Device to measure pH within an incubator

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Biomedical Engineering 201

March 2, 2001

Client: Dr. Theresa Duello

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Problem Statement:

To design a device to measure the pH of the bathing media surrounding *in vitro* cultured bovine embryos within a CO_2 incubator. The device cannot alter the incubator in any way, and should also be able to accurately measure the pH within tenths of a pH level.

Introduction:

Current studies involving embryonic development are focusing on the effects of hormonal introduction during their gestation period. However, to determine the validity of the hormonal influences in these studies, all other factors such as temperature, gas concentration, and pH levels of the bathing media must remain constant. While temperature and gas concentrations are easily determined, measuring the pH level of the bathing media is difficult. This measurement is necessary because changes in pH levels of the bathing media can influence both the tested hormonal effect on the bovine embryos and their developmental reactions *in vitro* [2]. Bovine embryos are very sensitive to changes in intracellular pH above the approximate resting value of 7.2 [3]. This design is intended to take continuous pH readings of the bathing media without affecting the embryos themselves, or affecting the current devices used for incubation.

There are three logical steps in the eventual outcome of the design. First, a method must be devised to measure the pH levels of a bathing media inside the incubator without altering the incubator itself in any way. The accuracy of the measurements must be in tenths of a pH level, approaching the intended pH range of 6.80-7.60. Next, is discovering a method to measure the pH level in a small amount of bathing media (~25µL). Finally, these methods must ultimately be used without harming an embryo inside the small media volume. Integrating these methods with a computer system would provide an easy method of taking continuous readings and an easy method of storing data. Ideally, the goal of this design project would measure the internal pH of embryos and in between cells; however, this is an advanced methodology. While this has been used before, it would be difficult to develop considering the different design constraints that are present. For a full elaboration of restrictions of the design, the current product design specification is attached to this report. (See Appendix A).

Alternative Solutions:

The current devices used to measure pH are comprised of two components: a probe (which actually comes into contact with the solution), and a receiver (which interprets the data from the probe). The type of receiver is independent of the type of probe that is used (other than the compatibility of signals). The current models of available probes involve two main methods of determining proton concentration.

-**Glass bulb probes**. This probe involves a Silver electrode submersed in Silver Chloride solution. The electrode and solution are encased in a glass-like polymer to which only free protons can bind. When the probe is submerged in solution, free protons bind to the glass shell in an amount proportionate to their concentration (the PH). The charge on the glass shell is transferred via the saturated AgCl solution through the Ag electrode and into the receiver. The receiver then uses the potential difference between the glass shell and a standard solution to determine the amount of free protons in the solution.

-**Fiber optic** probes. This probe involves a fiber-optic cable that has a pH indicator surrounding the tip. The indicator is either covalently bonded to the polymers of the fiber-optic cable, or encased in a permeable membrane that allows for free diffusion of protons, but not indicator. As the pH in the solution changes, free protons bind to the indicator and cause it to change color. Thus, the intensity of the color is directly proportional to the amount of free protons in solution, and thus to pH. The fiber optic cable transmits the color to a receiver that quantifies the color variation, and interprets its correlation to pH.

There are many types of receivers available for either type of probe. The receivers are divided between digital or analog receivers, based on their method of data analysis. They are also available in either benchtop or battery powered models. The benchtop models are fairly large (~2"x9"x9") and come in either digital or analog models. The benchtop models also require an AC power source. The battery-powered models are smaller in size (~1"x3"x5") and completely standalone. However, due to size constraints the battery receivers are mostly digital signal receivers. Despite the receiver model, a cable is required to connect the probe to the receiver.

From these available devices, several designs were generated. Theses designs represent a broad range of combinations for these different components. Each design represents separate aspects beneficial to measuring pH of the cell media with minimal alteration to the incubator.

#1 Battery powered glass-probe pH meter

This design solution makes use of a battery-powered receiver and glass-bulb probe. Because the power source is internal to the unit, there is no concern about power cords going in and out of the incubator. A digital display on the meter provides a continuous readout of both pH and temperature (Figure 1). A mounting unit will be construc ted so that the probe may be held in a stable and consistent position above the sample, while the receiver rests on the floor or shelf of the incubator (Figure 2).

