APPLICATION OF A LOW COST AMPEROMETRIC SENSOR FOR DETECTION OF ALCOHOLS IN PROCESS RELATED HEADSPACE APPLICATIONS

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Abstract
A novel testing scheme to assure packaging integrity for liquid products was reoptimized using an amperometric sensor. The original method employed for this application uses a gas chromatograph with a surface acoustic wave sensor as the detector (GC/SAW) to monitor volatilized solvent vapors resulting from breaches in packaging integrity when subjected to moderate vacuum (20 in Hg). One common solvent in pharmaceutical products is ethanol. In the mode of “keeping it simple”, the new process design relies on the direct detection of ethanol using low-cost electrochemical gas sensor technology to replace the GC-SAW detector. The electrochemical sensor exhibits good selectivity to ethanol, thereby eliminating the need for a separation column, while the low detection limits allow for direct, near real time monitoring. The electrochemical sensor is an “environmentally green” technology requiring no high-purity mobile phase reagents, gases for the detectors or other consumable material. The instrument is both small and very low cost compared to GC based systems, and maintenance is simple.

Analytical and operational specifications between the two methods are illustrated and compared to validate the electrochemical method for the package integrity application. The use of the electrochemical sensor in concert with vacuum chamber testing was fully characterized for leak testing. The parallel development investigations reported herein supported the goal of using the simple vacuum chamber system as a diagnostic tool to provide assurance of lot-scale product market container integrity without the need for individual inspection.

Additionally, because of the success in using this sensor platform with leak testing, the scheme was further investigated for use with setting torque specifications for product vials as well as with a preliminary feasibility study to look at sensor’s utility with solvent drying operations.

Introduction
Regulated industries are becoming more proactive rather than reactive in gauging when manufacturing systems are operating outside their control range or specifications. Pharmaceutical manufacturing is leading this change. With the advances in process analytical technology, a process-based approach to quality is rapidly becoming the norm. (1-7) Recently, our laboratory developed and optimized a novel and efficient testing scheme to detect breaches in seal integrity for products dissolved or suspended in a volatile matrix. (8, 9) The test protocol was initiated as a feasibility investigation on how to empirically monitor the effectiveness of packaging seal operations. (8) The analysis method subjected each lot of containers of product solution to low vacuum, thereby drawing product from those containers unable to maintain seal integrity. This method was developed as a lot-scale method to supplement or replace current inspection protocols, whereby every product container fabricated and released
is inspected for leaks by applying pressure to each individual product container with a manually operated clamp. A manual inspection is then performed to verify that there was no pressure-induced product released from the packaging. The absence of released product confirms seal integrity. With the success of that work, the technique was scaled up from laboratory testing to the manufacturing floor. (9)

While analytical methods based upon the gas chromatograph/surface acoustic wave (GC/SAW) detection worked, a new approach to test for package integrity was designed that relies on the use of a single, real-time direct readout electrochemical gas sensor technology. A tiny, low cost instrument could replace the entire GC-SAW instrument. Switching to electrochemical sensor technology enabled equivalent detection capabilities at a fraction of the cost in a more user-friendly instrument package. Ethanol was chosen as the test system because it is a common solvent in the pharmaceutical industry and because electrochemical detection of headspace ethanol in aqueous systems and breathalyzers is widely used. (10-13) Later investigations illustrated that methanol and 2-propanol effectively respond with this sensor as well.

The selectivity of the electrochemical sensor eliminates the need for a separation column while ensuring unambiguity of the alcohol response. The excellent detection limit and other analytical specifications of the sensor provide adequate detection capability for the analyte of interest. As configured, the portable electrochemical sensor detector not only presented no safety concerns, but also operated as an “environmentally green” instrument requiring no mobile phase, gases needed by the GC detector, or other chemical consumables.

For portability and ease of use, the investigation used the Pocket CO™, a carbon monoxide instrument platform based on an amperometric gas sensor. (14, 16-19) The Pocket CO™ is a complete battery-operated instrument system and includes sensor control circuitry (potentiostat), microprocessor and software to transform electronic signals into concentration units, and digital display. Two sensor technologies were evaluated for ethanol vapor measurements using this platform. First, a minor modification of the standard carbon monoxide sensor was performed to render it sensitive to ethanol. Commercial electrochemical carbon monoxide sensors typically use an activated carbon chemical filter to remove interfering vapors. Removing the filter facilitates ethanol detection with this sensor. A second sensor was used that was specifically developed for higher sensitivity to alcohol without interference from CO. Replacing the chemical sensor in the Pocket CO™ with a sensor designed for alcohol improved overall analytical performance. (18) Our experiments showed that the alcohol sensor exhibited better detection limits, higher sensitivity, and faster response times than the CO sensor. Each sensor uses a unique and proprietary preparation technique for the sensing electrode and, therefore, each instrument has different catalytic activities. The electrochemical potential for alcohols is frequently more anodic than for CO. In this study, the only difference is the proprietary Pt for each sensor with no filter.

