What is virtual pH?

An introduction to the new Petri dish pH / CO_2 sensor

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FREEZING INCUBATING STORING MONITORING

Contents

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The effect of pH on biological systems is well understood and characterised. Specifically, H+ and OH- ions, in both liquid and gas can not only optimise protein/substrate interactions[1], but outside strict levels can cause permanent, irreversible damage to proteins. Unlike temperature, where biological systems show robust tolerance, a pH change of as small as 0.1 is sufficient to impair human embryo development[1]

For most *in vitro* culture systems, pH is regulated via the use of bicarbonate buffered media in combination with higher CO_2 gas levels to create an optimal pH level of around 7.3.

The narrow boundaries required for optimal cell growth mean validation and monitoring of the culture environment is important. As a crude measure of acidity, some culture media contain inert dyes (e.g. Phenol red) which will change colour in response to significant pH change.

This gives the scientist some indication of a dramatic pH shift in the culture system, but by the time warnings are apparent it may be too late for remedial action.

Traditional pH meters make absolute measurements of H+ concentrations in media, though they can present users with the issue of maintaining sterility and also are usually subject to daily calibration requirements.

More recently, sensors have become available which determine pH levels as colour changes on a substrate which is analysed. Such systems overcome the sterility issues mentioned previously, though can be subject to drift if the substrate become saturated. Furthermore, such systems tend to lack portability and carry a significant price tag.

Virtual pH measurement for cell culture systems

Virtual pH uses a different methodology.

The relationship between environmental conditions, such as partial pressure of CO_2, and the properties of the culture media are well understood and characterised. This relationship can be expressed mathematically by the Henderson-Hasselbalch equation:

$$\mathbf{pH = pK_a + \log_{10} [HCO_3^- / (0.0003 \times P_a \times \%CO_2)]}$$

Essentially, this formula determines pH by using the following parameters:

- pK_a is the colog of the acid dissociation constant or in other words, a quantitative measure of the acid strength.
- HCO_3^- is the concentration of bicarbonate and is expressed here in mmol/L.
- The colog of the acid dissociation constant and HCO_3^- values may not be readily available from a media manufacturer in which case suitable coefficients can be calculated from the target pH and CO_2 concentration; these values should be available from the media manufacturer.
- P_a is the atmospheric pressure and refers to the altitude at which your laboratory is situated. This affects the actual number of CO_2 molecules present in a fixed volume and therefore has a direct impact on the resulting pH of a media solution.

Users can choose to express the output of the sensor as either a percentage of CO_2 or as a pH value.

The pH recovery time constant is a property of the culture media and dish configuration. In essence, this is a time constant taken for the pH to react to a change in CO_2 levels. One time constant is the time it takes for the media to reach 63% of its final value in response to a change in CO_2 level; approximately 5 time constants will be required for the pH to reach 99%. This information should be available from the media manufacturer.

Monitoring CO_2 and virtual pH

All commercially available media is validated to determine the pH level in response to CO_2 gas concentrations. Below is an example of the CO_2 / pH response for the Blastocyst media from Origio.

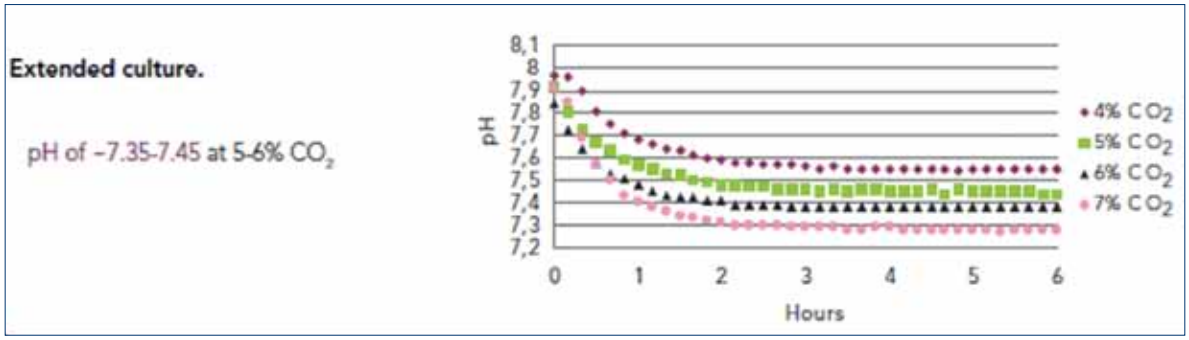

Figure 1. A graph detailing the pH response in the Origio blastocyst culture media at different CO_2 concentrations

In order to model the pH response to CO_2, culture and environmental conditions, parameters can be entered via the 'Configure' tab of the Petri dish pH / CO_2 free app.

Figure 2. Configure tab of the Petri dish pH / CO_2 application allows input of parameters used to determine pH in an established culture system

Once appropriate inputs have been made in the configure tab, the sensor can be placed in the culture environment and the CO_2 and corresponding predicted pH determined.

Figure 3. Monitoring of both the culture chamber CO_2 levels and corresponding predicted pH following multiple lid openings in a benchtop incubator.

Size Matters

Continuous monitoring of CO_2 levels in large incubators is commonplace. A variety of commercially available infra-red (IR) based sensors are available for use as static sensors. Additionally, portable hand held devices can be used to validate gas levels in big box incubators.

With the growing popularity of smaller, benchtop incubators for high value culture applications, the problem of measuring chamber CO_2 and resulting pH values became apparent. Various approaches have been employed to try to address this issue, including the passive sampling of chamber gas to determine the CO_2 level.

Figure 4. A Planer BT37 benchtop incubator with side mounted IR CO_2 sensor sampling the chamber culture environment

Alternative approaches include the introduction of a fluorescence based pH sensor directly into one of the culture chambers. While this does have the advantage of directly measuring the immediate culture environment, it requires machining holes into the chamber. Combined with the expense of the commercially available sensors, this is neither a quick nor inexpensive process, leaving the user with a reduced capacity culture chamber and the ability to determine pH in only part of the incubator.

The new Petri dish pH / CO_2 sensor is equivalent to a 35mm petri dish in size and includes an ultra-thin ribbon cable connector. This allows the sensor to be placed either statically as a fixed point sensor or used as a CO_2 validation tool for multiple laboratory devices.

Figure 5. The small size of the Petri dish pH / CO_2 sensor allows monitoring of small culture environments

Conclusion

It is well understood that accurately replicating *in vivo* conditions, such as micro nutrients, temperature and acidity, promotes better *in vitro* cell culture. As previously mentioned, many cell types are relatively tolerant to temperature change, even allowing scientists to freeze them in liquid nitrogen, but biological systems are much less tolerant to pH change. This makes pH an invaluable monitoring end point to ensure culture success, though once a significant pH change is observed, it is often too late to ensure a successful outcome.

By monitoring the CO_2 in an established culture system and inferring the resulting pH, users have an early warning mechanism allowing intervention if CO_2 levels are reduced (and CO_2 levels have the most profound impact on resulting media pH levels). Used as a fixed continuous monitoring sensor or a validation tool in variety of growth environments, the Petri dish pH / CO_2 sensor provides a new way to ensure the best possible *in vitro* conditions.

1 Swain J.E. Is there an optimal pH for culture media used in clinical IVF? Human Reproduction Update, Vol 18, No.3 p.333-339, 2012

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