Conclusion

Particle fines are an important part of understanding SPE techniques. They are present in silica based SPE devices available because they are present in the manufacturing and derivitization steps used to add functional groups to silica particles.

The absence of particle fines from eluates will help:

- Extend HPLC column life
- Decrease the maintenance time for injector systems
- Reduce the plugging of fine bore tubing
- Lower operating pump pressures

These factors all become part of the overall efficiency affecting analytical methods. The Empore™ 96 well plate product consistently produces extracts with significantly lower concentrations of particle fines. The patented PTFE matrix successfully confines the sorbent particles while still allowing for increased particle surface area to be available for adsorptive interactions. The low bed volume of this membrane-based, disk product allows for very low elution volumes to be utilized, enhancing both method throughput and sensitivity.

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An Evaluation of Particle Fines Present in Solid Phase Extracted Eluates From 96-Well Plates

Abstract

Particle fines appearing in eluates following solid phase extraction (SPE) can be a major factor influencing HPLC instrumentation down time. High-throughput bioanalysis relies on the use of 96-well plates and automation to advance the number of samples processed in the shortest amount of time. When loose particles are introduced into the HPLC systems via the eluates, these particles can lead to several problems, including plugging of fine bore tubing, scoring on the stator surfaces of injector systems and increased HPLC system backpressures. Any of these can lead to an increase in equipment maintenance, downtime and costs.

Two different commercially available "packed particle bed" 96-well plate products and a commercially available 96 well-plate "disk" product were measured against the CDS Analytical, LLC™ Empore™ 96-Well Extraction Disk Plate to determine the amount of particle fines produced in the eluate. All products tested were silica-based and subjected to identical laboratory conditions. An independent laboratory performedall data collection, data processing and image capture.

Introduction

Processing and preparing specimens for introduction into analytical separation systems can comprise a higher percentage of the analyst's available time today than any other single step in the analytical process. The three primary objectives of sample preparation remain constant:

1) Provide the sample component of interest in a suitable solution for the separation technique selected.
2) Provide the sample component of interest in solution free from interfering substances intrinsic to the matrix.
3) Selectively concentrate the component of interest to enhance detection or measurement.

In order to ensure that the extracts prepared are of the best quality to meet these specific goals, many scientists are turning to Solid Phase Extractions (SPE). This technique is based on the selective partitioning of one or more components of interest between two phases, one of which is a solid sorbent.

Reversed-phase sorbents are commonly employed in separation techniques where samples containing high aqueous content are submitted for analysis of drug and drug metabolites. Reversed-phase sorbents typically have chemically bonded

functional groups attached to silica particles. The majority of SPE extractions today are completed on silica based products using selective bonded phases.

Particle fines, residual silica particles left over from the manufacturing and derivitization process when making bonded phases, are significant because they impact the surface area of the SPE devices. Typically, the larger the surface area available, the higher the typical recoveries will be. These same silica fines may create problems when found in final eluates injected into chromatographic systems. These silica particles appear as fines, small particles not retained by polyethylene or polypropylene frits employed in the manufacturing process of some SPE devices, and can be a major factor adversely affecting HPLC systems.

Fines may also be found in SPE products, which do not rely on size exclusion frits to retain the silica based reverse-phase solid support sorbents. Disk technologies may be effective in reducing the number of fines contained in eluates depending on the process used by the manufacturer.

The proprietary process employed in the CDS Analytical, LLC™ Empore™ membranes successfully reduces the number of overall fines found in eluates, while retaining the advan- tage of low elution volumes. These thin membranes contain chromatographic par-ticles that are immobilized within an inert matrix of polytetrafluoroethylene (90% sorbent: 10% PTFE, by weight). The PTFE fibrils lock the particles in the mem- brane, effectively reducing the number of particulate fines found in eluates. The result is a clean eluate ready for injection on to chromatographic systems.

This poster will compare the concentration of fines found in different commercially available silica-based SPE 96-Well Plates. Two “packed particle bed” 96-well plate products, and a 96-well plate “disk” product will be compared with the CDS Analytical, LLC Empore High Performance Extraction Disk Plate.

Experimental

Materials:
ChromAR HPLC grade Methanol Lot # H080 KXKV (Mallinckrodt)
Sample A - 15mg C18 96-Well Disk Plate
Sample B - 10mg C18 96-Well Plate (packed bed)
Sample C - 25mg C18 96-Well Plate (packed bed)
CDS Analytical, LLC Empore High Performance C18SD Extraction Disk Plate Experimental Blank – External “blind” solution sent for Microscopy

Typical SPE Method

The guidelines for each SPE product was examined and the following method developed in accordance with the manufacturer’s recommendations. All test products were used in the same manner, used the same lot number of solvent, and identical collection device. All collection trays and glass vials used for sample handling and transport were rinsed with 18.2 Megohm.cm Millipore™ deionized water and lot specific HPLC grade methanol prior to use.

