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Author: Dr. Michael Forbush

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ATS Source Wellplates

Introduction

The ATS Acoustic Transfer System from EDC Biosystems can use a variety of different source wellplates for different applications. The wellplate is a key component for optimizing performance of the acoustic system. Some wellplates provide improved dispensing capabilities over others when used as a source plate. There are physical properties of wellplates that effect whether a plate can be considered for use as an acoustic source plate. The shape of the well bottom, the material it is made of, and the thickness of the bottom of the plate are some of the specific key properties that must be considered for acoustic dispensing.

This paper describes applications and wellplate attributes that effect these applications both positively and negatively.

General Considerations

Without any knowledge of the application the following considerations can be made:

- How much volume per each well dispensed does a user want to transfer?
- Is the sample rare and expensive?
- How will the user fill a plate?

Higher density plates such as 1536 and 3456 plates require specialized ways to fill the plate because of the low final volumes. The ATS has a large range in volume capability, however there is a limitation due to the focal distance of the acoustic beam. The diameter of the well affects the depth the well may be filled and will limit the actual volume the well may contain. For example, a 1536 wellplate may be limited to 8-10uL of total volume, but the dead volume will be a small amount. A 96 and 384 well density wellplates may be able to be filled higher in both total volume and well depth in millimeters.

High Throughput Screening

Considerations

When determining a wellplate for use in high throughput screening, a key consideration is the ability to store compounds dissolved in DMSO at low temperatures. DMSO is used because of its ability to dissolve a large number of compounds at high concentrations. This feature also means that DMSO is corrosive to some materials used to make wellplates. Low temperatures will make some plastics brittle and warp thin wall structures.

Cyclic Olefin Copolymer (COC) - Preferred material for acoustic dispensing

Two types of COC wellplates

Two Piece Wellplates

A two-piece wellplate is made by fusing a sheet of COC film to the bottom of a wellplate scaffold during the molding process. If the molding process is done in such a way to keep the bottom material taunt and the cooling does not result in warping, then the result is a very thin uniform bottom. COC plates manufactured with these control conditions are ideal for acoustic transmission.

Potential Manufacturing Control issue

Manufacturing control issues with the fusing process may result in two problems. One issue is a flexure condition of the film in each well due to the material not being taunt during the molding process. The result may be a curved surface, which may affect the focus of the acoustic beam. A random curvature of this well bottom throughout a plate will result in higher CVs. A process to limit this condition is for the manufacturer to use a slightly thicker film, however this may result in slightly higher CV’s when compared to a properly fused thin film bottom.

Single Piece Wellplates

A single-piece plate means that the entire plate is molded as one piece. This generally results in a thicker wellplate bottom, which may result in slightly higher CVs than the fused thin film bottom of two-piece plates.

The COC Advantage

Using COC plates in the ATS for high-throughput screening applications offers the combination of acoustic transparency and DMSO resistance. Acoustic transparency yields lower CVs (<5) of less than 5% *(units?)* for both accuracy and precision.

The COC Disadvantage

COC is slightly less DMSO resistant than Polypropylene. Some compounds have been shown to bind to the COC. Freezing COC plates can cause wellplate warping which may affect the COC film fused to the bottom of the plate. The thickness of the plate will become a factor if the plates are stored in a freezer. The storage and thawing of the plates will cause the plates to become warped and this curvature will effect the direction in which a drop will be ejected. In the worst case the film bottom of a two-piece plate may become separated from the main plate structure.

Recommended Plate

- **Greiner 384 COC, PN 781 801**

This Greiner plate has been tested with the Artel measurement system. It has demonstrated very low CVs over the largest volume range for any 384 COC wellplate when used as the source plate in an ATS system. The plate is a one-piece design and can hold a large volume of liquid. The plate has large well bottom diameters allowing the ATS to utilize its full focal length in depth, thus extending the usable range for these wellplates. The plate has a thin bottom thickness that allows for even more access to the liquid in the well.

High-Density Polypropylene

Polypropylene and High-density Polypropylene (HDP) are resistant to DMSO. Standard polypropylene is not ideal for acoustic dispensing because its normally spongy nature absorbs acoustic energy. HDP, on the other hand, allows a greater percentage of the acoustic energy to pass through. Some HDP wellplates can be made to work with the ATS system, however, CVs of volumes transferred from HDP plates is higher than from COC plates. This is due to less uniformity of the well bottom of HDP plates when compared to the COC plates. HDP plates have thicker well bottoms than COC plates. This is an additional contributing factor for higher CV's.

