AUTOMATED DROPWISE PEPTIDE CYCLIZATION USING A GX-281 LIQUID HANDLER WITH ORBITAL SHAKER

TECHNICAL NOTE TN233

TECHNICAL FEATURES

- Dual orbital shakers in left and right orientations
- Corrosion resistant probe

TECHNICAL BENEFITS

- Allows 'workable' dropwise reactions for the busy laboratory
- Improves yield and quality of the peptides

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INTRODUCTION

In the drive to obtain better and more suitable drug candidates, peptides have come to the forefront of the drug discovery world over the last decade. Peptide-based drugs can inhibit protein-protein interactions in the body more effectively than small molecules due to their larger size. This allows the drug to be more specific in its target use, often leading to fewer side effects or unwanted consequences.

Early peptide drugs were based on biologically isolated natural hormones such as Insulin in the early 20th century, followed by the further discovery of hormone-based peptides that had therapeutic uses throughout the 20th century and the development of the first synthetically made hormone-based peptides, such as Oxytocin.

Peptide drugs have been used in a wide range of therapeutic areas, such as urology, respiratory, pain, oncology, metabolic, cardiovascular, and antimicrobial applications.

With the turn of the 21st century and advances in peptide chemistry, thanks to new production, modification, and analytical technologies, it has been possible to create more customized peptide drugs that are no longer simply hormone mimics or composed only of natural amino acids.

With the ability to apply design to the creation of peptide therapeutics, the focus then turned to cyclic peptides. The rigidity of cyclic peptides increases binding affinity and selectivity toward target molecules, improved hydrolysis resistance, and potentially better membrane permeability. Being able to design a cyclic peptide also offers the ability to determine the linkage method, be it end-to-end, end-to-sidechain or sidechain-to-sidechain, offering further improved scope and potential (Figure 1).

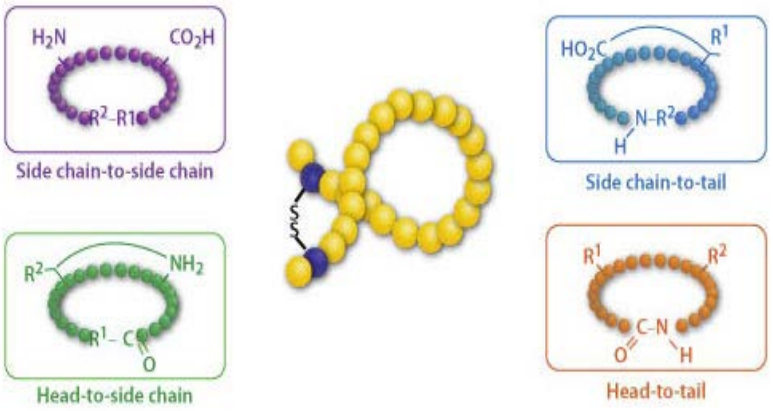

Figure 1

Structural example of peptide cyclization (Image from www.biosyn.com)

In the laboratory, peptides are generally synthesized, purified, and then cyclized, and finally repurified as necessary. Purification of peptides is done by means of preparative HPLC, using a system such as a Gilson VERITY® HPLC Purification System, which allows for fully automated purification of multiple peptides at once. But often, the cyclization step is done by hand. This manual cyclization step is time-consuming and can easily become the rate-limiting step of the whole workflow leading to delays in peptide production.

Manual cyclization can also expose staff to some particularly nasty chemicals which are often used in cyclization procedures. Additionally, completing this step manually doesn't allow for the same level of optimization that an automated process can offer. Yield and final purity of the product at this stage is often improved by drop-wise addition of the reagents with continuous swirling or mixing, but this is something that is often foregone in a manual process in favor of getting the job done quicker.

Figure 2

Gilson GX-281 Liquid Handler

Automating a drop-wise cyclization process using a liquid handler with a large rack capacity and incorporating automatic orbital shaking is an ideal solution to this workflow bottleneck. The Gilson GX-281 Liquid Handler (Figure 2), with the addition of the Orbital Shaker module, is perfect for this use, as demonstrated in this Technical Note.