The 96 well eluates were collected in a collection tray precleaned with methanol. These combined eluates were poured directly into a solvent-cleaned glass vial for transport. The collection tray was then rinsed with 5.0 mL methanol and this additional methanol was added to the collection vial. A second 5.0 mL rinse of methanol was added to the collection tray. The collection tray was then placed in a sonic bath for 15 seconds to dislodge any residual particles and transferred to

Discussion

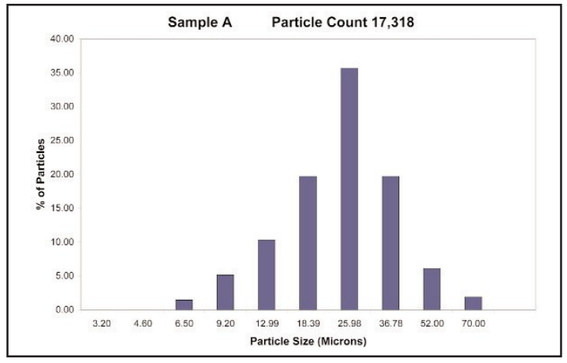
Particle “fines” remain an important aspect of SPE. They affect the number of theoretical plates available within SPE devices, which in turn affect flow rates, recoveries, capacity and the elution volume necessary for the final extract. The number of theoretical plates of separation for an SPE device is relatively low compared to typical HPLC columns. However, the number of theoretical plates available in SPE devices is greatly affected by the amount of residual fines. The number of theoretical plates will increase by the square root of 2 (1.4 times) for every halving of particle size.

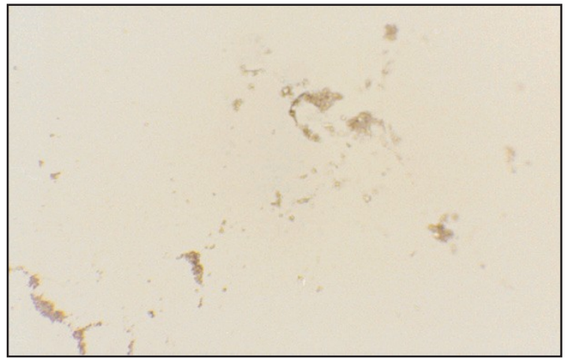
The number and distribution of particle fines present in the eluates tested correlated well with the visual observations recorded in Table A. A slight to moderate haze visible to the eye in the eluates coordinated well with the actual recorded elevated particle counts. For the packed particle bed products tested, the number of particle fines increased as the size of the particles decreased. This may be due to the selective sized frits used in the packed bed products. However, if smaller frits were used to reduce the fines present, then the effective flow rate through the plate could be compromised. Particle sizes for SPE devices are typically from 40 to 80 microns in diameter. This particle size distribution within packed particle bed products creates areas where channeling of sample flow can occur.

Both disk-based products tested had lower particle counts. Disk-based products rely on increased surface area as well as their ability to eliminate channeling to obtain high recoveries while retaining acceptable capacities. The disk-based product’s increased available surface area results in good mass transfer characteristics. This allows disk-based products to achieve high recoveries using less particle mass. The lower the particle mass required for capturing analytes of interest, the lower the amount of elution solvent required. Therefore, disk based products can offer lower elution volumes while still retaining high recoveries.

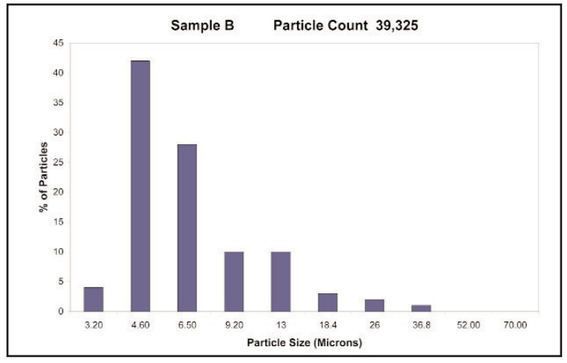
Particle fines appearing in eluates can be a major factor influencing instrument downtime. The cleanliness of the extract is an important consideration affecting the throughput achievable for a given method. When fines are visible to the unaided eye, our investigation provides you with a guide for estimating the number of fines present in your eluate. Failure to adequately remove these visible fines can lead to increased HPLC system backpressures, possible scoring on the stator surfaces of the injector rotor, as well as plugging of HPLC columns and fine bore tubing. The injector stator surface scoring leads to leaks that change the delivered pump pressures, causing changes in retention times and increased need for injector system maintenance. Fines that migrate to the HPLC column will cause a gradual increase in column backpressures with compromised column performance.

