The 7800 Visual Imaging And Patching Chamber, For Upright And Inverted Microscopes.

Abstract

The CI7800 was tested to investigate its operational limitations in achieving thermal stability and control under a multitude of conditions. In order to assess the effect of thermal exchange with the laboratory environment the chamber was placed in a worse case scenario, subjecting it to the thermal contributions of air-conditioning, personnel movement and drafts.

1) Static testing revealed a skewed temperature distribution in the bath which could be reduced by coverage with a lid. The skewed distribution was removed in the presence of perfusion (1ml/min).
In the presence of perfusion:
2) bath temperature was maintained at the physiological temperature of 37.1±0.25 ^{o}C (SD) during a 3 hour stability test. The chamber heater had a time to half rise of 291s.
3) The addition of an immersion objective causes an offset in bath temperature but does not affect stability, and so can be compensated for in physiological experimentation.
4) The chamber responds to a 10^{o}C command temperature increase in 180s. The rate of cooling is dependent on the ambient environment.
5) Carbogen gas (95%O_2, 5%CO_2) perfusion (10ml/min) lowers the temperature gradient between the bath and the environment. Encapsulation and gas perfusion provides the optimum physiological conditions but this is not a suitable scenario for electrophysiological experimentation because it limits electrode access.
6) The faster perfusion rate of 2ml/min produced lower bath temperatures but greater thermal stability, in comparison to the slower perfusion rates of 1 and 0.5ml/min, which increased bath temperature at the cost of thermal stability.
7) Perfusion tubing with a greater bore size to wall thickness (OD: 1.1mm and ID: 0.6mm) enables more efficient heat transfer from the heat exchanger to the bath.
8) The contribution of the CI7800 to signal noise is dependent on the temperature set. At bath temperatures within the physiological range a contribution of 0.07pA was observed.

The CI7800 showed a high level of temperature control and thermal stability given the exposed testing environment. This level of performance is well within research requirements and could be further improved when incorporated as part of a sensitive imaging or electrophysiological recording apparatus.

The 7800 Visual Imaging And Patching Chamber, For Upright And Inverted Microscopes.

Aim: To determine the limitations and operating conditions for the CI7800.

Introduction

The importance of maintaining a specimen at physiological temperature when undertaking imaging or electrophysiological experiments is becoming increasingly recognised. In view of this, Campden Instruments have designed the 7800 unit and chamber to allow scientists greater control over the temperatures that their specimens are subjected to, thereby achieving the goal of maintaining a slice/specimen in optimum conditions for longer.

The imaging and patching chamber is based on existing designs from the Forsyth lab, and incorporates a heat exchange system and feedback control to ensure accurate temperature measurement and stability of bath temperature. The device includes a separate temperature probe for in situ measurement of bath conditions. These temperature control devices have been calibrated to national standards and the sensitivity of these feedback sensors allows a greater temperature resolution, to two decimal places.

This report will present the results of this investigation in six parts, describing:

1. The performance of the system under static conditions, no perfusion.
2. The performance of the system with perfusion.
3. The effect of the addition of an Immersion Objective (IO).
4. The Responsiveness to command to change temperature.
5. The effect of gas perfusion.
6. The effect of perfusion rate.
7. Heat transfer properties
8. Signal to noise ratio of the unit.

These will be followed by a discussion.

Methodology

All tests were carried out in a laboratory environment. The temperature range across the environment would naturally change throughout the day. The conditions of each test were duly noted and will be described in the results.

For the majority of the experiments the chamber was mounted on the stage of an upright top-down microscope (Micro Instruments LTD, UK) (diagram 1). The bath was fed by perfusion (Campden instruments, UK. Cole Parmer, US) as part of a system powered by a ‘Minipuls3’ peristaltic pump (Gilson, FR). A constant flow of perfusate into and across the bath was maintained by the suction needle outflow (Campden/Forsythe). This removed the waste perfusate to a waste vessel, and kept chamber volume constant. The surrounding bank of the bath chamber was swabbed with sigmacote® (Sigma, UK) to aid hydrophobicity and maintain the solution in the bath. The perfusion tubes were positioned according to the instructions in the