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Figure 1. Battery Powered pH meter from Jenway, Inc.

The Model 3071 pH Meter from Jenway is one example of an available Battery-Powered pH reviever. The receiver is designed for most glass bulb probes and uses a 9V battery for its power source.

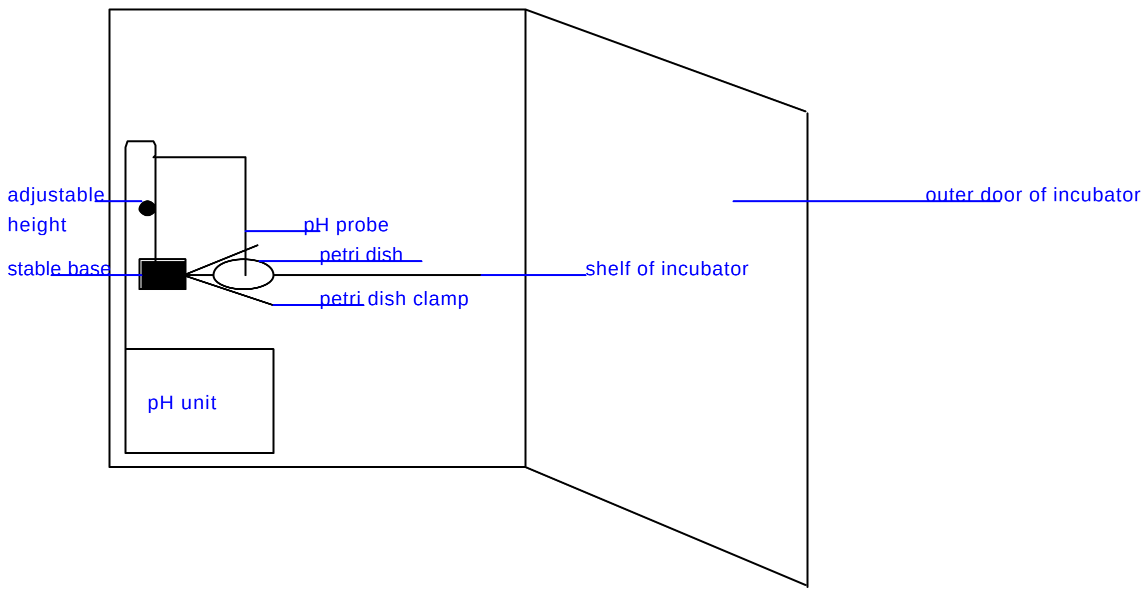

Figure 2. Battery- Powered pH Meter sketch.
The receiver unit is located on the floor of shelf of the incubator, while cables connect it to the probe resting in the media. A stand with a clamp and base provides support for the probe and holds both the probe and dish in such a way that the probe is completely submersed in media. The probe mount has a mechanism for adjusting the height and the probe's radial position over the culture dish.

#2 Micro Glass Bulb with Benchtop Receiver

A micro-glass bulb probe (Figure 3) is used in conjunction with a benchtop receiver (not necessarily limited to benchtop model). The small size of the probe allows for a more consolidated method of mounting on the culture dish. However, the design utilizes a benchtop receiver that, while cheaper, requires cables to run between the inside and outside of the incubator. If cost is ignored, this design could easily utilize the battery-powered receiver to keep the entire device within the incubator. The Micro probe would be mounted over the media via a conical plastic stand (Figure 4). The stand fits over the culture dish and places the probe in a uniform radial position. The probe's depth is variable as well to allow complete adjustment of the probe's location.

Figure 3: Micro pH Electrode. The Model 98-03 Micro pH electrode from Thermo Orion is one example of an available Micro-glass bulb pH probe. The probe utilizes an Ag/AgCl electrode-solution combination and is compatible with most glass-bulb receivers.