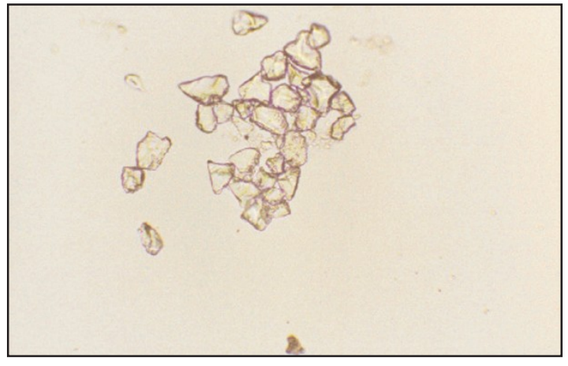
Removal of visible fines requires additional method steps usually involving additional filtration or centrifugation. Since both of these steps involve additional time and expense, initial selection of the SPE device becomes more significant. Selection of the optimal product format considering the inherent potential for fines to be present in the final elution extract may play a significant role in the achievable method throughput and your overall instrument performance.





Sample A





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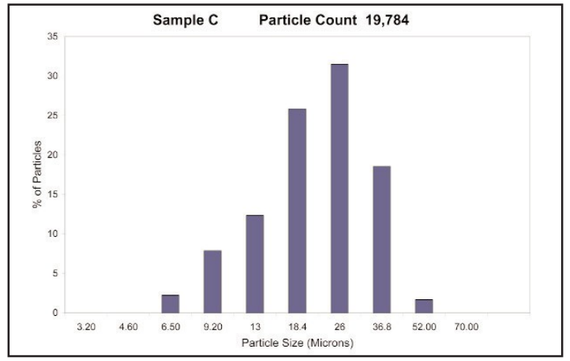
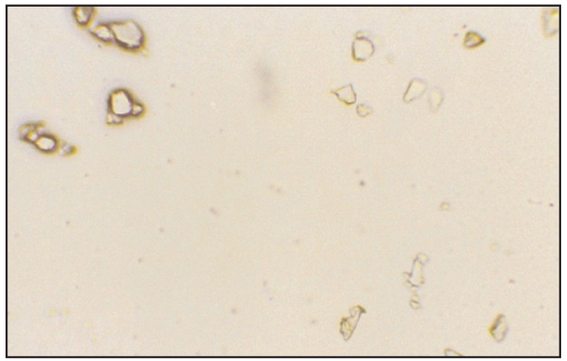
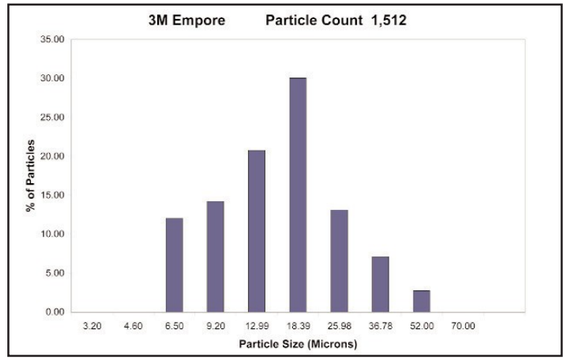




