

Enhancing solid-phase disk extraction performance with design of experiments

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Solid-phase extraction (SPE) disks created using designed experiments are prepared by blending polytetrafluoroethylene (PTFE, generic for Teflon™ [DuPont, Wilmington, DE]) with particle adsorbents to make thin membranes from which the disks are produced. Ranging in size from 6 to 90 mm in diameter, the disks are used to capture everything from pesticides in drinking water to drugs in biological samples.

Several months ago, various PTFE membranes were reformulated to make faster-flowing disk products that would be less prone to plugging. SPE disks that plug during extractions can result in incom-

plete or inconsistent analyte recovery, costing laboratories valuable time especially if an entire analysis procedure must be repeated. mentation method was needed to rapidly evaluate the effect of more than one process variable at a time. Design-Expert® software (Stat-Ease, Inc., Minneapolis, MN) was used because of its straightforward capability to design a multi-variable experimental protocol.

SPE disks

Empore SPE disks are formulated using PTFE and various solid-phase sorbent particles, such as C18-derivatized silica or resin-based sorbents. The chemistry of PTFE disks is similar to conventional SPE cartridges manufactured by other companies using loose

adsorb analytes more efficiently. Smaller bed volumes are achieved, meaning less solvent is required for processing, resulting in more concentrated samples. Advantages include: 1) reduced solvent and elution volumes, 2) elimination of a solvent evaporation step, 3) higher sample throughput, 4) improved recovery efficiency, and 5) eliminated channeling effects.

96-well plates

Empore extraction disks are an important component of the company's 96-well plates. In this application, disks are punched from a large membrane and placed in an array of 96 wells, forming what is known as a 96-well plate. These plates are used in hospitals, pharmaceutical companies, and bioanalytical laboratories to analyze blood and urine specimens, conduct drug screens, or perform drug metabolism studies. An individual well in a plate of 96 is one analysis, meaning 96 specific samples can be prepared per plate (*Figure 1*).

A standard plate of 96 wells measures approx. 5 in. × by 3 in., with each well holding up to 1.0 mL of solution. The bottom of each well contains a prefilter and an Empore extraction disk. Small retaining rings secure each disk in place at the bottom of every well. Customers fill the plates manually with micropipets or insert them into robotic machines that prepare and extract the plates according to preset software routines. Typically, robotic instruments place a small amount of sample into each of the 96 wells, then

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The 3M™ Company (St. Paul, MN) had previously developed a successful Empore™ extraction disk with a process patented in 1989, but the company saw the need for an improvement to increase customer satisfaction. Both new and established customers wanted a durable disk to rapidly extract analytes without the problems often associated with competitive SPE products. The goal in improving Empore technology was to redesign the disk to make a strong, fast-flowing product capable of consistently yielding high recovery rates.

Because of the large number of variables encountered in making Empore SPE products, an experi-

ment method was needed to rapidly evaluate the effect of more than one process variable at a time. Design-Expert® software (Stat-Ease, Inc., Minneapolis, MN) was used because of its straightforward capability to design a multi-variable experimental protocol. particles, but there are differences. The PTFE tightly enmeshes the small sorbent particles, providing high uniformity in the particle bed and eliminating channeling effects that may occur when loose-packed particles are used in cartridges and plates. With this design, the disks

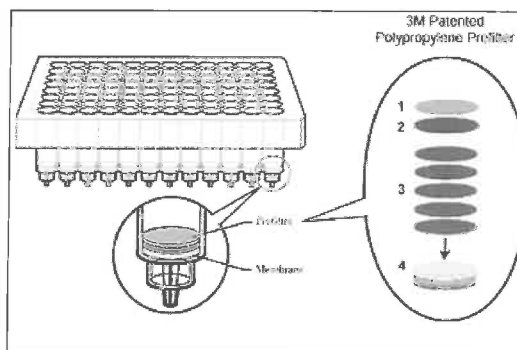


Figure 1 An improved 96-well extraction plate patented by 3M provides high analyte recovery while maintaining excellent flow characteristics.

apply a vacuum that draws liquid through the disk. As the drug of interest is drawn through each well, it is captured by the Empore membrane. In a subsequent step, solvent is added to the well to remove or elute the drug analyte from the membrane. The eluted material is placed into a collection plate (also 96 wells) before being injected into HPLC or LC-MS instrumentation for analysis.