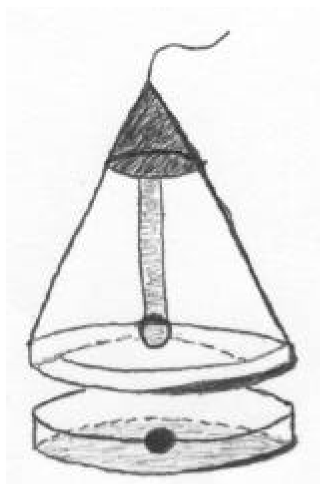

Figure 4: Micro-Glass Bulb Probe Design Sketch.
The mounting unit holds a detachable probe over the culture dish with a conical-shaped structure. A template on the dish is available to center the media droplet in order to insure that the probe remains in radial contact with the media.

#3 Fiber optic electrode

This design utilizes the fiber-optic probe (Figure 4) and a benchtop receiver. A small hole is made in the side or bottom of the dish and the fiber optic probe is inserted. A small cable exits the incubator and inserts into the receiver. A benchtop receiver is used in this design to allow for data storage of the pH readings (Figure 5).

Figure 4: Fiber-optic pH probe.

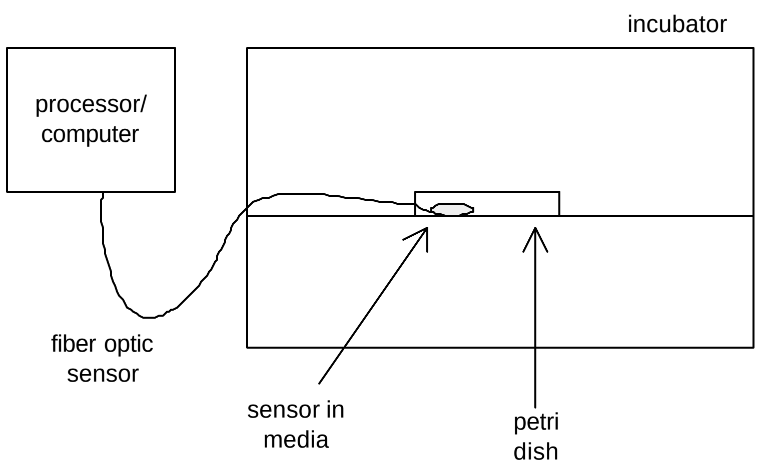

Figure 5. Schematic of petri dish fitted with fiber optic pH sensor.
By using a small petri dish, the probe can be directly fitted to the side or fastened to the dish in a manner that the tip is submerged within the media sample. Although wires do exit the incubator, the addition of a processor or computer provides an advantage to thoroughly analyze and track data.

#4 Fiber Optic / 96-well Plate Design

This design is similar to the previous solution in that it also makes use of a fiber-optic sensor to determine the sample's pH. With the introduction of a 96-well plate, multiple samples can be monitored with more ease and efficiency. Each well containing media, embryos and mineral oil may be fitted with a fiber-optic sensor (Figure 6). The lid of the plate contains ports/holes for the sensors to go through and remain stable within the well. The sensors are relayed to a processor or computer outside of the incubator to analyze and track data.

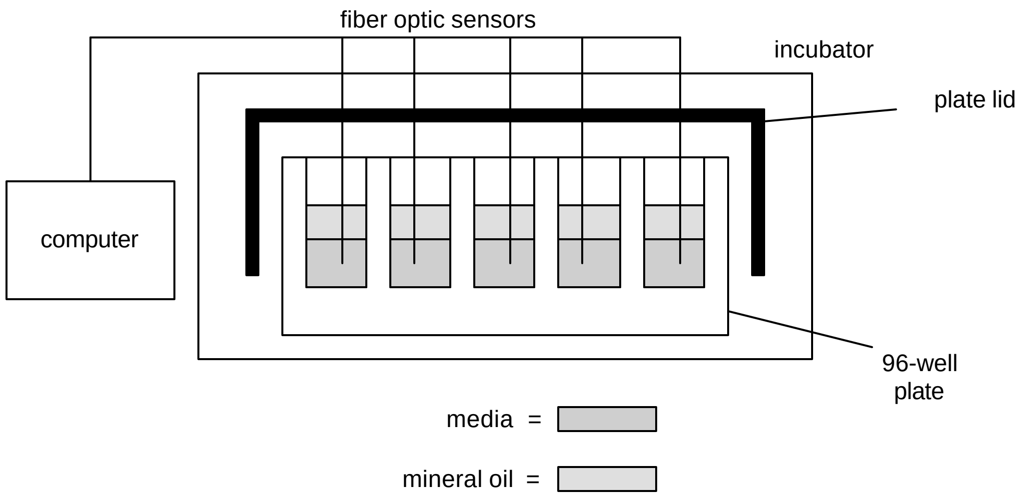

Figure 6. Sketch of 96-well plate fitted with fiber optic pH sensor.
The sensors are held securely in place by fittings directly above the well, within the lid of the plate. One sample to 96 may be monitored simultaneously. Additionally, the use of a computer or processor allows for continuous feedback of data.

