Accepted Manuscript

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PII: S0165-9936(14)00195-2
DOI: http://dx.doi.org/doi: 10.1016/j.trac.2014.08.002
Reference: TRAC 14315

To appear in: *Trends in Analytical Chemistry*

Please cite this article as: Edouard S.P. Bouvier, Stephan M. Koza, Advances in size-exclusion separations of proteins and polymers by UHPLC, *Trends in Analytical Chemistry* (2014), http://dx.doi.org/doi: 10.1016/j.trac.2014.08.002.

Advances in size-exclusion separations of proteins and polymers by UHPLC

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HIGHLIGHTS

- Size-exclusion chromatography (SEC) for characterization of proteins and polymers
- Key challenges in developing UHPLC stationary phases for SEC
- Key UHPLC instrument requirements for performing SEC
- Recent UHPLC applications of SEC

ABSTRACT

The separation of molecular compounds based on their capacity to access the intra-particle pore volume of chromatographic media, which is dictated by the relative size in solution of those compounds, has been commonly known as size-exclusion chromatography (SEC) or gel-permeation chromatography (GPC). Conventionally, these two terms have been applied to the analysis of biomolecules and polymers, respectively. Over the more than half-a-century history of size-based separations, there has been a series of advancements, starting from the earliest soft-gel particles and culminating within the past few years in the use of sub-2-µm particles in ultra-high-performance liquid chromatography (UHPLC). The intent of this review is to provide a concise synopsis of the advancements of both chromatography columns and instrumentation for protein and polymer size-based separations. Also, this review presents brief summaries of the application of UHPLC technology for these classes of analytes.

Keywords:
Biomolecule
Chromatography
Gel-permeation chromatography
GPC
Polymer characterization
Protein aggregate
SEC
SE-UHPLC
Size-exclusion chromatography
UHPLC

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1. Introduction

Size-exclusion chromatography (SEC) and gel-permeation chromatography (GPC) are two names for the same technique, the only difference being application area. SEC is predominately used to describe size-based separations of biomolecules, while GPC typically refers to separation of synthetic and natural polymers.

In this article, we discuss some of the more recent trends in the area of SEC separations. Historically, the technique was considered to be a low-resolution, time-consuming separation