Diagram 1. The Set-up

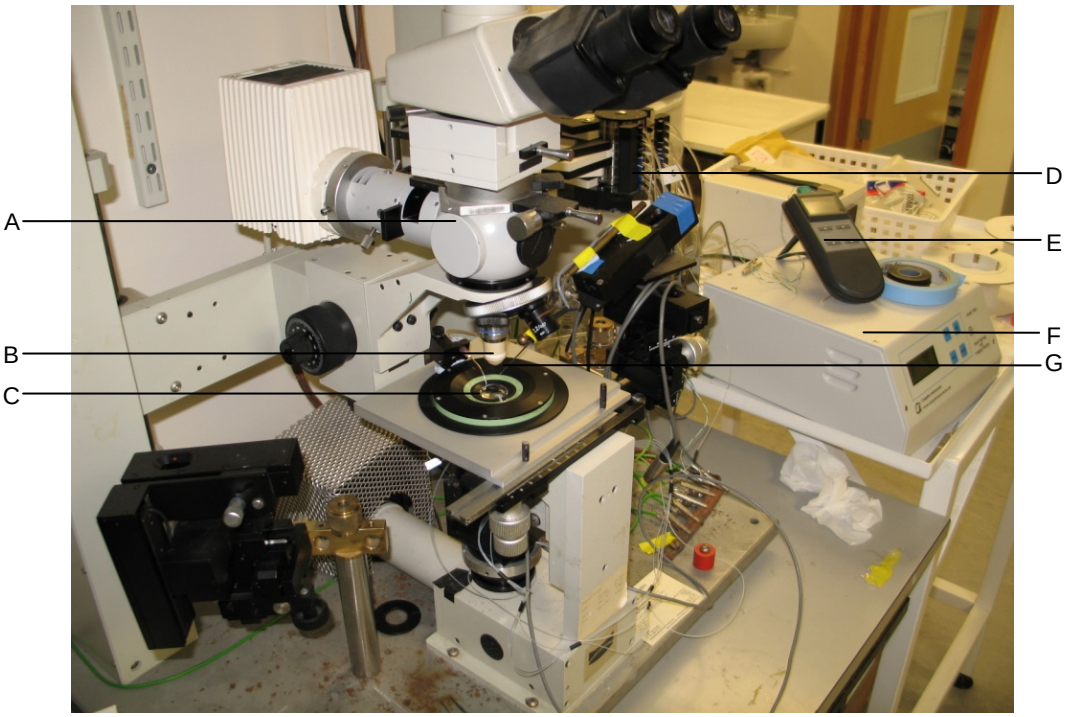


Figure 1 displays a photograph of the set-up. The letters point out the key features: A) The upright top-down microscope (Micro Instruments LTD, UK). B) The Plan 40x immersion objective (Zeiss). C) The chamber of the CI7800, with its central bath. D) The Minipuls3 peristaltic pump (Gilson, FR). E) The secondary hand-held temperature probe (Comark, UK). F) The CI7800 control unit. G) The CI7800 temperature probe, anchored to a micro-manipulation system.

operating manual. A small amount of silicone was used to prevent capillary action backflow and to stabilise the tubing. The 7800 was set up in this way to mimic the conditions of an electrophysiological rig.

To prevent unnecessary variability between tests certain basic parameters to the testing were insured:

- The perfusate level in the chamber bath was maintained at an ideal flat level, with minimal volume undulation. This was achieved by correct laminar flow ensuring a four to one extraction of air to perfusate by the extraction needle.
- The bath probe was centred and raised 0.5mm from the bottom of the bath. This clearance ensured temperature measurement of the perfusate and not the chamber bottom.
- In this report physiological temperature is defined as 37^{o}C. A deviation of ±1^{o}C is viewed as acceptable for *in vitro* physiological experiments.
- In some of the experiments the temperatures of the bath areas were measured with a handheld temperature probe (Comark, UK) for ease of manoeuvrability. Standardisation of the handheld device with the CI7800 probe shows a -0.1^{o}C difference. This was considered negligible, and will therefore not be referred to here-after in this document.

1. Static tests: showed that a lid and warmed gas reduced heat loss.

To observe the temperature distribution in the bath under the simplest conditions, isotherm observations were carried out in the absence of perfusion. The chamber temperature was set so as to try and maintain temperature within the physiological range. Figure 1 shows the results of three isotherm tests: a) static, b) static with gas, and c) static with gas and lid. The boxed arrows at the edges of the Isotherm plots denote the positions of the inflow perfusion tubes (white), and outflow needle (shaded). The crosses through the boxes describe the omission of perfusion.