MATERIALS AND METHODS

The drop-wise cyclization of peptides was performed using the following automated liquid handling system.

GX-281 Liquid Handler:

The Gilson GX-281 is a single-channel liquid handler with a metal washable probe. It is based on a liquid-filled fluidic system with system wash solutions delivered by either a traditional syringe pump or by the unique Gilson solvent system pump. The solvent system pump is a continuous flow pump that allows for an unlimited delivery range of solvent volume, while maintaining suitable accuracy for typical laboratory-scale liquid handling procedures. The continuous flow nature of the pump allows for increased rinsing speeds, subsequently reducing the overall sample processing time.

The GX-281 Liquid Handler was chosen from the GX range of liquid handlers because of its greater bed size. This allows for larger racks to be fitted and a greater quantity of samples to be processed in a single run.

A Gilson GX-281 Liquid Handler was used for this application with the prep solvent system, 175 mm rinse stations, and a 1.5 mm OD non-septum piercing probe.

Orbital Shaker:

For the cyclization reaction to proceed successfully, there needs to be sufficient mixing of the cyclization reagent with the purified peptide while the reagent is being delivered. The Gilson solution to this in the automated system is to use an Orbital Shaker module. Depending on the capacity requirements of the laboratory purchasing the system, this can be purchased as either a single rack version or a dual rack version. The orbital shaker can also be purchased with a left-handed motion or a right-handed motion for installation to the left or right side of the liquid handler, respectively.

Racks:

The GX-281 system allows for a large variety of racks which in turn can hold a huge range of different tubes, vials, bottles, and microplates. In a typical peptide synthesis laboratory, peptides are collected from a purification system into graduated 50 mL centrifuge tubes which can be held in a Gilson Code 222 rack. Using two dual Orbital Shakers, four of these racks can be placed onto the system, providing a system capacity of 108 peptides per run.

The two remaining rack positions on the GX-281 can then be used for reagents and for analysis vials if the resulting cyclized peptides require subsequent analysis. An example tray layout for the GX-281 can be seen in Figure 3. The transfer of a small sample of the cyclized peptides to these analysis vials from the centrifuge tubes can be programmed as a separate additional method or included as a final function of one continuous method, as required. In the example configuration referred to in this technical note, the analysis vials are standard 2 mL glass vials commonly used with HPLC and GC. The rack for the GX-281 that holds these vials has a capacity of 96, so a continuous method that cyclizes the peptides and then goes on to transfer samples of these to the analysis vials will be limited to a maximum run size of 96 peptides and 12 of the peptide tubes will remain empty.

Alternative racks can be used; for instance if the analysis is performed using deep well microplates, then a code 205 rack can be used and 2 x 96 well plates can be held allowing for the full 108 peptides per run to be performed with sampling to the analysis plates.