Optimizing design of experiments (DOE)

DOE is an effective statistical method that applies systematic experimentation to all factors or variables simultaneously. It provides information about how factors interact in a way that one-factor-at-a-time (OFAT) cannot determine. OFAT's major downfall is that it holds all factors constant while testing only one at a time. This technique is an artifact from the days of Thomas Edison, who used the method to conduct thousands of single-variable tests while developing the light bulb.

The solution for an optimized extraction disk formula required analyzing how process variables or factors were interacting, an ideal scenario for DOE. The method's sophisticated statistical technique shows how interconnected factors respond over a wide range without the necessity to test all possible values directly.

Using Design-Expert, 3M conducted a series of reformulation experiments. In this effort, experimenters studied different Empore disk formulations for membranes

of various thickness. The project determined the effects of formulation changes on the membrane by analyzing various responses such as flow rate and other responses. Four formulation factors were studied including: 1) organic solvent amount, 2) particle binding materials, 3) dough solids or water added, and 4) number of mill passes of Empore predisk dough through a proprietary mill process.

A design-building matrix within the Design-Expert software provided assistance in choosing the number of experiments to conduct (Figure 2).

After reviewing several experimental options, a four-factor, fractional-factorial, two-level experiment with one center point was chosen as the best approach. (A center point is an experimental run with all numerical factor levels set at the midpoint of their high and low settings.) A partial factorial design protocol yielded eight formulas plus one center-point formula. This was found to be a desirable number of experiments, considering the number of analytical tests that would subsequently have to be conducted on each membrane. Such tests were required in order to evaluate the effect of various formula changes generated by the software.

Two-level (high and low) testing used in DOE is highly effective. A powerful statistical engine within Design-Expert software fits response data to mathematical equations. The resulting equations serve as models that predict what will happen for any given combination of values. The DOE soft-

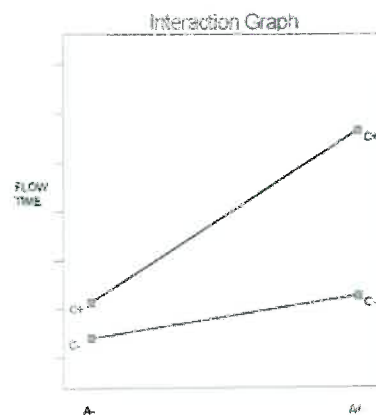


Figure 3 Factor A and C interaction affects Empore disk product flow time.

ware uncovered the Empore formula's most dominant factor interactions and identified the variables that had the greatest effect on disk performance (Figure 3).

Conclusion

Designed experiments software is a powerful tool when several variables are to be studied at one time. The results of this study yielded new Empore formulas that provide faster flow rates with no membrane plugging problems while maintaining high analyte recovery. These benefits were obtained while maximizing physical disk strength and the integrity needed in a finished consumer product. High-performance membranes mean that customers can use Empore products with the assurance that they will provide the best possible performance in all applications. With the statistical assistance of Design-Expert software, the improved extraction disk now meets the needs of researchers worldwide with its faster-flowing, high analyte recovery capabilities.

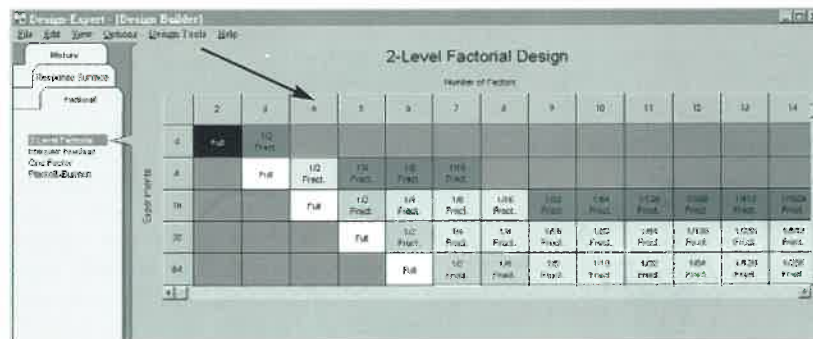


Figure 2 The design builder helped researchers set up a 2^{4-1} fractional-factorial design (see arrow). Color coding similar to a stoplight represents safe (green), cautious (yellow), and risky (red) design options. This 2^{4-1} DOE required careful attention (yellow). However, with half as many experiments (8) to be performed as a full factorial (16), monetary and time savings were substantial, justifying the decision.

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