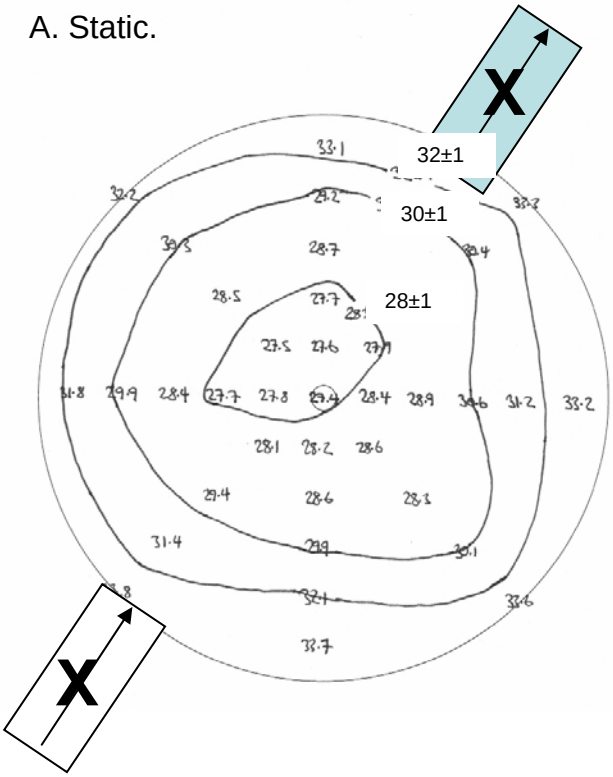
A maximum temperature of 55^{o}C was set. The lab environment was moderately busy. Measurements were taken at 2mm intervals from the centre of the chamber after centring. In the gas tests, Carbogen gas (95% O_2, 5% CO_2) was perfused into the heat exchanger, across the heating unit and then out across the top of the bath solution, at a rate of 10ml/min. The aim of using the warmed gas was to lower the temperature gradient between the bath and the environment.

1A.The static test (figure 1A) produced a skewed temperature distribution centred at 27.6 ^{o}C; greater temperatures were obtained at the chamber edge, with an observed maximum temperature of 33.8 ^{o}C. The ambient temperature was 23.5 ^{o}C and the bath volume (B.V) was 0.42ml.

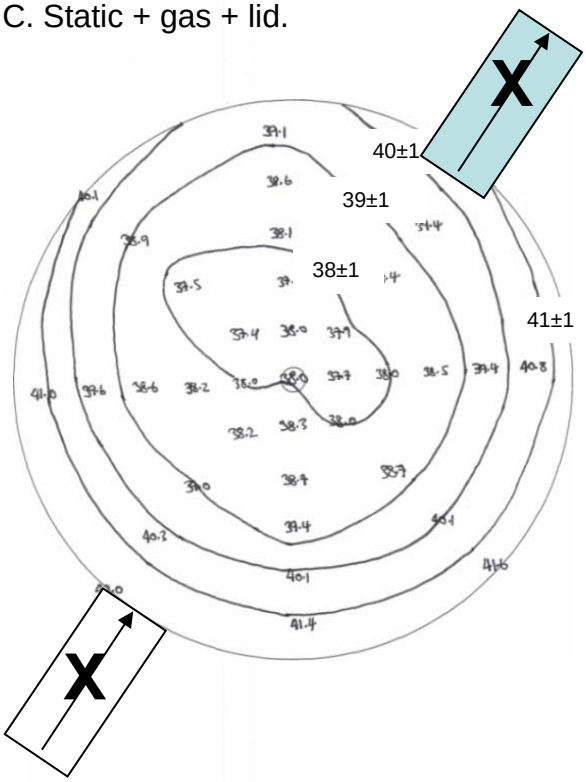
1B. The static + gas test (figure 1B) produced a less skewed temperature distribution similar to test ‘a’. The temperature at the centre of the bath was 28.1 ^{o}C. Greater temperatures were also observed at the edge of the bath with an observed maximum of 43.6 ^{o}C, almost 10 ^{o}C higher than in test ‘A’. The ambient temperature was 23.6 ^{o}C and the bath volume was 0.31ml.

