Compound libraries – cost of ownership

The value of high quality storage and processing of small molecule libraries has recently received increased interest due to a better understanding of the physical properties of DMSO. Automated equipment can now provide storage and processing fully contained in an inert environment that greatly extends the sample lifetimes. A cost analysis based on a review of purchasing and operating decisions for various automated sample storage and processing solutions demonstrates a wide range of possible returns on investment.

By Dr W. Steven Fillers

Multiple freeze/thaw cycles do not lead to compound degradation or precipitation[1,2]. That is the conclusion from an increasing number of recent studies of small molecules stored in DMSO in which the samples contained low water content[3,4]. The implications of these results are large because much of the effort in compound library management has been oriented towards minimising the number of freeze/thaw cycles. The desire for storing large numbers of one-time-use replicates has created a market for expensive consumables and very large storage facilities. In many cases the automation requirements for specific consumables has driven requirements for massive reformatting of libraries and radical changes in laboratory procedures. While change is hopefully for the best, there are always costs associated with time lost during the transition due to retraining and inventory shifts. In severe cases, there may be costs related to decommissioning and removal of outmoded equipment and/or the construction of additional facilities.

The reasoning behind decisions to allocate these expenditures almost exclusively points back to a single physical limitation in the choice of the storage solvent. DMSO is very hygroscopic. So hygroscopic that exposure to the average atmosphere in a laboratory for just a few hours will result in significant absorption of water into the sample. The detrimental effects of water in the samples has received considerable public discussion[5-7] and will not be detailed here, however, the primary negative effect, being a change in sample solubility exacerbated by freeze/thaw transitions[8], has been generally accepted. Thus, in order to avoid sample losses associated with precipitation, several methods for sample storage have developed. Implicit in the methods is an acceptance of water accumulation in the DMSO during the lifetime of the sample. This decision, based on current processing requirements, is so strong that some researchers add water to their DMSO in order to avoid future shifts in solubility. The decision is based on an understanding of the limitations of changing the process steps that allow water to be absorbed into the DMSO.

DMSO will absorb water up to about 30% by weight in laboratory atmospheres maintained at 50% relative humidity at 20°C. The function is hyperbolic with the first 10% increase occurring in as little as six hours[9]. Reviewing established process steps for sample handling and attempting to minimise contact with the atmosphere is not a trivial task. In some organisations, it is a moot case because the samples have gained water during solubilisation, distribution and shipment to the final recipient. Additionally, there are rarely any quality control demands related to water content made on incoming samples. Many screening methods involve hours of sample exposure during each processing event such that, after just a few screens, the samples have reached maximum water content. There are laboratories that are fortunate to be located in regions of low relative humidity. Unfortunately, there are as many or more groups that operate in high humidity environments especially during the summer months.

Today, controlling the process environment is more important than making improvements to the storage environment because more compromise to the integrity of the sample occurs during processing than occurs during typical storage. That said, there are important issues concerning storage conditions that bear consideration. The primary material employed for storing DMSO solubilised samples is polypropylene. Polypropylene is very resistant to DMSO and it can be molded into a wide variety of container geometries. It can also be utilised to manufacture films and container caps for sealing purposes. Polypropylene is permeable to most gasses and to water[10], a property that diminishes the effectiveness of argon purges and one that can lead to eventual accumulations of water and oxygen in the sealed sample. Permeability is directly proportional to storage temperature and inversely proportional to container/seal thickness. Management of this property during long-term storage requires inert atmosphere surrounding the container. Low humidity storage is effective in minimising water uptake but does not limit oxygen transport. Chemical compounds that have been dried down into films on the bottoms of polypropylene containers may be especially susceptible to reactions due to their large surface area and high concentration. Effective management of long term storage and processing of samples solubilised in DMSO requires minimising the water and oxygen content during the entire lifetime of the sample.

Colder is better. Chemical reactions occur at slower rates as the temperature is lowered. The desire of many researchers to avoid freezing has prompted them to store at +20°C because the freezing point of DMSO is +18°C. As stated earlier, DMSO is difficult to maintain in a completely anhydrous state. There is a marked freezing point depression upon addition of water to DMSO[11]. At 20% water, DMSO will freeze at -30°C. That means that samples could be stored at -20°C and remain in a liquid state, however the reduced temperature would limit chemical reactions much better than storing at +20°C. And, as mentioned above, gas permeability of polypropylene is lower at reduced temperatures. The samples in the solution may add to the reduction in freezing point. Liquid samples have been observed in 50%DMSO/50% water at -80°C. A process limitation might be that the installed storage management automation might not operate effectively at reduced temperatures.

Researchers have noted precipitation in DMSO of samples that were freeze/thawed[12]. It is reasonable to assume that these samples had 10% or greater water content because they were repeatedly exposed to laboratory conditions. Precipitation in samples containing 6% or less water has not been observed in multiple freeze/thaw cycles[1-3]. The solubility of each chemical compound in DMSO and water will determine the performance of a given library but, in general, samples soluble at room temperature in pure DMSO at 10mM or lower will not precipitate on multiple freeze/thaw cycles.

