Lessons with Protein Separation ZenMasters

Large Molecules vs. Small Molecules

Chad Eichman, Ph.D.
BioPharm Global Marketing Manager

The pharmaceutical industry has a long-standing relationship with small organic molecules. Synthesis gurus of the past, especially the ZenMaster of organic synthesis, Robert Burns Woodward, built a foundation for the industry to create elegant chemical syntheses. The revolution of organic synthesis transformed the pharmaceutical industry by allowing companies to produce any desired molecule to deploy in nearly all therapeutic areas. Decades of drug development revolved around these small molecules, which vary in complexity but typically weigh less than 900 Dalton (Da). Aspirin, for example, weighs 180 Da. More complex molecules such as breast cancer therapeutic paclitaxel comes in at 854 Da. Most small molecule drugs are chemically synthesized, but more complex small molecules, like paclitaxel, are produced recombinantly through cell cultures. Once generated, most of these drugs are packaged into ingestible tablets that are taken orally to distribute throughout the body. The mechanism by which small molecules act is usually through cell penetration, which prompts a desired cellular response. Overall, this classic approach in drug development is a mainstay in the pharmaceutical industry and continues to grow each year.

While the growth of small molecule development continues, biologics, or biopharmaceuticals, have emerged as the next-generation of therapeutic molecules in the pharmaceutical industry. Insulin, the first biologic drug, has been implemented in the treatment of diabetes for nearly a century. Biologics are significantly larger and more complex than their small molecule counterpart. These large molecules range from ~3,000-150,000 Da and their use as drugs requires injections instead of the pill format. Insulin, for example, weighs 5,808 Da, whereas adalimumab (Humira®) weighs 144,190 Da. Because of their large structure and complexity, biologics are not chemically synthesized, but are only recombinantly produced by engineered cells. Biopharmaceuticals are usually derivatives of natural human proteins, which make them ideal for targeted cellular therapy. Unlike small molecule drugs, which penetrate cell membranes including healthy cells, biopharmaceuticals act through external cellular binding to induce the desired cellular response. Moreover, the molecules are capable of site-specific cellular binding which means they do not interfere with healthy cells, ultimately making them more attractive drugs.

Regardless of the above differences, small molecule drugs dominate the market and account for over 90% of the top 200 prescribed drugs in the U.S. in 2016.[1] However, in terms of cost, biopharmaceuticals are drastically more expensive, as demonstrated by Humira®, which generated $18.4 billion in sales in 2017.[2] Correlated to the high consumer cost is a significant increase in research and development costs to generate a biopharmaceutical. One driver for the increased cost of development is because the analytical characterization of biologics is incredibly challenging. Pharmaceutical companies need to characterize biologics in multiple ways including purity, stability, and function. For purity assays, unlike small molecules, which can be structurally confirmed through high resolution methods like X-ray crystallography and NMR spectroscopy, biologics routinely require a combination of various high and low-resolution techniques in order to validate structure. Moreover, during the recombinant production of these large molecules, a multitude of post-translational modifications (PTMs) are generated. These modifications directly influence the function of the protein and are therefore the most critical aspect of protein characterization to consider.

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The daunting task of identifying post-translational modifications is made a bit more Zen through advances in chromatography and mass spectrometry. Scientists rely on classical chromatographic methods such as size exclusion chromatography (SEC), reversed phase (RP), and ion-exchange chromatography (IEX), but also on newer separation modes including hydrophilic interaction chromatography (HILIC) and hydrophobic interaction chromatography (HIC). These methods are implemented in a variety of techniques that are stringed together to complete the full picture of each biologic characterization. Utilizing efficient chromatographic separations in combination with high resolution mass spectrometry (MS) gives scientists an essential element of identification. These high-resolution methods are often utilized to observe even the most minute PTMs. Both chromatography and mass spectrometry technologies are increasing in resolution and efficiency as research progresses. With the increase in pharmaceutical R&D efforts shifting toward biologics products, analytical companies are also creating novel solutions for biologics characterization. The result of these combined efforts will hopefully lead to a more streamlined and rapid development of biopharmaceutical products for the advancement of human health. Ultimately, a more Zen-infused world, if you will.