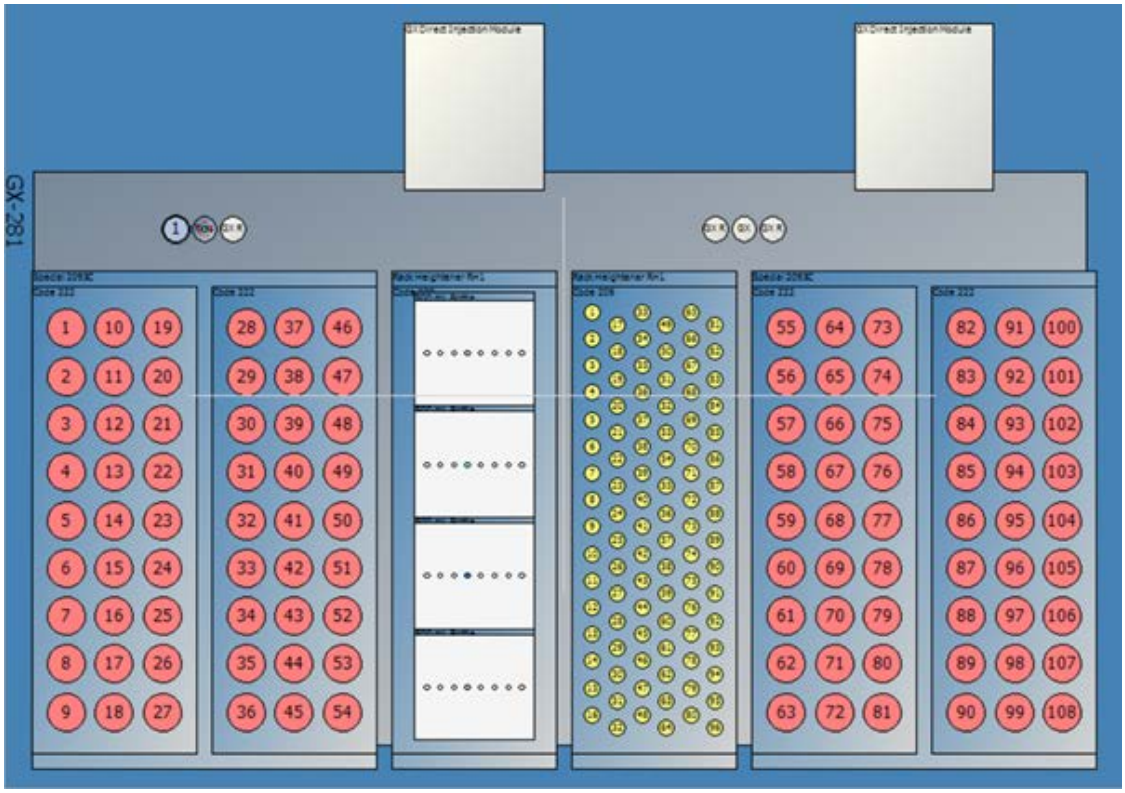

Figure 3
GX-281 Liquid Handler Tray Layout

Software:

The GX Liquid Handler was controlled using Gilson TRILUTION® LH v4 Software. The software provides full and flexible control of the Liquid Handler, Solvent System pump, and Orbital Shakers. This allows for methods to be created that control the volumes of reagents dispensed, the speed of dispensing, the shaking speed, etc.

Method:

The TRILUTION LH software allows methods to be set up using ‘drag and drop’ from a list of liquid handling ‘tasks’. The tasks are pre-written and supplied as part of the software package. Additional ‘custom’ tasks can be written to further optimize the method if desired, although this is not a common need. For the application described here, custom tasks are required for the control of the Orbital Shakers. These custom tasks are available and can be supplied upon request, by contacting techsupport@gilson.com for further details.

Using this custom task along with the other standard TRILUTION LH tasks, it is possible to write a method that will replicate how this process is done by a laboratory scientist. In addition to this, it is also possible to maximize the strengths of the automation platform and perform this process in a more iterative way with smaller additions of reagents and more regular shaking. This can lead to better yield than if the process was done in the typical manual way.

Additionally, the TRILUTION LH Software allows for the peptides to be cyclized using either batch processing or sequential processing. This allows the laboratory to fit the system into their overall workflow the way that they want and to focus on the priorities that are most desired, be it throughput, yield, or fast completion of priority samples etc.

CONCLUSION

For any peptide laboratory who are synthesizing large numbers of peptides and then going on to purify and cyclize them, the GX-281 system described in this tech note will be of great value. With the GX-281 system, it is possible to automate the cyclization process and take this work away from laboratory staff, allowing those staff more time to pursue other tasks more deserving of their focus.

It also helps improve safety in the laboratory environment as the staff are less exposed to the chemicals involved and less at risk from potential mishaps such as spillages and glassware breakages.

Finally, the cyclization process can be better optimized with the added control provided by an automated system. Offering the potential for better final product yield and more consistency from day to day and project to project.