Enabling Technologies for Low Volume Dispensing

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Abstract

Robust dispensing of fluids in low nanoliter volumes is a technically demanding process requiring tight control of multiple variables. Degassing of fluids is one critical element in ensuring consistent, high quality dispensing. The BioDot helium degassing system reduces dissolved gases to negligible levels and can maintain them at those levels over the course of extended dispensing runs using the BioJet Plus dispensing system, resulting in consistent 10 nL dispense volumes.

Introduction

Robust dispensing of fluids in low nanoliter volumes is a technically demanding process requiring tight control of multiple variables. The primary system components contributing to an accurate and reproducible low volume dispense are:

i. The dispenser
ii. The environment.
iii. The fluids in the system.
iv. The substrate to be dispensed onto.
v. Programming and process design factors.
vi. Dispenser cleaning and maintenance factors.

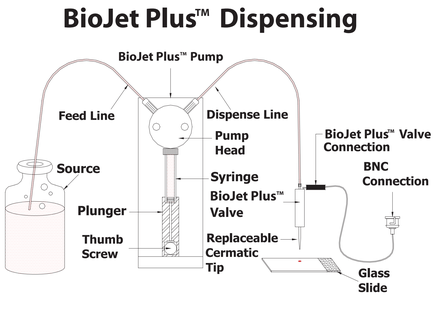
BioDot's dispensing technology exhibits a high level of reliability for small volume dispensing under the proper conditions. Key among these conditions is the removal of dissolved gases from the fluid to be dispensed and from any backing fluids used in the system.
This poster demonstrates a method for degassing reagents which results in consistent low volume dispensing.

The BioJet Plus™ Dispensing System

The BioJet Plus™ technology combines the high-resolution displacement capabilities of a syringe pump with a high-speed micro solenoid valve. This combination permits the non-contact dispensing of nanoliter volumes.
In a typical dispensing system, multiples of these syringe/solenoid channels are placed together. Two modes of liquid handling are possible: Continuous (bulk) dispensing and aspirate/dispense.

Continuous dispensing involves pulling reagent or solvent from a reservoir into the syringe and then dispensing it through the micro solenoid valve. Aspirate and dispense mode involves filling the system with a backing fluid, dipping the tip of the valve into a sample, using the syringe to aspirate the sample into the tip, and then dispensing the aspirated sample.

The BioJet Plus dispensing system is a hydraulically driven system that requires a fluid medium to be present from the syringe to the



microsolenoid BioJet Plus valve. The dispensing process involves the following steps:

1) The syringe displaces a given amount
2) The valve is opened for a short period of time (milliseconds)
3) Fluid is released from the valve and travels to the tip
4) The fluid increases its linear velocity as it passes through the tip orifice and ejects as a drop (or stream if the amount of fluid is large).

One valve actuation results in one drop.

The key to a correct volume being dispensed in a given drop from the BioJet Plus system is the steady-state pressure (SSP) in the dispensing system. This pressure has several important features:

- It is achieved by the displacement of fluid by the syringe pump
- The SSP is displacement (drop size) dependent, increasing with increasing displacement (drop size)
- The SSP is determined by the system compliance, which is dominated by entrapped air bubbles.
- Once the SSP is established, the amount of fluid displaced by the syringe pump will equal the amount dispensed

The BioJet Plus dispensing system can be modeled as an electrical circuit with the pressure acting as the voltage, the flow rate as the current, the system compliance as capacitive elements, the valve, tip, and feed lines as resistive elements, and the valve as a switch. This model shows the syringe pump as a current source, which provides an advantage over a pressure source (e.g. gas pressure) in that any changes in resistance will not affect the flow rate. In contrast a pressure source will be affected by changes in resistance in the system.

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De-aeration of the backing fluid is critical to maintain the fluid path free of any air bubbles.
The presence of any air bubbles would ruin the steady-state pressure thus leading to inaccurate dispensed volumes.

Several methods are currently employed by scientists to de-aerate their solutions, including vacuum with agitation, helium sparging, and gas filtration through membranes. The efficiency of each method has been studied and reported in numerous publications. The inert gas sparging method stands out as the most efficient method, but its cost can be prohibitive for some companies. BioDot has thus designed its own degassing procedure and equipment to lower the level of dissolved gas inside a fluid and to maintain this low level through a typical dispensing experiment.