Evaluation of Designs:

The battery powered glass-probe pH meter has certain advantages and disadvantages toward a future design. Since the power source is completely internal, there is no concern for a cord running out of the incubator. The digital display also provides continuous readouts for the experiment. The glass bulb probe is relatively inexpensive and accurate to the design requirements. However, it is unknown at this time if the glass probe will negatively affect the embryos in their development. Also, the glass probe may not be small enough in order to effectively measure the pH levels of such a small volume of media. Additionally, the internal design may produce conflicts with reading the data because the incubator door may need to be opened every time a reading is recorded.

The next design is the micro glass bulb with laptop receiver. The small size of the probe allows for easier access to the bathing media. Additionally, the smaller size may allow accurate measurements of the small volume possible (a problem with the previous design alternative). The laptop computer could take continuous reading of the bathing media and provide a means of data storage. This use of the laptop computer requires running cables from inside to incubator to the computer itself. This may pose a problem with not altering the incubator in any way. Secondly, this design is assuming that a cost factor is ignored.

The third design option includes a fiber optic electrode and laptop receiver. The fiber optic probe provides the ideal probe size for the volume of media that the design is going to be working with. Also, it has an acceptable accuracy range for the design specifications. The laptop can provide a means of continuous readings and data storage for the experiment. Unfortunately, the use of the laptop computer may require some kind of alteration to the incubator. Additionally, more testing is also necessary for the probe to discover its influence on the embryos in the media.

The final design alternative is similar to the previous design but it incorporates a 96-well plate instead of the current use of a petri dish. With this design, pH measurements of multiple samples can be taken. Additionally, the media can be placed in the same location for each sample ensuring that the probe is in contact with the media. Unfortunately, this method of multiple sampling is extremely complicated and may be beyond the scope of the client's requirements.

Design Matrix

	Battery Powered pH meter	Micro Glass Bulb	Spectrometer	Fiber Optic Electrode	96-well plate
Alteration of incubator	+	+	S	-	-
Capable Continuous Readouts	+	+	+	+	+
Degree of Accuracy	+	+	S	+	S
Interaction with embryos	S	S	+	S	S
Multiple Samples At once	-	-	-	-	+
TOTALS					
-	1	1	1	2	1
+	2	2	2	1	2
0	1	1	1	1	1
Total Score	1	1	1	-1	1

Table 1. Design Matrix. Each design idea is evaluated using the same criteria. The criteria are not ranked in order of importance. A (+) indicates that the design fully meets the criteria, (-) indicates that the design fails to meet the criteria, (0) indicates neutral (neither meeting, nor failing the criteria), and (S) means that more research/testing is necessary to determine the result.

Proposed Direction and Possible Problems:

While the above solutions each have merit, it would be premature to choose one design as presented and proceed in the design process due to a lack of information. By combining similar design solutions, closer scrutiny can be paid to the advantages and disadvantages of each design.

After design solutions are combined, a concerted effort must be made to address the lack of information in some aspects of the design project. Quantitative data concerning a variety of topics must be gathered in order to obtain a suitable design solution. By creating subgoals, different types of data will be collected at different points in the semester. First and foremost, a method should be found to determine the pH within the incubator (of any volume). Next, the pH of a small volume of liquid should be measured within the incubator. Finally, the pH of a media sample, containing embryos, will be measured. By building upon prior steps, the overall task of measuring the pH of the culture media is reduced to a more manageable series of targets. It is important to note that flexibility

In order to first measure the pH of a sample within the incubator, necessary information such as the cost of a battery-powered pH meter, the shelf-life of such a unit, and how the data will be displayed and stored should be investigated and evaluated. During the evaluation of this first subgoal, it is important to remember that some forward thinking is necessary in this process. Although the immediate goal is to measure a large volume sample within the incubator, a solution should be chosen which possesses some flexibility in terms of necessary volume size.