Figure 1. Static Isotherm tests show that a lid and warmed gas reduced heat loss.

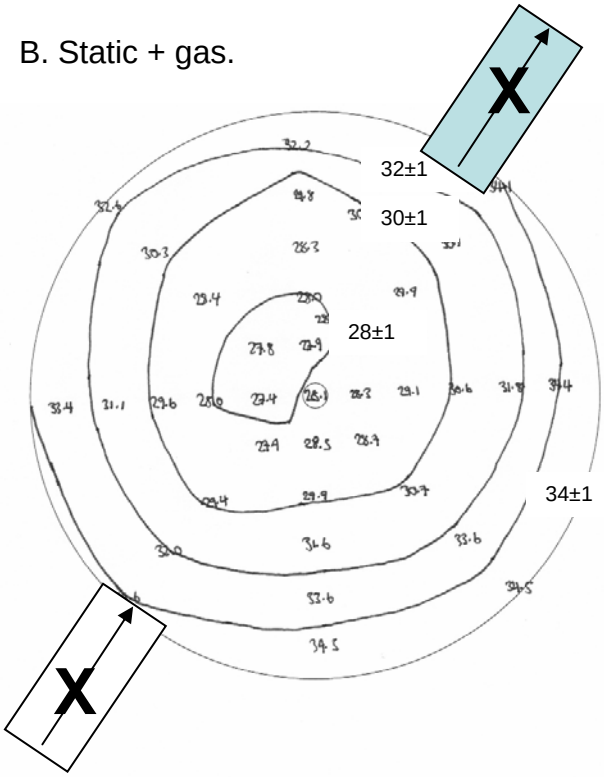
A. Static.



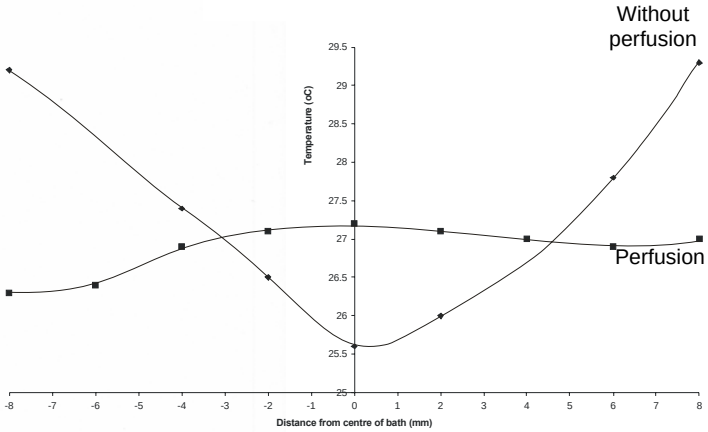
C. Static + gas + lid.



B. Static + gas.



D. Temperature distribution across chamber with/without 1ml/min perfusion. Command = 30°C. Ambient temperature= 23.0±0.2°C. B.V= 0.5ml



1C. The static + gas + lid (figure 1C) test produced bath temperatures that were greater than the physiological temperature range desired. The temperature distribution across the bath is more regular than in tests ‘a’ and ‘b’. The temperature at the centre of the bath was 38 ^{o}C, with the edge of the bath measuring 41.4 ^{o}C. This test emphasises the effect of encapsulation on temperature control and stability. The ambient temperature was 23.3 ^{o}C and the bath volume was 0.53ml.

1D. The static versus perfusion plot (figure 1D) exemplifies the effect of perfusion versus static flow on temperature distribution in the bath. Measurements were taken across the bath in the same plane, for direct comparison. The skewed temperature distribution of the static test, which shows a temperature difference of nearly 4^{o}C between bath edge and centre, is rescued by 1ml/min perfusion. The perfusion test maintains a uniform temperature range across the bath. The test was carried out in a moderately busy laboratory, with an ambient temperature of 23.0±0.2^{o}C. The chamber command was 30^{o}C and the bath volume was 0.5ml.

2. Tests With Perfusion

During imaging or electrophysiological experiments it is often necessary to constantly perfuse the chamber. This not only prolongs specimen survival during testing but also enables greater temperature control, and pharmacological manipulation. The following tests were carried out under perfusion as this was deemed a more experimentally relevant condition.