Methods

General

A dissolved oxygen (DO) probe from TOPAC (distributor for Jenco electronics) was used to approximate the amount of air dissolved in solution. The lower detectable amount of dissolved oxygen with this probe is 0.5 ppm. All experiments were conducted on freshly de-ionized water or on freshly prepared and filtered 0.01 M PBS. The DO measurement required the atmospheric pressure at the time of the measurement and the salinity of the solution. On each measuring day, the pressure was recorded from a weather forecast web site. The salinity was set to zero for fresh de-ionized filtered water and to 8.3 ppt for fresh and filtered 0.01 M PBS solution.

The flask used for this experiment was cleaned with a KOH/H2O/EtOH basic solution, then with a diluted hydrochloric acid solution. The flask was then rinsed thoroughly with de-ionized and filtered water, and then with reagent alcohol. The flask was dried in an oven at 95 ?C for 1 hour.

A 0.01 M PBS solution was prepared by dissolving 25 mL of filtered 10X PBS solution into 225 mL of de-ionized and filtered water.

The cap used for this experiment accommodates both the oxygen probe and the HPLC fitting connected to the tubing in an air-tight manner. No air was introduced to take the DO measurement, as the DO probe was be constantly inside the closed flask.

For each experiment, the calibration method used was a 100% water-saturated air environment. The DO probe was held inside an empty bottle containing a wet sponge. The wet sponge provided a water-saturated atmosphere. Since the atmosphere was saturated in water, the partial pressure of oxygen in the liquid water was equal to the partial pressure of oxygen in the gaseous water. The calibration temperature was slightly different from the measurement temperature due to the calorific capacity of the water. All DO measurements needed to be taken while stirring otherwise the DO is not stable. Stirring was achieved either through sonication or with a magnetic stirrer.

A 3-way valve was installed between the flask containing the backing fluid, the Helium source, and the vacuum source. This allowed to switching between vacuum and helium without introducing any air to the flask.

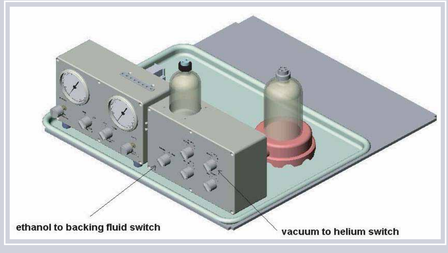


Figure 1:
Schematic of the BioDot vacuum/helium degassing system Degassing Method

Degassing Method

Step 1: Vacuum Degassing with stirring
At the beginning of the day, the backing fluid was degassed under vacuum with stirring. The efficiency of this step depends greatly on the strength of the vacuum provided by the vacuum pump. This step is completed once all visible air bubbles inside the fluid have disappeared. Depending on the vacuum and the volume of liquid, typical times are from 0.5 – 3 hours.

Step 2: Helium Blanketing under pressure
The vacuum was switched off and the Helium switched on to reach 3psi on the helium degasser gauge. The helium was switched off and the vacuum switched on for one minute. The vacuum was switched off and the helium switched on for 2 minutes

Step 3: Iteration of steps 1 and 2
Steps 1 and 2 were looped 5 times.

Step 4: Low pressure helium blanketing during dispensing.
The vacuum was switched off and the helium switched on. Once it reached equilibrium, the Helium pressure was lowered to 0.5 Psi. The pressure was kept at 0.5 Psi for the remainder of the dispense run.

Step 5: Purging air from lines with ethanol
The fluid lines were switched to the Ethanol bottle. The fluid path was purged several times. The fluid lines were switched back to the backing fluid and the fluid path was purged several times with the backing fluid. The ethanol/backing fluid washes were repeated three times prior to the start of dispensing.

Step 6: Begin the dispensing experiment

Dispensing Method

The AD3200 unit was set-up with one dispensing channel. A program was written to bulk dispense 1500 drops of 10 nL each onto 50 slides. The program executed a washing and pre-dispensing (2x20 drops) loop after every slide (1500 dots). The open time was set at 250 microseconds. The humidity was set at 80%.

