides to either the side chain of asparagine (N-linked) or serine/threonine (O-linked) in the polypeptide backbone [2]. The market for therapeutic proteins has increased significantly since the introduction of the first therapeutic monoclonal antibody product in 1986 (Orthoclone OKT3, for prevention of kidney transplant rejection). Currently (statistical data from 2014) 212 biopharmaceutical products have been approved by the FDA (Food and Drug Administration) and/or EMA (European Medicines Agency) for treatment of different diseases. Just the monoclonal antibody products that are expected to enter the market by 2020 will represent world-wide sales of ca. $125 billion [3,4].

In the production of recombinant glycoproteins, the biosynthesis of covalently attached oligosaccharides (glycans) is often the most difficult to control, therefore their glycosylation pattern should be carefully monitored during every stage of the manufacturing process to ensure quality, safety, and efficacy. Even minor changes in process conditions can modify the enzymatic glycan synthesis and consequently the physicochemical and biological properties of the final product [5]. In the production of glycoprotein based biopharmaceuticals based on the Quality by Design (QbD) paradigm one of the important targets is the modification of the carbohydrate moieties to enhance efficacy. For example, the two major effector functions of monoclonal antibodies are antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), both influenced by the glycosylation of the final product. Therefore, tight characterization of their carbohydrate structures is already crucial in clone selection, but also later at various stages of the development process [6]. The presence or absence of various sugar residues on monoclonal antibody N-glycan cores increases the so called microheterogeneity, which also affects stability and effector functions. Namely, terminal galactose (Gal), N-acetylglucosamine (GlcNAc), and mannose (Man) residues affect the CDC activity, while terminal sialic acid, mannose, core fucose (Fuc), and bisecting GlcNAc affect the ADCC function [7–9]. Therefore, regulators prefer the Quality-by-Design approach instead of the rigid pharmaceutical manufacturing process with variable product quality. The QbD paradigm introduces the concept of quality into the manufacturing process from the start, which consistently delivers a product with the desired quality attributes for the best clinical performance (efficacy and safety) [10]. In this case the raw material and process parameters should be continuously monitored and controlled to assure consistent product quality. As a matter of fact, biopharmaceutical companies get more robust manufacturing processes by implementing the QbD concept [11,12], leading to higher productivity and product homogeneity, and also greater therapeutic efficacy through the development of biopharmaceuticals with the desired glycosylation patterns [13]. By all means, the profile of the glycosylation pattern along with the microheterogeneity information should be determined to produce safe, good quality, consistent biological drugs for human use. Consequently, the demands for state-of-the-art glycan analysis methods have been continuously increasing as better and better technologies are developed. Regulatory agencies worldwide require robust, information-rich, reproducible and preferably orthogonal methods for glycosylation analysis at various stages of the development process from clone selection to product release to ensure accuracy and consistency of the final drug product [5]. In a recent review, Planinc et al. demonstrated the importance of glycosylation analysis in the field