2A.The calibration analysis supports a linear relationship between the chamber temperature and the bath temperature.

Firstly a calibration test was performed to characterise the bath temperatures observed at set command temperatures (figure 2A). Chamber temperatures conferring bath temperatures within the physiological range (30 ^{o}C to 40 ^{o}C) were measured. The curve shows the results of the chamber temperature vs. bath temperature in an exposed test in a moderately busy laboratory. The perfusion rate was 1ml/min. The ambient temperature was 28.8±0.3 ^{o}C (SD), and the bath volume was 0.82ml.

At the lowest commandable chamber temperature (30^{o}C) a bath temperature of 27.1 ^{o}C is observed. At the greatest temperature command (54 ^{o}C) a bath temperature of 40.2 ^{o}C is observed.

The linear relationship between the chamber temperature and the bath temperature is of the form $y = 0.5514x + 10.888$. It should be noted that commands in the upper temperature range of between 52 ^{o}C and 54 ^{o}C took over 10minutes to stabilise at 54 ^{o}C.

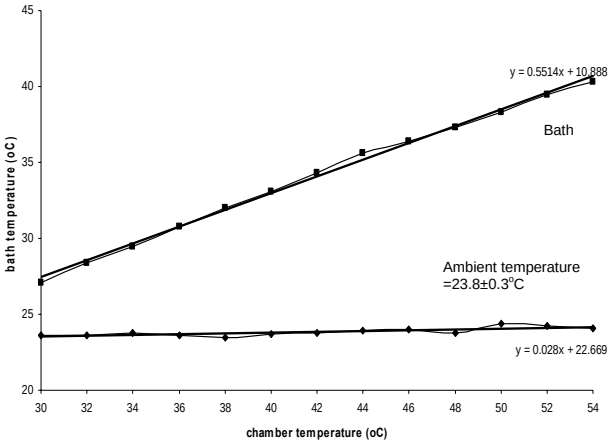
2B. Physiological temperature is maintained over 3hour stability test.

The ability of the 7800 to maintain a bath temperature at a physiological temperature (37 ^{o}C) for an extended period of time was measured by way of a stability test (figure 2B). The bath was set to approximately 37.0 ^{o}C by setting the temperature command to 47.3 ^{o}C. The chamber temperature also registered 47.3 ^{o}C. Ambient and probe

Figure 2

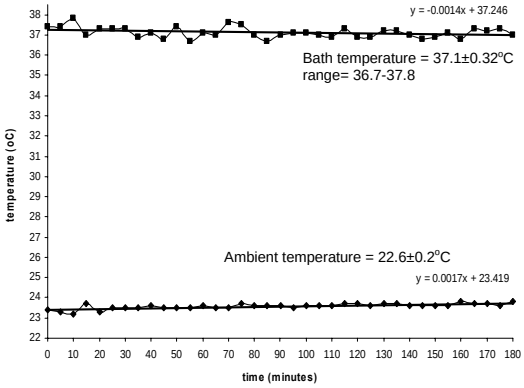
A

Calibration curve for an increase in bath temperature with perfusion (1ml/min). Lab traffic: moderately busy. B.V=0.82ml.



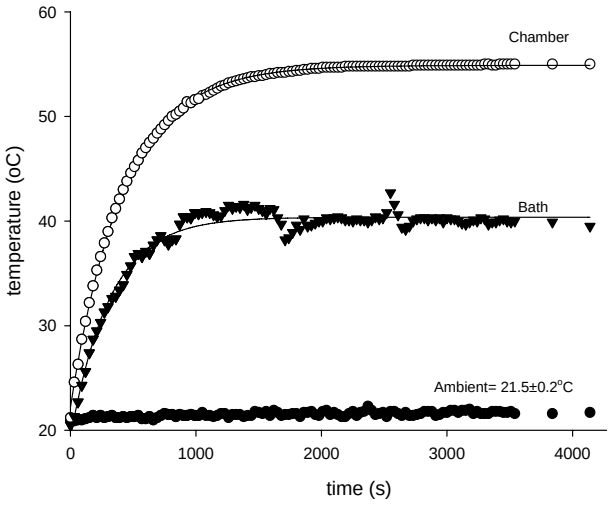
B

3 hour environment stability test. No lid.
Lab traffic: moderately busy. Perfusion rate: 1ml/min. B.V= 0.456ml