The HDP Advantage

HDP is more durable than COC when freezer storage is a consideration.

The HDP Disadvantage

Higher CV's (<10) compared to COC (<5) due some absorbed loss of acoustic energy, reduced well uniformity, and thicker well bottoms. HDP impedes acoustic energy, resulting in higher CVs in accuracy and precision than COC plates.

Recommended Plate

- **Greiner 384 high density polypropylene 781 201 - 906**

The plate is a one-piece design and can hold a large volume of liquid. The plate has large well bottom diameters allowing the ATS to utilize its full focal length in depth extending the usable range for these wellplates.

High Throughput Screening Conclusion

The ideal plate for high throughput screening will depend on the storage requirements and transfer precision. A one-piece HDP plate may be the best for storage, while a well-manufactured two-piece film bottom COC plate would result in the best CVs in volume transfer. A single piece COC plate may be a good compromise between these two extremes.

Aqueous Solutions – The Hydrophobic & Hydrophilic Effect

While high throughput screening of compounds dissolved in DMSO comprises a large portion of acoustic transfer applications, there are a large number of applications involving aqueous solutions. And, because of the polarity of water molecules, water tends to react with surfaces in two specific ways. Water reacts with a surface in a range of hydrophobic to hydrophilic behaviors. This behavior may result in making it very easy or very difficult to dispense from a given wellplate. The addition of ions in buffers, glycerol or surfactants will also affect this behavior in aqueous solutions.

Typically a more hydrophilic behavior will result in better CVs, because a hydrophilic solution will tend to pull the fluid's meniscus out evenly in all directions. This results in a flattening of the fluid surface, which results in a more consistent dispensing.

One way to improve this hydrophilic/hydrophobic nature is to improve the micro-nature of the wellplate surface. Manufacturing tiny groves in the surface of the plate by using a plasma stream, which burns these groves improves this.

One exception to this rule is the use of proteins or other biological materials. Proteins and cells may adhere to the micro-groves created by this process.

There are other techniques to limit this effect, one of which is photo-micro-grafting polyethylene glycol methacrylate (PEGMA) to a COC surface.

Plasma treated plates will improve the meniscus properties of an aqueous solution in a plate. A large volume plasma treated plate should be the best plate for these applications, however EDC has limited experience with such a plate. The main use of plasma treated plates is to grow cell cultures in the wells. Only plates that are marketed for this purpose are easily obtained from manufacturers and limits our testing.

Aqueous Solutions Recommended Plates

- **Greiner 384 Low Volume COC w/plasma treatment, p/n 788 876**

This is a low profile plasma treated COC plate improving the uniformity of the aqueous meniscus resulting in lower CVs.

- **Brooks Automation Aurora 384 Low Volume w/plasma treatment, p/n 1012-XXXXXX**

This is a plasma treated COC plate improving the uniformity of the aqueous meniscus resulting in lower CVs.

Arraying

The success of using the ATS for an arraying application is specific to the wellplate and solvent used. For all applications the ideal situation is to get the source wellplate and the target as close together as possible. Error in placement is mainly due to the fluid surface flatness. Any variation in the meniscus is translated into error in the trajectory angle. Moving the source fluid surface as close as possible to the destination surface will reduce the error in the drop placement. This may be done by using the shortest plates available and bringing the destination surface as close as possible to the source plate. Several prototypes of specialized arraying wellplates have been tried, but at this time there is no commercially available ideal arraying wellplates.

Recommended Plates

- **Brooks Automation Aurora MaKo 27830 COC, 1536 plate, w/plasma treatment**

  PN 1061–XXXXX

This plate is a plasma treated 1536 plate. The smaller well diameter yields very uniform menisci from well to well resulting in very good placement of the array droplets.

- **Greiner 384 Low Volume COC plate w/plasma treatment, p/n 788 876**

This plate is very short (8.5mm) allowing the plate to be placed close to the target destination.

Conclusion

The ATS Acoustic Transfer System from EDC Biosystems is capable of using many different micro-titer wellplates and there are advantages and disadvantages to using most of them. It is important to understand the expectations and use for each of the various plate types before determining the “best” plate for any application.