Table 1
Storage system costs

CREATION
- Capital
- Floor space
- Construction/Renovation
- Integration
- Process change
- Install downtime
- Training

MAINTENANCE
- Access/FTE support
- Consumables
- Sample loss
- Service

EXPANSION
- Capital
- Floor space
- Construction/Renovation
- Decommission
- Install downtime

The question becomes one of a decision between the cost of a process that can maintain low water content over the lifetime of a sample verses the cost of processes that manage higher water content of the samples.

Sample storage is expensive. Construction of a storage area is a capital cost. Allocating high value laboratory space to create a storage facility represents a loss of alternate productive activities. Although each square foot of laboratory space has a high intrinsic cost, the immediate user of the space often does not need to account for the expenditures associated with the creation of the space. This cost is often hidden from the consideration of the overall cost of a system especially when the facility is created within existing space. Construction of external buildings has high visibility with attendant fiscal responsibility. Installing new automation equipment can cause down time for other laboratory activities. The larger the storage project the larger the disruption in laboratory activities. Very large projects often adversely affect more than one laboratory and can have significant impact on an entire site.

More storage costs more money. Management approaches that rely on storing many copies of a sample will have increased costs over those that store a few or only one copy. If the requirement for a large facility is envisioned for the future, capital outlay will occur immediately upon construction of space that will not be fully utilised until the future is realised. If the requirement was overly optimistic, the surplus space represents a loss of capital and space resources. Another hidden cost of a storage system is the cost of expansion. A large storage system cannot be decommissioned until a newer, larger space is functioning. That translates into needing more than double the space of the original facility to create the next generation. Modular expansion of storage facilities obviate this conundrum. In addition, modular storage units may be purchased as requirements for additional needs are realised and surplus space is minimal. Modular storage units inherently offer increased redundancy. Redundancy may be consid-

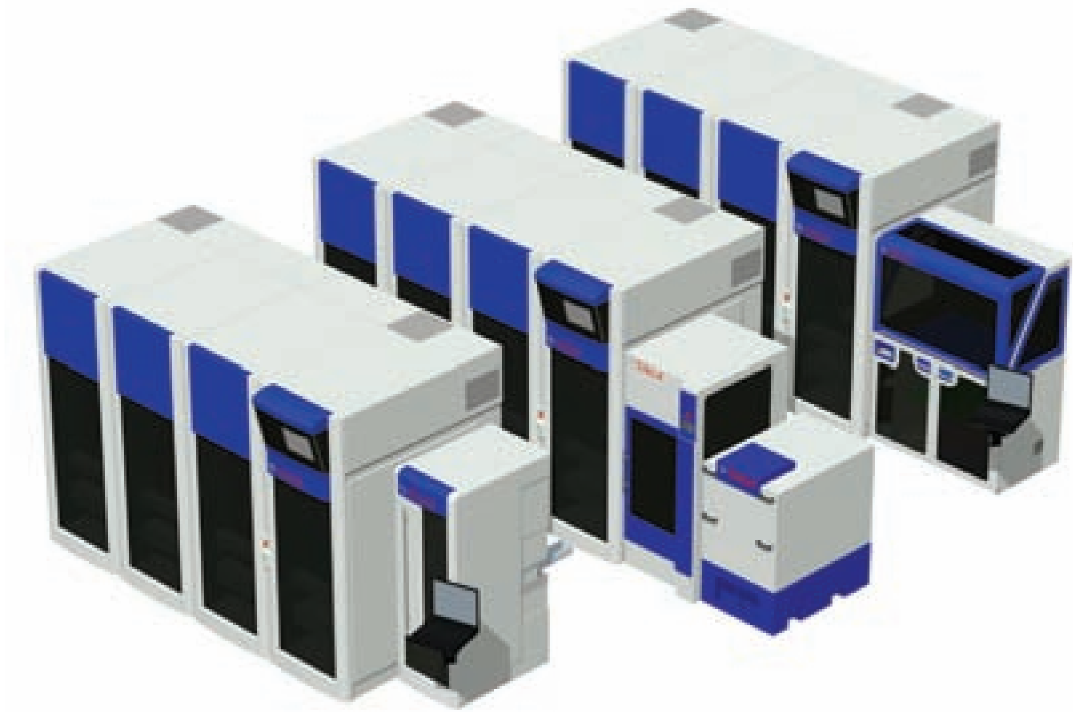

Figure 1
Multiple modules may be assembled to store and process microplates and/or microtubes. The connected modules share storage samples via internal exchange track and tracking data is shared between modules via imbedded LAN. Inert atmosphere can be maintained throughout all sample storage and processing operations. The system is expandable to meet requirements as they are realised. Total storage system costs are minimised by on-demand sample replication, expanded user access and simplified installation

ered a cost if one never needs it and may be considered a prudent expense if it is utilised.