Sensor performance was validated by laboratory measurements designed to exactly mimic the product seal integrity test protocol. Critical sensor analytical parameters, including selectivity, linearity, response time, stability and reproducibility, were calculated and compared to both the alternative analytical method and to the required specifications for the package integrity test. For topical and liquid products, one issue that is routinely encountered is setting the proper cap torque on the finished product. If the cap is under or over tightened, the seal integrity may become suspect. In drug development, poor seals can affect perceived stability of a product line resulting in the loss of thousands of dollars. Because of the success found with the leak testing platform, a simple process to empirically determine torque specification setting was devised. This methodology can be used to verify the proper torque setting for a new production line and avoid the significant expense and regulatory issues of leaking vials in the future.

Another potential application of this sensor platform is solvent testing for bulk product and intermediates. Active Pharmaceutical Ingredients (API) are routinely dried in ovens are the end of their synthesis and sometimes at some intermediate steps requiring isolation. This process entails placing the API in a drying oven and placing the chamber under vacuum and above ambient temperature to drive off the solvents used during the synthesis.

Oftentimes this drying chamber is purged with an inert gas to facilitate the efficiency of the vacuum in removing/sweeping the evaporated solvent form the headspace. In the laboratory, the synthesis chemist monitors the drying of the API for degradation can occur if the process runs too long. In addition, the chemist wants to efficiently move the project along and complete the drying step as quickly as possible. Because of this, intermittent sampling with subsequent gas chromatographic testing is often taken to verify that enough residual solvent has been removed in order to proceed to the next step or release testing. If the residual solvent level is deemed too high the process is repeated until suitable solvent levels are found.

The process would be much more rapid if the chemist could determine the solvent level in the drying oven headspace without intermittent sampling. Such rapid, online response can be achieved with a low cost chemical sensor. Though analytical technologies exist to monitor the headspace or intermittent testing, these technologies do not combine the sensitivity, portability
and low cost of a chemical sensor. (8) For example, GC-MS and quantitative NMR have been used or proposed for this testing. Both techniques require significant investment in capital to achieve their objectives and are not portable when applied to the numerous drying ovens concurrently in use each day in synthesis laboratory operations.

Experimental Sensors

The amperometric sensors in this investigation used the Pocket CO™ Carbon Monoxide Instrument platform. The CO sensor was operated with the carbon filter removed. For improved ethanol measurements, the Pocket CO™ was modified by installation of a proprietary alcohol sensor in place of the CO sensor. (19) The Pocket CO™ and sensors were obtained from Transducer Technology, Inc. (Newark, CA), now part of KWJ Engineering (Newark, CA).

Chemicals

The ethanol used with this study was ACS grade and denatured with methanol and isopropanol. The hexane used was LC grade. Both chemicals were obtained from Sigma-Aldrich (St. Louis, MO).

Volatileization Chamber

All experiments were performed in a Labconco fiberglass Vacuum Desiccator Cabinet, which was 1 cu. ft in volume. (VWR International, Chicago IL) Vacuum was supplied to the chamber via a house line. Volatilizing ethanol samples was facilitated with a custom resistor-based heater powered by a 12V Lead Gel battery. A 10 Ω resistor connected to a 4 channel RF relay board (Carl’s electronics, Oakland CA) installed inside the chamber was used as a heat source. The relay board could activate the resistor heater while the chamber was under vacuum through the use of a remote RF wireless transmitter. A vial filled with 0.25mL of ethanol was heated by the resistor heater to approximately 40° C in less than 90 seconds.

