

GUIDED WAVE RADAR:

A NOVEL TECHNIQUE FOR NON-INVASIVE VOLUME MEASUREMENT IN DISPOSABLE BIOPROCESS BAGS

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DISPOSABLE BIOPROCESS bags are increasingly being used in biopharmaceutical operations for buffer storage, buffer and media prep, bioreactors, product pooling and storage of bulk drug substance. Disposables offer certain advantages over stainless steel tanks: elimination of CIP/SIP, reduced labor, reduced validation requirements and increased operational flexibility.

Most bag films are multi-layer with a polyethylene or ethyl vinyl acetate fluid contacting layer. Small bags ($\leq 20\text{L}$) can be handled and stored in a simple tray, but larger bags are typically housed in a bioprocess container—a plastic or stainless steel bin that supports the filled bag. Bioprocess containers greater than 500L are often stationary while smaller bins are typically portable. New sensor technologies for pH, DO, conductivity, temperature and pressure have recently been developed that are compatible with disposable bag systems. However, one technology that has lagged behind is volume measurement for portable bioprocess containers.

There are several existing methods for volume measurement, including load cells, pressure transducers,

graduation marks and floor scales, but all have limitations. Load cells are extremely accurate, but not a robust option for portable systems. Pressure transducers located on the base of bioprocess containers are susceptible to damage and their signals tend to drift over time. In an unpublished study at Genentech, the pressure transducer's signal drifted up to 5% over 24 hours for a 2500-L bioprocess container system. This phenomenon is not well understood at the moment. Graduation marks have limited accuracy since the volume is read subjectively by the operator and automated measurement technology does not currently exist. Floor scales are not always available in the operation area, are relatively expensive and may present a safety risk when transporting heavy containers on and off the scale, especially if the scale has a ramp.

An alternate, novel method for non-invasive volume measurement in bags is guided wave radar (GWR), which will be the focus of this article. An approximate equipment price comparison of these various volume measurement options is provided in Table 1.

probe (Model 7XF-E) was made of 316L stainless steel with a ½-inch diameter and 50.3-inch length. The transmitter was a Magnetrol Eclipse Enhanced Model 705. The probe was bent to a right angle and routed through a hole drilled into the back left corner of the bioprocess container (Figure 1). The vertical section of the probe extended from the top of the bin to the base, maximizing the volume measurement range. Two plastic spacers were used to create a 3-cm gap between the probe and the back wall of the bioprocess container, improving contact between the bag film and probe. This also eliminated interference between the GWR's electromagnetic pulses and the back wall. The GWR transmitter was mounted on the outside of the container.

Table 1. Equipment prices for volume measurement options (2008 prices, w/o installation)

Measurement Method	Equipment Price
GWR	\$2,400 ^a
Floor Scale	\$11,400 ^b
Pressure Transducer	\$1,800 ^c
Load Cells	\$2,600 ^d
Graduation Marks	\$0

^a Magnetrol Eclipse Guided Wave Radar Level Transmitter (705-510A-110) with 50" 316L SS probe
^b Mettler Toledo Vertex 4' x 4' 304L SS floor scale with guard rails, ramp, and transmitter
^c Rosemount 3051S 2" tri-clamp pressure transducer with transmitter
^d Kistler Morse LD3-02K-X-015-X-X (4 load cells)

Guided wave radar is based upon the principle of time domain reflectometry (TDR). TDR is a measurement technique used to determine the characteristics of electrical lines by observing reflected waveforms. Pulses of electromagnetic energy are transmitted down a probe and the pulse is reflected when it reaches a liquid surface. The distance to the reflecting surface is determined by the return time of the pulse to the source [1].

GWR technology is commonly used for fluid volume measurements in traditional stainless steel tanks. However, GWR can also be used non-invasively for disposable bioprocess bags. If the probe is in close contact with the external surface of a filled bag, the electromagnetic pulse from the probe can penetrate the plastic film barrier to detect the air/liquid interface without being in direct contact with the liquid. Therefore, in bioprocessing applications where sterility is important or non-disposable instruments cannot contact the fluid, GWR may be a viable option.