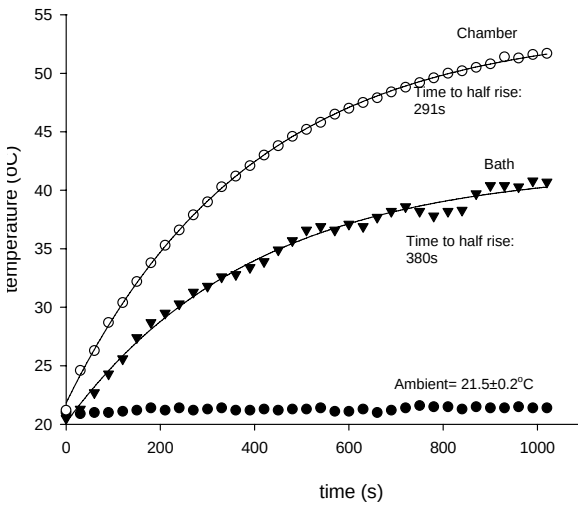
C

Power-on to maximum temperature. Lab traffic: moderately busy. Perfusion rate: 1ml/min. B.V=1ml



D

Expanded version of first 1000 seconds of 2C.



temperatures were recorded at 5 minute intervals for a total of 3hours. The test was undertaken in an exposed environment in a busy lab.

The results show that the mean bath temperature was 37.1±0.25 ^{o}C (SD) over the test period, with an overall temperature range of between 36.7 and 37.8^{o}C. This range is within the limitations for physiological experimentation defined in the methodology. Interestingly there is a shallow decline in bath temperature over the 3hour period, exemplified by the trend line. There is also a maintained increase in ambient temperature, although this is also small, again this is exemplified by the trend line. The bath volume was 0.456ml. The ambient temperature was 23.6±0.13 ^{o}C.

2C. Chamber heater has a time to half-rise of 291 seconds.

The performance of the 7800 on heating from the lowest temperature command (30^{o}C) to the highest temperature command (55 ^{o}C) from the initial powering-on of the unit was assessed (figure 2C). This was achieved by turning on the 7800 and sampling temperatures at 30 second intervals, until the command temperature was reached, and the probe temperature stabilised.

An exponential rise to maximum curve was plotted to the data using the formula $y= y_o + a\ (1-e^{-bx})$. The chamber time to half-rise was 291s, and the probe time to half rise was 380s. The ambient temperature was 21.5±0.2 ^{o}C. No lid or immersion objective was used. The lab traffic was moderately busy, and the bath volume was 1ml.

The data in this test reveals a relatively slow response to changing command temperature. The chamber took 3390s to reach the commanded 55 ^{o}C, and it took a further 150s to stabilise at this temperature. The bath temperature stabilised at 40.3 ^{o}C at maximum chamber command, a -14.7 ^{o}C difference to the chamber.

3. The addition of an immersion objective

Electrophysiological and imaging experiments often necessitate the use of an immersion objective (IO). The extent to which the addition of a Plan 40x IO (Zeiss) affects temperature stability due to its action as a heat sink was assessed in the following experiments.

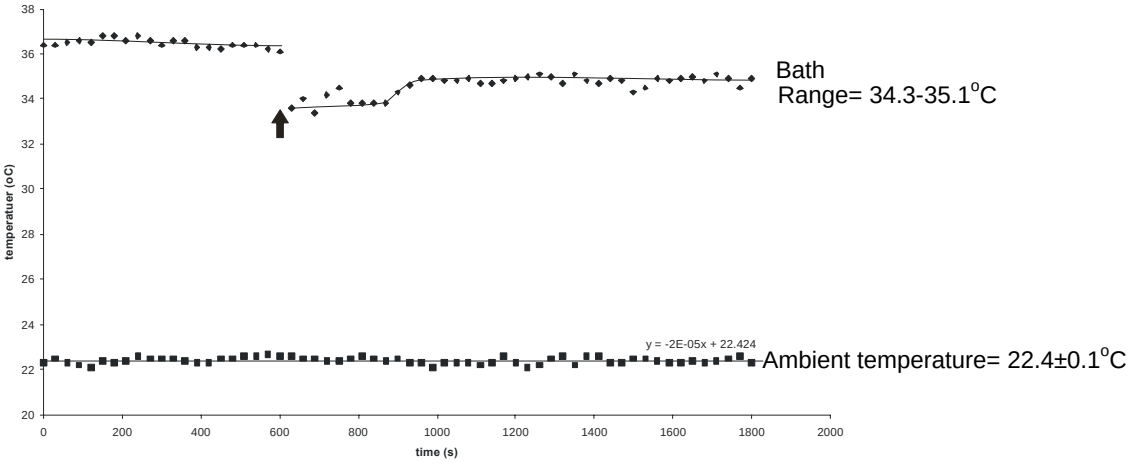
3A. IO addition causes an initial drop in bath temperature, but this recovers over time.

