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APPLICATION NOTE

A Simple Gradient Reverse-Phase Liquid Chromatography method for Bovine Serum Albumin (BSA) using NanoPak-C All Carbon HPLC column

Background. Bovine serum albumin (BSA) is among the most widely used proteins in protein formulations and novel therapeutic delivery systems. BSA's well-defined properties and versatility make it a valuable tool for researchers developing new protein-based therapeutics and delivery systems.

Reliable and accessible methods for BSA characterization are beneficial and essential for research involving protein formulations and delivery systems. Accurate BSA quantification is the key to determining the optimal concentration for desired effects, such as stabilizing a therapeutic protein or optimizing drug delivery. It ensures the quality and consistency of protein formulations during development and manufacturing. It is crucial for interpreting data from experiments involving protein-protein interactions, release profiles from delivery systems, and immune responses to vaccines containing BSA.

HPLC is a valuable technique for characterizing and quantifying BSA. NanoPak-C All Carbon media offers tunable pore size and surface area suitable for reverse phase separation of proteins. BSA's hydrophobic region can interact favorably with the hydrophobic carbon surface, leading to better protein conformation and stability during chromatography. This note summarizes the optimal HPLC method for detecting and purifying BSA. The technique uses a UV detector, available in most analytical labs.

Probe Analytes

BSA: Sigma A2153: 5mg/mL in DI water pH 5.1

Instrumentation

HPLC Conditions	
Column	Nanopak-C All Carbon 150 x 4.6 mm, 6um
Mobile phase	Mobile Phase A: 0.1% TFA in water Mobile Phase B: 0.1% TFA in Acetonitrile Gradient: Time %B 0 35 6 75 7 35 Run Time: 10
Injection volume	20ul
Flow	1ml/min
UV detection	280nm

Results

Figure 1 shows a representative chromatogram of BSA. Baseline separation of BSA with good peak shape was observed within 5 minutes. The results indicate that the carbon microbead's pore size is appropriate for large macromolecular proteins like BSA, which has a size of 66,000 daltons. Pore size for separation varies depending on the substance being examined. If the pore size is too small, the molecules will not pass through, resulting in insufficient resolution. On the other hand, if the pore size is too large, the separation will be poor. Therefore, achieving good resolution requires choosing a pore size that matches the size of the molecules. The results demonstrate that the optimized gradient HPLC method presented in this note is suitable for characterization and purification of BSA in biological samples.

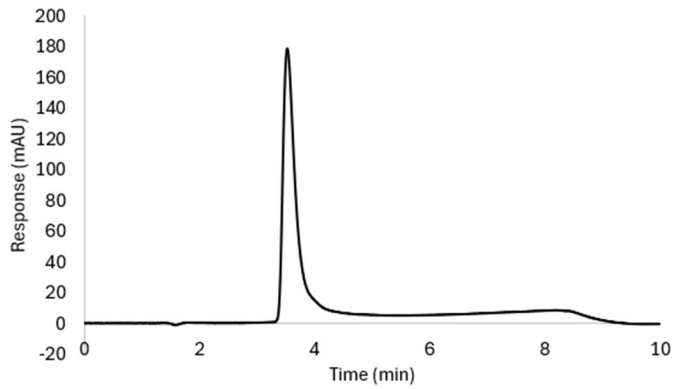

Figure 1. Representative chromatogram of BSA.