EXPERIMENTAL PROCEDURE

To test the accuracy of a GWR system with bioprocess bags, we installed a GWR probe and transmitter on a ConeCraft 500-L 304L stainless steel portable bioprocess container. The

GWR Calibration Procedure

A 500-L gamma-irradiated Sartorius-Stedim Flexel 3-D bag with Stedim-40 film was installed in the 500-L bioprocess container. Stedim-40 is a 200 +/- 25-µm thick film constructed from the co-extrusion of seven layers, including ultra-low density polyethylene (fluid contact layer), ethyl vinyl alcohol, polyamide, polyethylene terephthalate, and several tie layers. The bioprocess container with bag was transported to a Mettler Toledo Vertex floor scale (+/- 0.1-kg accuracy; calibration range: 0.0-800.0 kg), which recorded the tare weight. The bag was slowly filled with purified water through the bottom inlet line. The GWR probe was calibrated by recording the GWR output current (mA) and the corresponding floor scale weight using a total of 17 strapping points. The bag film surrounding the GWR probe was manipulated during each measurement to eliminate creases and folds.

Water Fill Studies

A 500-L gamma-irradiated Sartorius-Stedim bag was installed in the bioprocess container and tared on the floor scale. While resting on the floor scale, the bag was slowly filled with purified water to 475L and subsequently drained. GWR and floor-scale volumes were recorded every 25L while filling and draining the bag. This procedure was repeated two times for a total of three runs (Runs #1-3) and a fresh bag was installed after each drain. The bag film was manipulated between each reading to eliminate creases and folds in the area sur-



Figure 1. GWR probe installed in filled bioprocess container

rounding the GWR probe, which otherwise could have contributed to error in the GWR reading. The floor scale provided a stable, level surface so variability due to floor slope was not a factor. Average GWR readings for these three runs, including 95% confidence intervals and percent deviation from floor scale readings, were determined.

Cell Culture Harvest Studies

The GWR system was also tested in pilot plant operations where we performed eleven fills with Chinese Hamster Ovary harvested cell culture fluid (HCCF), each time using a fresh bag. The fill volumes varied from 165.4L to 445.1L. After each fill, the bioprocess container was transported to the floor scale and the net weight of the liquid was recorded, tak-

ing into account the tare weight of the bioprocess container and empty bag. The volume of liquid measured by GWR was also recorded. The bag film surrounding the GWR probe was not manipulated in any way prior to recording the volume. The results were tabulated and the percent deviation between the floor scale and GWR measurements was calculated (Table 2). The conversion of floor-scale weight to volume was based on an HCCF density of 1.017 kg/L.

OBSERVATIONS AND DISCUSSION

Water Fill Studies

The following observations were made from the water fill studies:

- Variability in GWR measurements was minimized by manipulating the bag film surrounding the GWR probe and at the corners of the bioprocess container. Without tugging on the corners of the bag during the fill, the bag does not always fill uniformly, especially at fill volumes less than 250L. Also, by not adjusting the bag film surrounding the GWR probe, the film would trap pockets of water as it folded over, leading to erroneous signals. If the fold extended upward, the liquid level at the GWR probe was above the bulk liquid level and the GWR reading was artificially high. If the fold extended downward, the GWR reading was artificially low. The best way to mitigate this issue was to manually manipulate the bag film to remove the fold.
- The GWR accuracy improved as the fill volume increased, likely due to greater static head pressure inside the bag. The increased pressure naturally

Table 2. Measurement Comparison: Cell Culture Harvest

Run #	Weight from floor scale – pallettank + bag (kg)	Volume from floor scale (L)	Volume from GWR (L)	% Deviation (GWR vs. floor scale)**
1	168.2	165.4	156	5.7
2	260.4	256.0	251	2.0
3	263.9	259.5	248	4.4
4	287.1	282.3	275	2.6
5	392.1	385.5	384	0.4
6	405.8	399.0	399	0.0
7	416.2	409.2	403	1.5
8	422.5	415.4	420	-1.1
9	447.3	439.8	446	-1.4
10	447.9	440.4	440	0.1
11	452.7	445.1	444	0.3

**% Deviation = (Vol. floor scale – Vol. GWR) / (Vol. floor scale) x 100

eliminated major creases and folds and helped draw the bag film tightly against the inside walls of the bioprocess container and GWR probe, leading to extremely accurate and consistent GWR readings between 250-475L.