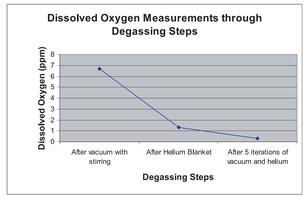
The fluid path of the unit was initially degassed with ethanol (EtOH). The dispensed fluid (PBS, 100X) was filtered prior to entering the fluid path with an UpChurch filter (10 microns) mounted on the tubing inside the bottle. The fluid was constantly stirred using a small stirrer plate (Sargent Welch, color squid magnetic stirrer) and a T-shaped stirring bar in PTFE (Emsdiasum).

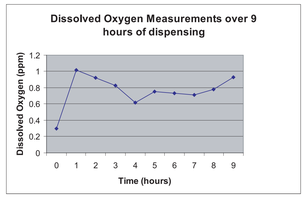
The bottle containing the backing fluid had been thoroughly cleaned using a base bath, an acid bath, and an alcohol bath. The dispensing tip was cleaned in an ultrasonic acid/alcohol bath and rinsed in an ultrasonic water bath prior to each experiment.

Dispenses were judged visually over the course of the experiment. The failure criteria for this dispense experiment was the absence of a spot or balling of the fluid on the tip.

Results

1. Dissolved oxygen levels before and during dispensing





Note: Helium blanket at 0.5Psi was used throughout the 9 hour period. It was observed that the DO level would double within 5 minutes if there was no blanket of helium over the fluid and the system was open to the air.

2. Dispensing

Observation of dispensing of 1150 spots onto each of 50 slides over the course of a 6 hour run resulted in 0 observed failures.

Number of drops dispensed	Volume	Total Run Time	Failures
57,500	10 nl per drop	6 hours	0

Discussion

Low volume dispensing is an extremely demanding process, involving optimization of dispense methods, overall process design, environment, substrate and reagent design.

Degassing of solutions is one extremely important component to ensuring robustness of dispensing in manufacturing and R&D environments, particularly at volumes below 50nl. The expression of bubbles at any point in the system can result in missed dispenses, incorrect volume dispenses, balling of fluid at the dispenser tip, and satellite drops on the product, all of which ultimately lead to increased dispense CV and/or loss of product. The degassing method used in a dispensing system such as that described herein must meet several important criteria:

- The ability to lower dissolved gas levels to negligible levels in bulk dispensed or backing fluids.
- The ability to maintain negligible levels of dissolved gases over the duration of a manufacturing run while not disturbing the dynamics of the dispensing process.
- The ability to handle high volumes of fluids BioDot has developed a degassing system and method which fulfills these requirements and results in improved performance of the BioDot dispensing system at the edge of its typical dispensing envelope.

Conclusion

- Demonstrated ability to reduce dissolved gas levels to negligible levels and to maintain those levels over an extended period
- Consistently dispensed over 57,000 drops of 10 nL each
- Switching between vacuum and helium degassing methods can be achieved without disconnecting any tubing, thereby preventing re-gassing of the system fluids.
- A slight overpressure of helium in the closed vessel connected to BioDot's syringe pump prevents any backpressure from occurring during the experiment. Backpressure would typically happen because fluid is withdrawn in large quantity from a closed vessel. Backpressure severely impacts the dispensing quality, causing cavitation in the syringe pump, and missed dispenses.
- Using this system, fluid lines can be purged with ethanol, lowering the surface tension in the system and allowing for good cleaning of the lines and expulsion of extraneous air from the lines, and subsequently purged with the degassed backing or dispensed fluid, without adding air back into the fluids being dispensed.
- The low pressure of helium used during this procedure allows users to operate with small, commercially available helium tanks if desired. This results in a reduction in concerns over safety with users inexperienced in handling large helium tanks, as well as reduced cost and smaller system footprint.
- The constant stirring of the fluid prevents the formation of a gradient of gas inside the fluid. This gradient would cause the formation of gas bubbles, which in turns would severely impacts the quality of the dispensing. Efficient stirring is achieved with a T-shaped stirring bar.
- BioDot's current design integrates all aspects of the degassing problems into one compact unit, small enough to be mounted on any of BioDot's dispensing platforms