References:

1. http://njardarson.lab.arizona.edu/sites/njardarson.lab.arizona.edu/files/2016Top-200PharmaceuticalPrescriptionSalesPosterLowResV2.pdf
2. http://njardarson.lab.arizona.edu/sites/njardarson.lab.arizona.edu/files/2016Top-200PharmaceuticalPrescriptionSalesPosterLowResV2.pdf

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bioZen™ Products - Powered by Biocompatible Hardware

bioZen Columns (mm)								Biocompatible Guard Cartridges*	
Phases	**50 x 2.1**	**100 x 2.1**	**150 x 2.1**	**250 x 2.1**	**50 x 4.6**	**150 x 4.6**	**300 x 4.6**	**for 2.1 mm**	**for 4.6 mm**
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bioZen 3 µm Peptide PS-C18	—	—	—	—	00B-4771-E0	00F-4771-E0	—	—	AJ0-7606
bioZen 1.7 µm Peptide XB-C18	00B-4774-AN	—	00F-4774-AN	—	—	—	—	AJ0-9806	—
bioZen 2.6 µm Peptide XB-C18	00B-4768-AN	—	00F-4768-AN	00G-4768-AN	00B-4768-E0	00F-4768-E0	—	AJ0-9806	AJ0-9808
bioZen 3.6 µm Intact C4	00B-4767-AN	—	00F-4767-AN	—	00B-4767-E0	00F-4767-E0	—	AJ0-9809	AJ0-9811
bioZen 3.6 µm Intact XB-C8	00B-4766-AN	—	00F-4766-AN	—	00B-4766-E0	00F-4766-E0	—	AJ0-9812	AJ0-9814
bioZen 1.8 µm SEC-2	—	—	—	—	—	00F-4769-E0	00H-4769-E0	—	AJ0-9850
bioZen 1.8 µm SEC-3	—	—	—	—	—	00F-4772-E0	00H-4772-E0	—	AJ0-9851

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Australia
t: +61 (0)2-9428-6444
f: +61 (0)2-9428-6445
auinfo@phenomenex.com

Austria
t: +43 (0)1-319-1301
f: +43 (0)1-319-1300
anfrage@phenomenex.com

Belgium
t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
f: +31 (0)30-2383749
beinfo@phenomenex.com

Canada
t: +1 (800) 543-3681
f: +1 (310) 328-7768
info@phenomenex.com

China
t: +86 400-606-8099
f: +86 (0)22 2532-1033
cninfo@phenomenex.com

Denmark
t: +45 4824 8048
f: +45 4810 6265
nordicinfo@phenomenex.com

Finland
t: +358 (0)9 4789 0063
f: +45 4810 6265
nordicinfo@phenomenex.com

France
t: +33 (0)1 30 09 21 10
f: +33 (0)1 30 09 21 11
franceinfo@phenomenex.com

Germany
t: +49 (0)6021-58830-0
f: +49 (0)6021-58830-11
anfrage@phenomenex.com

India
t: +91 (0)40-3012 2400
f: +91 (0)40-3012 2411
indiainfo@phenomenex.com

Ireland
t: +353 (0)1 247 5405
f: +44 1625-501796
eireinfo@phenomenex.com

Italy
t: +39 051 6327511
f: +39 051 6327555
italiainfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
f: +31 (0)30-2383749
nlinfo@phenomenex.com

Mexico
t: 01-800-844-5226
f: 001-310-328-7768
tecnicomx@phenomenex.com

The Netherlands
t: +31 (0)30-2418700
f: +31 (0)30-2383749
nlinfo@phenomenex.com

New Zealand
t: +64 (0)9-4780951
f: +64 (0)9-4780952
nzinfo@phenomenex.com

Norway
t: +47 810 02 005
f: +45 4810 6265
nordicinfo@phenomenex.com

Portugal
t: +351 221 450 488
f: +34 91-413-2290
ptinfo@phenomenex.com

Spain
t: +34 91-413-8613
f: +34 91-413-2290
espinfo@phenomenex.com

Sweden
t: +46 (0)8 611 6950
f: +45 4810 6265
nordicinfo@phenomenex.com

Switzerland
t: +41 61 692 20 20
f: +41 61 692 20 22
swissinfo@phenomenex.com

United Kingdom
t: +44 (0)1625-501367
f: +44 (0)1625-501796
ukinfo@phenomenex.com

USA
t: +1 (310) 212-0555
f: +1 (310) 328-7768
info@phenomenex.com

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Corporate Office USA
t: +1 (310) 212-0555
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info@phenomenex.com

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