The effect of the physical addition of the IO to the bath was assessed by maintaining the bath at a physiological temperature before adding the IO (figure 3A). The chamber command was 47^{o}C. Following a period of stabilisation sampling began at 30s intervals. After 600s of sampling, the IO was added to the set-up and its effect was analysed. The ambient temperature was 22.4±0.1 ^{o}C, in a lab with low traffic. The bath volume was 0.42ml.

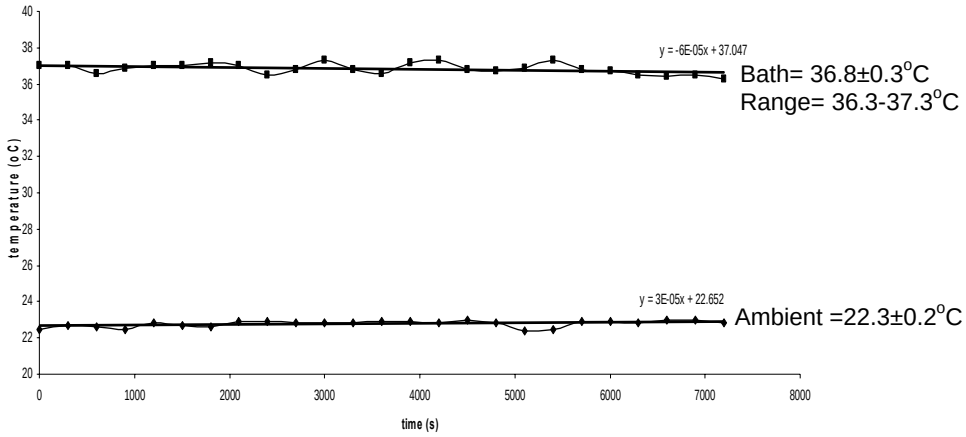
The results show that prior to the addition of the IO the bath was operating within a temperature range of 36.8 and 36.1 ^{o}C. On addition of the IO (at t=540s), bath

Figure 3.

A Effect of adding an immersion objective. The set temperature was 47^{o}C. Lab traffic: low. Perfusion rate= 1ml/min. B.V=0.42ml



B 2hr stability test with immersion objective. Lab traffic: moderate. Set temperature= 47.5^{o}C. Perfusion rate= 1ml/min. B.V= 0.62ml



temperature dropped to 33.6 °C. The bath temperature recovers as the IO warms, and by 1200s the temperature has reached 34.8 °C and stabilises within a range of between 35.1 and 34.3 °C until the end of the test at 1800s. This range is over 1 °C cooler than prior to addition of the IO.

This cooling effect would need to be compensated for in an experiment and could be achieved by setting a higher chamber temperature.

3B. A 2 hour stability test with IO displays a well maintained physiological temperature.

After defining the effect of the IO on bath temperature, its effect on temperature stability was then measured. This was achieved by conducting a 2 hour stability test in the presence of an IO, sampling at five minute intervals. The chamber was set to 47.5 °C to achieve a physiological bath temperature. The bath + IO set-up was left to stabilise for thirty minutes before testing. The ambient temperature was 22.8±0.2 °C, in a moderately busy laboratory. Bath volume was 0.62ml.

The IO test showed small fluctuations throughout the test. A line of best fit was plotted with the form $y=-6E^{-5}x + 37.047$. The mean temperature was 36.8±0.3 °C, with a range of 36.3-37.3°C, which is within an acceptable physiological temperature range. The trend line showed a small degree of temperature decline over time, similar to the degree observed in the 3 hour exposed stability test.