Test Procedure

Headspace vapor samples were prepared from 0.25 mL of 1% ethanol in hexane solution placed in a vial, chilled to 0°C, and placed inside the vacuum chamber. The sample was subjected to the following steps:

1. Place sample in 1 ft3 vacuum chamber
2. Evacuate to 20 inches Hg (~70sec)
3. Activate heater, if required (volatilizes standard)
4. Record sensor reading at 20 minutes
5. Release vacuum
6. Record maximum sensor reading at 22 min (Ambient Pressure)
7. Evacuate chamber with air (cleanout step)
8. Allow sensor to zero before starting next run

Results and Discussion

As a first test, it was shown that the electrochemical sensor could be operated within the product test chamber under vacuum without damage. It thus provided a feasible system for operation inside the manufacturing work area. This new approach improves safety and cost for seal integrity testing because: (1) flame-based GC detectors pose a potential safety hazard in the area due to volatile solvents, (2) a simple and safe sensor replaces GC systems, and (3) the portable sensor has fewer consumables, as well as lower capital costs, making the approach orders of magnitude less expensive. It is noted that the first concern listed above was addressed by the SAW detector, which also does not require a flame. The work can enable the goals of using the vacuum chamber system as a diagnostic tool to provide assurance of adequate integrity in product market containers without the need for expensive equipment or individual inspection.

Amperometric Sensors

The Pocket CO™ (Figure 1) was developed for personal monitoring of carbon monoxide. The unit is battery powered and configured with a digital output. This enables the unit to operate in a stand alone fashion with real time display of concentration. The concentration is measured each few seconds and the display is updated while the microprocessor logs data such that at the end of a run, each unit reports the TWA (time weighted average), concentration in ppm, the total exposure (ppm hours) and the maximum concentration and time into the run which the maximum occurred.

For simplicity, the ethanol investigation began using the Pocket CO™ as originally configured, except that the carbon filter was removed from the standard electrochemical CO sensor in order to make it responsive to alcohol. The sensors are small (Figure 2) but quite sensitive. The electrochemical half reactions for CO monitoring are:

Half cell electrochemical reactions for the CO sensor:

Anode (sensing reaction)  
CO + 2H₂O → CO₂ + 2e⁻ + 2H⁺
Cathode (counter reaction)  
½O₂ + 2H⁺ + 2e⁻ → H₂O
Overall Reaction  
CO + 2H₂O ↔ CO₂

Alcohols, as an interference to CO monitoring, are normally kept away from the sensor with the use of a carbon filter. However, with the filter removed, alcohols will respond on this sensor with the half reactions:

Proposed half cell reactions for the alcohol interference (ethanol as an example):

Anode (sensing reaction)  
CH₃CH₂OH → H₂CCHO + 2e⁻ + 2H⁺
Cathode (counter reaction)  
½O₂ + 2H⁺ + 2e⁻ → H₂
Overall Reaction

\[ \text{CH}_3\text{CH}_2\text{OH} \leftrightarrow \text{H}_3\text{CCHO} + \text{H}_2\text{O} \]

The Pocket CO™ with the carbon filter removed was a fairly good alcohol sensor. However, it had two limitations: (1) it was not as sensitive as desired and (2) the recovery kinetics following exposure were slower than expected and this limited the method’s turn around time. To improve upon these limitations, the Pocket CO™ was modified by replacing the CO sensor with a proprietary sensor (KWJ/Transducer Technology Division, Newark, CA) more specific for alcohols. While the reactions are equivalent; the sensitivity and kinetic’s of the analytical response are improved over the CO sensor.

Known interferences for this sensor would include electroactive inorganic gases (NO, H2S, SO2), hydrazines, arsines, thiols, and other alcohols. In a clean laboratory or process environment required for pharmaceutical quality controls, these interferences would be insignificant since they are typically not found in the tested product(s). No interferences were encountered in our headspace investigations.

Validation/Calibration

The Pocket CO™ comes calibrated for carbon monoxide exposure. Rather than recalibrate each unit for alcohol concentration, the readout was calibrated externally using an excel spreadsheet. For deployment, the instrument would be calibrated for the analyte of interest so that direct readout in ppm is on the display. To improve upon these limitations, the Pocket CO™ with the carbon filter removed was a fairly good alcohol sensor. However, it had two limitations: (1) it was not as sensitive as desired and (2) the recovery kinetics following exposure were slower than expected and this limited the method’s turn around time. To improve upon these limitations, the Pocket CO™ was modified by replacing the CO sensor with a proprietary sensor (KWJ/Transducer Technology Division, Newark, CA) more specific for alcohols. While the reactions are equivalent; the sensitivity and kinetic’s of the analytical response are improved over the CO sensor.

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CO Sensor Response to Ethanol

The basic analytical performance of the electrochemical sensor with each of the two sensor designs was evaluated to determine the viability of either sensor to identify breaches in package integrity.