- It was difficult to measure volumes under 50L accurately using GWR, especially during the fill. With a low static head pressure, the bag did not press tightly against the GWR probe or conform to any consistent shape, thus GWR liquid measurements within this range were typically inconsistent.
- Some of the largest deviations in GWR accuracy occurred within the range of 150L-200L. Since the GWR reading was consistently low for all four runs, accuracy may be improved by recalibrating within this range or incorporating additional strapping points.
- GWR consistency improved with the presence of an air pocket above the liquid surrounding the GWR probe. The air pocket prevented liquid from drawing into the upward fold and impacting the GWR signal. Under these conditions, the liquid level at the probe was fully representative of the bulk liquid level.
- Overall, the GWR was accurate and consistent as evidenced by the 95% confidence intervals. GWR appears to be a reliable method for liquid volume measurement as long as the calibration is done carefully.

Cell Culture Harvest Studies

The volume measured by GWR was comparable to the water fill studies for all 11 runs (Table 2). The difference between floor-scale and GWR measurements varied from 0L to 12 L and the maximum deviation was 5.7%. GWR accuracy improved at higher fill volumes, which is also consistent with the water fill studies. Accuracy could have been improved further by manipulating the bag film surrounding the GWR probe and incorporating an air pocket as described above.

GWR Limitations

There are limitations for this particular GWR system, such as the minimum and maximum volume detection limits. Because of the location of a bioprocess bag’s heat-sealed edges, the top of the bag does not remain flat as the bag approaches its maximum fill volume. Instead, the top becomes “dome-like” and the bag film and liquid pull away from the GWR probe, preventing accurate measurement. Under this scenario, the GWR reading would be artificially low. There are very few applications where bioprocess bags are filled to capacity because of the danger of overpressurization and bag failure. Furthermore, this issue can be resolved by using slightly oversized bags. The limitation of

maximum volume measurement of GWR must be assessed for each individual application.

There is also a minimum volume limitation for GWR since the bag must be in contact with the probe to obtain an accurate signal. A certain volume of liquid must be present in the bag for this to occur. This volume varies depending on the size of the bag and footprint of the bioprocess container. The minimum volume range may improve with different vessel geometries—for example, a tall, cylindrical vessel with conical bottom.


Another limitation of GWR is signal variability due to floor slope. Floor slope may be a factor for portable bioprocess containers whose volume is measured in more than one location. Since the GWR probe is not centered in the middle of the bag, any floor slope from front to back of the bioprocess container may result in some degree of error. Floor slope was not a factor for these experiments since GWR volume was measured on a stable, level floor scale.

A fourth limitation of GWR is that the bag must be manipulated to produce the most accurate and consistent volume readings, as seen in the water fill studies. Bag manipulation may not be realistic for large bioprocess containers (>1000L) in which the top of the bag is not readily accessible.

RECOMMENDATIONS AND FUTURE WORK

Based on the results of this study, GWR appears to be a viable option for non-invasive volume measurement of liquid in bioprocess bags. GWR is robust and ideal for portable bioprocess containers, it provides relatively accurate, consistent volume readings, and it is relatively cheap and easy to install on new and existing bioprocess containers. Also, the stainless steel GWR probe can be bent to conform to the geometry of almost any vessel.

We recommend using GWR for portable bioprocess containers in place of load cells or pressure transducers. Floor scales are also a reliable option but are expensive, are not available in all facilities, and they present a safety risk when transporting heavy containers on and off the scale. In addition, the floor scale may not be positioned next to process skids. Since the GWR probe is installed on the actual bioprocess container, volume can be measured wherever the bioprocess container is being used.

GWR may also be suitable for bioprocess containers greater than 500L volume. We recommend conducting additional studies to support use of this technology for larger bags. 

References

1. http://en.wikipedia.org/wiki/Time_domain_reflectometer