Sample C



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Results

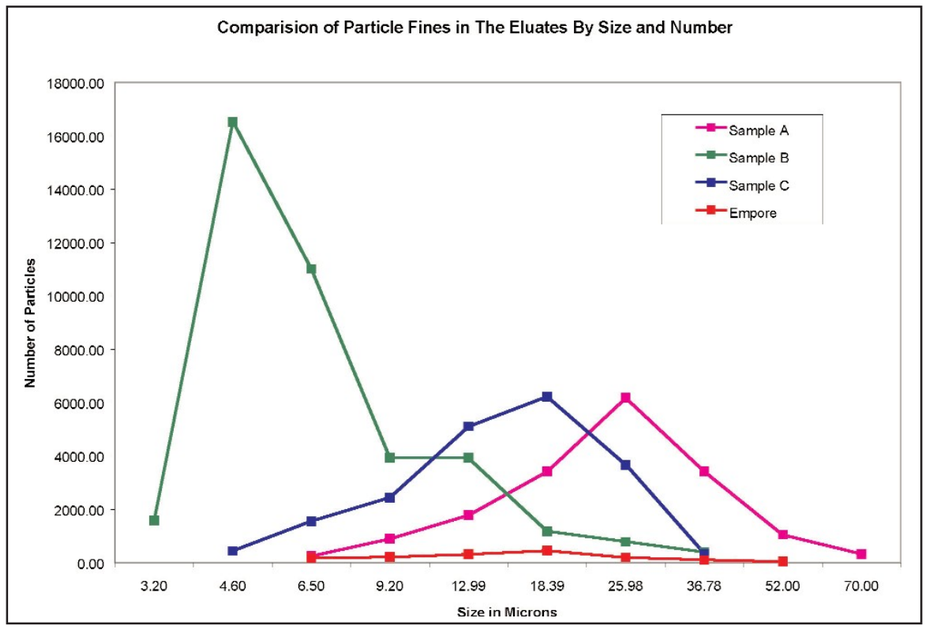
Table A is a summary of the eluates collected from the SPE 96-well plate products evaluated. Each summary was based on multiple product lots available. Three plates were used for each product tested and all data included in this table was derived from an average of the three plates tested.

	Visual	Average % Absorbance @ 550nm	Total Fines / Eluate
Blank	Very Clear	0 %	None Detected
Sample A	Sl to Mod Haze	0.15%	17,318
Sample B	Definite Haze	1.5%	39,325
Sample C	Moderate Haze	0.45%	19,784
Empore™	Clear	0.05%	1,512

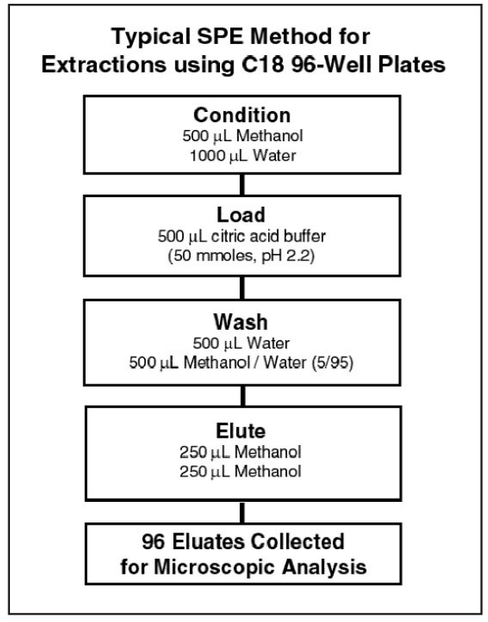
Table A

The previous graphs display the particle distribution in the final eluate as identified microscopically. Please note the number of particles is referenced here in the title bar for each sample and these graphs represent particle distribution only. The particle distribution was completed on the initial plate submitted for evaluation.

The following graph is a summary of the distributions and amount of particle fines for all tested samples.



Graph 5



the transport vial. All particle counts have been corrected to reflect the actual concentration present in the original eluate volume. The glass vial was then sealed and labeled for transport to the Microscopy Laboratory.

Microscopy

The microscopic technique for both qualitative (imaging & capture) and quantitative (counting & sizing) was transmitted bright field illumination. A certified microscopist in an independent laboratory performed all data collection and analysis. Qualitative analysis included visual inspection by the unaided eye. The samples were ranked according to amount of particulates seen and optical flow cell absorbance readings from the Shimadzu SA-CP4 particle size analyzer. Image capture was completed using a Cannon AE1 camera with an Olympus BH2 microscope. The photomicrographs were captured using transmitted bright field illumination with a magnification of 100X.

Quantitative

Particle Sizes and Distribution

Vertically drop shaking the vial several times re-suspended the particles and mixed each sample. A full sample was filtered through a 0.45 um nylon filter under low (~ 8 in Hg) vacuum. The vial and filter were rinsed with Methanol from a Teflon™ squeeze bottle. Each filter was transferred to an Analyte filter holder (Millipore™) and covered. The filters were dried and counted using transmitted bright field illumination.

Particle Concentrations

Total particle counts by volume were obtained by mixing the vial and pipetting (Finn pipette) 6.6 ML onto a KOVA Glasstic slide (10 counting grids per slide) and the counts were performed in quadruplicate. Representative photomicrographs were captured for each product tested.