The Pocket CO™ with a modified CO sensor was calibrated from 0 to 125 ppm ethanol. For package integrity tests, vapors in the 10 to 25 ppm range would be viewed as a strong indicator of damaged seals. Testing was performed using two independent sensors. The sensors were linear within the 0 to 125 ppm concentration range. The first CO sensor yielded a correlation coefficient of \( r = 0.9972 \) and the second CO sensor had a correlation coefficient of \( r = 0.9985 \). (Figure 3) The precision analysis at 50 ppm ethanol (\( n = 4 \)) for the first sensor yielded a 7.8% RSD and a 2.0% RSD for the second sensor. While the net sensor responses looked promising, the sensors were slow to return to background. For multiple samples, this would not be efficient. In addition, the lower detection limit of sensor indicated that determinations of less than 20 ppm ethanol were going to be difficult.

Modified Sensor Response to Ethanol

The test procedures were repeated with the alcohol sensor installed in to the Pocket CO™. The sensor was calibrated at two ranges, a high level and low level of ethanol. From 0 to 75 ppm the alcohol sensor yielded a correlation coefficient of 0.9943. (Figure 4a) From 2.5 to 18 ppm the alcohol sensor had a correlation coefficient of 0.9995. (Figure 4b) At 28 ppm, the alcohol sensor had a precision (\( n = 6 \)) of 4.2%. Not only was the detection limit dramatically improved by using the alcohol sensor, but also the sensor rapidly returned to background after exposure. Based on the exemplary performance relative to carbon monoxide electrochemical sensor, all further investigations proceeded using solely the alcohol electrochemical sensor. The lower limit of detection was ~1 ppm in these experiments.

Analysis of Product Vials Using the Modified Sensor

The capability of using sensor based analytical methods to identify breaches in sealing包包装 integrity by detecting escaping alcohol in the headspace was assessed using the Pocket CO™ modified sensor. For this evaluation, ethanol was sealed in a variety of sample vials and tested in the chamber for leakage. (Figure 5). Because this investigation was primarily designed for a topical product matrix in glass vials, the experiment used several vials with various seal issues: (1) a simple snap on vial, which was made for aqueous samples and expected to leak in the presence of a volatile organic matrix, (2) a screw cap vial, which was made for volatile samples and not expected to leak, (3) a screw cap vial intentionally misthreaded, and thus with the seal integrity suspect and it was expected to leak, (4) a screw cap vial that had its septa punctured with a needle, which is not expected to leak and finally, (5) a screw cap vial that had its septa punctured with needle multiple times and was expected to leak due to the suspect seal integrity.

Each vial was tested at room temperature (Table 1) and at an elevated temperature (Table 2). The resulting data shows that the system easily detected leakage on capped vial, poorly capped and integrity breached septa. Significantly, no evidence of solvent volatilization to the headspace was observed for the properly capped alcohol solution. The latter result is equally important; we did not want the system to be so challenging that properly sealed/packaged samples would yield a positive response. With these results, samples showing a response of less than 50 concentration units were considered to show that no leakage was induced. As expected, using the higher temperature conditions made it easier to distinguish leakage from background. Where feasible, future further testing will use the higher temperature challenge.
Table 1. Leak Challenge Results – Vial at room temperature.

<table>
<thead>
<tr>
<th>Vial</th>
<th>Sensor Response (Units)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 min</td>
</tr>
<tr>
<td>snap on vial</td>
<td>0</td>
</tr>
<tr>
<td>screw cap vial</td>
<td>0</td>
</tr>
<tr>
<td>screw cap vial-bad thread</td>
<td>49</td>
</tr>
<tr>
<td>screw cap vial- bad thread</td>
<td>152</td>
</tr>
<tr>
<td>screw cap vial- 2x needle</td>
<td>11</td>
</tr>
<tr>
<td>screw cap vial- &quot;multi&quot; needle</td>
<td>144</td>
</tr>
</tbody>
</table>

Table 2. Leak Challenge Results – Vial at 40°C

<table>
<thead>
<tr>
<th>Vial</th>
<th>Sensor Response (Units)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 min</td>
</tr>
<tr>
<td>snap on vial</td>
<td>offscale</td>
</tr>
<tr>
<td>screw cap vial</td>
<td>0</td>
</tr>
<tr>
<td>screw cap vial-bad thread</td>
<td>515</td>
</tr>
<tr>
<td>screw cap vial- bad thread</td>
<td>offscale</td>
</tr>
<tr>
<td>screw cap vial- 2x needle</td>
<td>7</td>
</tr>
<tr>
<td>screw cap vial- &quot;multi&quot; needle</td>
<td>offscale</td>
</tr>
</tbody>
</table>