In terms of the second subgoal, information regarding the minimum sample amount necessary for recording a result and the reliability and accuracy of such results will be gathered. Once moving onto the final subgoal, an analysis must be made of possible interactions of the system with the embryos in their culture environment.

References:

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Appendix A: Product Design Specification

Preliminary Design Specifications

February 13, 2001

pH Monitor for *In Vitro* Bovine Embryo Incubation Labs

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Design Statement: To design a device to measure the pH of the bathing media surrounding *in vitro* cultured bovine embryos within a CO_2 incubator.

Client Requirements:

- Does not interact with or disturb the embryos
- Does not alter the existing incubator
- Accurate to one tenth of a pH unit

Design Requirements:

Performance: The pH meter will obtain continuous readouts of bathing media surrounding *in vitro* bovine embryos. The device must not interact with or disrupt the embryos. The readings must be accurate (to the tenth of a pH unit). The measured pH will also reflect the average pH of the bathing media. The device will take readings from cultures held in a CO_2 incubator and will either fit inside the incubator, or have leads that run into the incubator (without inhibiting or changing the incubator).

Safety: The device will be properly insulated to avoid undesired electric discharge. The entire device, or the section in contact with the bathing media, can be sterilized to avoid contamination of the embryos.

Accuracy and Reliability: The device will continuously measure pH between 5 and 9 with accuracy to a tenth of a pH unit .

Life in Service: The meter will be used long enough to obtain a reliable reading, and readings will be recorded daily to biweekly.

Shelf Life: The device will function for a comparable pH meter lifetime (~2 to 10 years).

Maintenance: The pH meter must be calibrated on a regular basis (~bimonthly). Calibration includes testing and resetting the meter in correspondence with the pH of known solutions (i.e. 5, 7, and 9). The cables and connections of the meter will be checked regularly (~annually). The device must be sterilized between each use (~ethanol wash, disposable sections, autoclave, etc.).

Operating Environment: The operating environment is a lab setting. The incubator is approx. 3’ x 2’6” x 3’.

Ergonomics: The device’s readout will be easy to understood and read (i.e. LED display). Controls for making the reading will be easily access and understand.

Size: The pH meter will either to fit within the incubator (approximate dimensions given above), or have an attachment small enough to run into the incubator without affecting the function of the incubator.

Weight: The device must be light enough as to sit in or on the incubator.

Materials: The section of the device in direct contact with the media will be composed of materials that will not alter the bathing media or interact with the embryos. The section of the device external from the media can be composed of nay materials that do not effect the incubator environment.

Aesthetics, Appearance, and Finish: Must be relatively easy to use.

Quantity: The client requires only one device for the time being.

Target Product Cost: Unknown for now until we get a better idea of equipment that we are going to use and what is acceptable and unacceptable for the project from talking to our client. As of now our cost is restricted to that of the BME budget (~$200).

Standards and Specifications: The device must be approved by Dr. Duello and must be tested extensively as to determine if there is any interaction with the media or the embryos.

Customer: The client (Dr. Duello) suggested the idea of a small microprobe in measuring the pH levels. She stated that the ideal meter would read the pH within the embryo, but the pH in the surrounding media would be sufficient. She would like the meter to make the reading within the incubator as to avoid moving and possibly traumatizing the embryos. The type of dish that the embryos are cultured in is variable and the client even suggested that another shape of culture dish might prove easier to make a measurement in. She specified that the device must not alter the incubator's function/performance in any way.

Patient Related Concerns: Device must be easily sterilized between measurements of different cultures of bovine embryos.

Competition: There are many existing methods for measuring pH levels in solution of which three are three major types of measurement used (glass bulb meters, pH indicator dies, and spectroscopy/laser meters). There are devices commercially available for each method. However, many of these devices either slightly interact with the media that they make their readings from, or require a greater volume of solution to read than is present in the embryo media drops. Thus, the client's specifications are not met entirely by any of the already existing devices that are commercially available.