Storing large numbers of one-time-use samples requires large purchases of consumables. Cherry-picking libraries are especially lucrative in this regard. The requirements for creating unknown subsets from a library includes the long-term storage of copies of compounds that are never picked. Unpicked samples expire in storage and the spaces that result from the samples that are picked continuously decrease the density of the storage facility unless defragmentation housekeeping is continuously performed. Cherry-picking and on-demand replication from a single source container represents the highest form of efficiency in storage space utilisation.

Integration of a storage system with the downstream sample automation hardware and the corporate data software often becomes the most costly and the final hurdle of upgrading a storage process. It is difficult for all requirements to be realised before the integration begins so that some discovery occurs during the construction of the new system. Integration costs increase as the number and source of the instrumentation units increases. Lack of both communications and operations standards leads to the creation of a multitude of issues to be resolved during this phase. Of critical importance is assigning the responsibility for the continued operations of the integrated equipment. Careful contractual agreements should be put into place before, during and after the completion of an integration project. Costs associated with tracking down the party responsible for resolving a warranty problem are considerable when viewed as lost processing time. Limiting the number of vendors providing hardware and software to a project will minimise the integration time and capital costs. A single vendor solution for a fully integrated system will eliminate all of the costs associated with attempting to join disparate hardware and software.

Limited access to the library is a cost to an organisation. Any intermediate processes between the end user and the library represent an organisational cost. The organisational cost is usually offset by the reduction in risks associated with not allowing the end user to have direct access to the library. Risks of misfiling and mishandling samples is usually avoided with automated systems, however the replication process often requires manual intervention and even well-trained technicians make mistakes. The cost of each resource required to support the delivery of samples to the end users can be partial or complete justification for adopting automation. Fully integrated processing that enables the end user to direct the automation to create a copy of the required samples minimises risks and lowers the costs of the support structure. Access to automated ordering can be regulated by user access and priority codes.

References

1 Cheng, X, et al (2003). Studies on repository compound stability in DMSO under various conditions. J. Biol. Screening 8:293-304.

2 Heaton, Z, et al (2001). The effect of freeze thawing and storage time on degradation of compounds at 4°C under humidity control using experimental design. SBS 7th Annual Conference and Exhibition p188.

3 Semin, D (2004). A multidimensional approach towards optimizing collection stewardship. Comments made during presentation at MipTec 2004.

4 Oldenberg, K (2004). Compound dissolution in 96/384-well plates using high throughput sonication. Comments made during presentation at ALALabFusion2004.

5 Lipinski, CA (2002). Storage of samples in DMSO: issues and potential solutions. Presented at SBS 8th Annual Conference and Exhibition, Sample Management Special Interest Group.

Continued on page 90

Continued from page 89

6 Yaskanin, D (2003). Quality perspectives for compound storage. Presented at LRIG-mid-Atlantic, January 2003.
7 Connelly, JA (2004). Stability of compounds stored in TekCel Plate Management System: Application of new approaches for ultra-high-throughput analysis of compound stability. Presentation at LRIG-mid-Atlantic February 2004.
8 Lipinski, C (2004). Compound solubility and HTS screening. Presentation at Miptec 2004.
9 Fillers, WS (2002). The problem is the process: Sample preparation vs. sample integrity. Presentation at ISLAR2002.
10 Cooksey, K, et al (1999). Predicting permeability and transmission rate for multilayer materials. Foodtechnology 53: 60-63.
11 Rasmussen, DH, MacKenzie, AP (1968). Nature 220, 1315.
12 Kozikowski, BA, et al (2003). The effect of freeze/thaw cycles on the stability of compounds stored in DMSO. J. Biol. Screening 8: 210-215.

A single aliquot of a compound that could be stored and sampled infinite times without any degradation or precipitation represents the lowest cost storage scenario. It can be approached by creating an inert environment for all storage and processing steps and by storing at temperatures that freeze low water content samples. Modular construction of small processing units minimises construction costs, laboratory downtime during integration and maximises the value of capital expenditures. Direct access to an automated replication system by end users removes processing overhead costs and provides the most responsive mechanism for delivering samples to the customers.

A fully integrated system that includes managing the storage and processing of microtubes and microplates is already being used by a wide variety of companies and is depicted in **Figure 1**. Any storage modules may be scaled to meet the current needs of the customer. Each module fits through standard laboratory doorways and easily connects to the adjacent modules. Storage sample containers and data are communicated between the modules. Samples may be reformatted from microtubes to microplates for storage or processed directly into assay plates. The system is capable of maintaining inert atmosphere throughout all modules and the storage units may be regulated down to -20°C. End users may send request lists of barcode IDs through e-mail to the storage system requesting replications in a specified container format and schedule the delivery for a specific time. User permission levels for access and processing priority override may be granted. This single-vendor, fully integrated solution minimises many of the costs associated with the creation, maintenance and expansion of sample storage processing facilities. DDW

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ADVERTISEMENT INDEX