Table 3. Monitoring of API Solvent Drying.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Initial Sensor Reading (units)</th>
<th>Final Sensor Reading (units)</th>
<th>Headspace GC Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>Off-scale</td>
<td>14</td>
<td>0.22%</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Off-scale</td>
<td>14</td>
<td>&lt; 0.05%</td>
</tr>
<tr>
<td>2-Propanol</td>
<td>Off-scale</td>
<td>10</td>
<td>&lt; 0.05%</td>
</tr>
</tbody>
</table>

Figure 1. The Pocket CO™ Carbon Monoxide Instrument (used with permission from Transducer Technology, Inc.)
Figure 2. T-Series CO & alcohol sensors used in the Pocket CO™ (used with permission from Transducer Technology, Inc.)
Figure 3. Alcohol Detection using the CO Sensor

![Graph showing alcohol detection using the CO Sensor](image)

Figure 4. Alcohol Detection using the Modified Sensor

a) High levels of ethanol
b) Low levels of ethanol

**Figure 5.** Vials filled with ethanol and various stages of seal integrity.
**Figure 6.** Torque study with market containers filled with ethanol.

![Ethanol Sensor Torque Study](image)

**Torque Study**

To challenge the torque settings, individual vials were filled with ethanol (worst case) and capped using a calibrated torque wrench (Model 751LDIN from CDI Torque Products, Industry CA) to precise torque settings in a range of 1 to 8 in-lb. Each of these vial settings were prepared in triplicate sets and stored at 25°, 40° and 60° C for one week. The vials were then transferred to the vacuum chamber system for seal integrity testing. Since each vial was prepared individually by hand, the use of triplicate vials at each setting should provide a needed variance to evaluate each torque setting. As illustrated in Figure 6, the vials capped at a torque less than 5 in-lb had a potential to leak. The vials could safely be packaged at a torque range for 5-8 in-lb. Thus, the chamber system effectively verified the torque range for this vial and matrix prior to development as well as prior to the start of expensive stability studies.

**Monitoring of API drying**

The effectiveness in monitoring the drying of an API in several alcohol solvents was investigated in our laboratory using the modified sensor. The API under investigation was slurried in alcohol and filtered. A representative sample (5 g) was placed in a vacuum oven (VWR 1415M oven with a Edwards E2M vacuum pump) at 45°C, 18 in Hg vacuum and a slight sweep gas (air) to pull the solvent from the chamber. As expected, when the sample was placed in the chamber, the sensor immediately went off-scale due to the evaporation of the solvent. As the cake dried and the solvent exited the chamber, the sensor readings came back on scale and subsequently decreased. As seen in Table 3, the Modified Pocket CO™ effectively monitored the drying process and indicated when the solvent drying was complete. Using headspace gas chromatographic testing, each solvent was confirmed to be below its pharmaceutical specification. (15)

The limitation of the amperimetric sensor is that the residual solvent under investigation needs to contain an electrochemically active functional group for detection. Furthermore, the sensitivity to each individual functional group meeting this criterion may not be suitable for headspace monitoring. Many of the solvents routinely used and tested in the pharmaceutical industry do not meet this requirement, such as hexane and acetone. Thus, the further development of the sensor platform is needed but a simple modification such as including a second sensor could make the technique virtually universal in application.
It should also be noted that the loss on drying technique is suitable approximately 80% of the time. It is not uncommon for residual solvents to be trapped in the crystal matrix of the API. When this happens, laboratory testing will report high solvent content even though vacuum drying has exhausted its capability of removing any more solvent. Recrystallization is required. In this scenario, besides a great deal of time being wasted redrying the sample in the oven, the process imparts a risk to the API through it being exposed to repeated drying environments. If a drying monitor, such as the modified sensor under study, was present to confirm the drying technique is not removing any additional solvent, the chemist can proceed to alternative steps (such as recrystallization) sooner.

Conclusion
Vacuum chamber testing is an effective empirical method for efficiently and accurately determining the presence of seal problems. The electrochemical sensor used in this study provided a low-cost, easy to implement analyzer for real-time monitoring of package integrity by headspace analysis. This system is now being optimized as a tool for setting performance standards, making quality improvements and improving the efficiency of packaging and synthesis operations. Future investigations will focus on using the chamber system to optimize tube sealing efficiency, providing feedback to set-up mechanics and continuing performance verifications following shutdown and change control events.

Literature Cited
15. Code of Federal Regulations, 21 CFR 211. 194 (a)