4. Responsiveness to change in temperature command

Altering bath temperature during testing is a necessary feature of many experiments, the ability to do this quickly and accurately can be crucial. Having investigated the temperature stability and maintenance of the chamber under the previous conditions, the following experiments aimed to analyse the responsiveness of the unit to changes in temperature command.

4.1A. The chamber takes 180s to respond to a Δ10°C heating command.

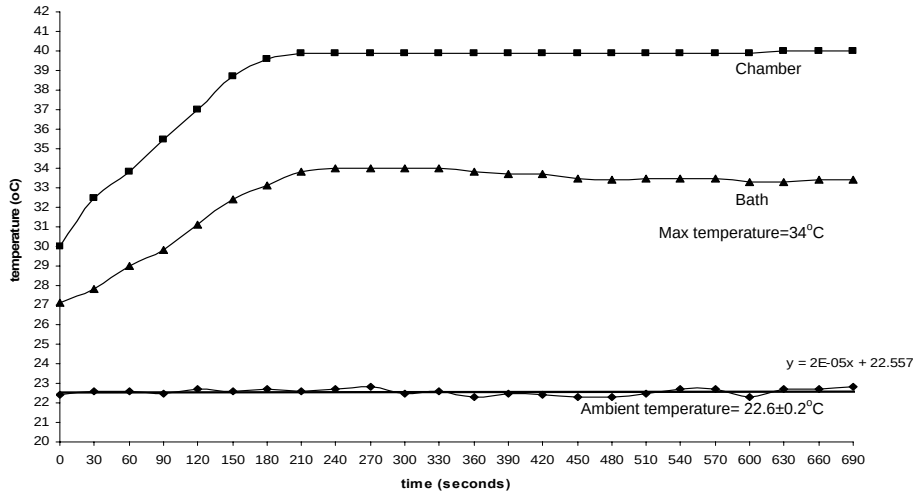
To assess the speed and efficiency of commanded temperature change a 10 °C increase in temperature was commanded and sampled at 30 second intervals. The test was firstly carried out in an uncontrolled environment; the ambient temperature was 22.6±0.2 °C; the lab was moderately busy. Perfusion rate was 1ml/min and the bath volume was 0.43ml.

A 10 °C increase command from 30 °C to 40 °C takes the chamber 180 seconds to rise to the near-target temperature of 39.9 °C. The data shows a positive hyperbolic trend. The bath temperature follows in accordance with a slight lag, revealing an almost sigmoidal shape. It peaks at a maximum temperature plateau after 210s, with a range of between 33.3 and 34 °C.

Figure 4.1

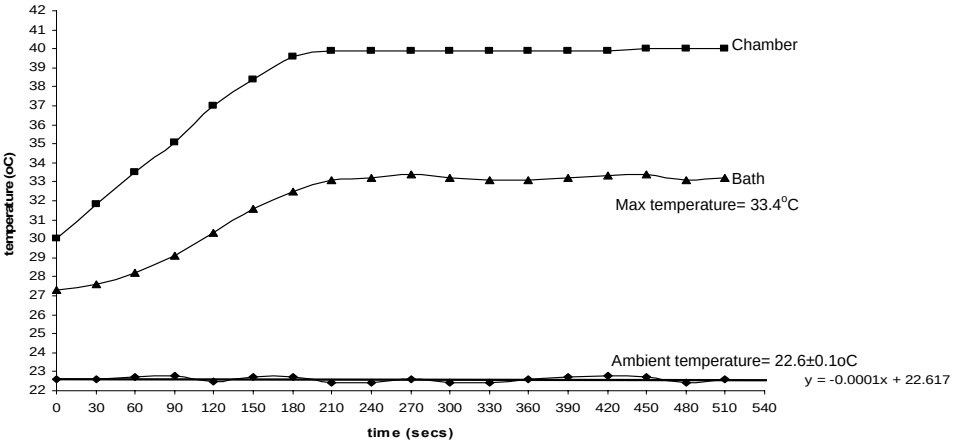
A

10 degree increase in temperature. Without lid. Lab traffic: busy, but calming down. Perfusion rate: 1ml/min. B.V= 0.433ml.



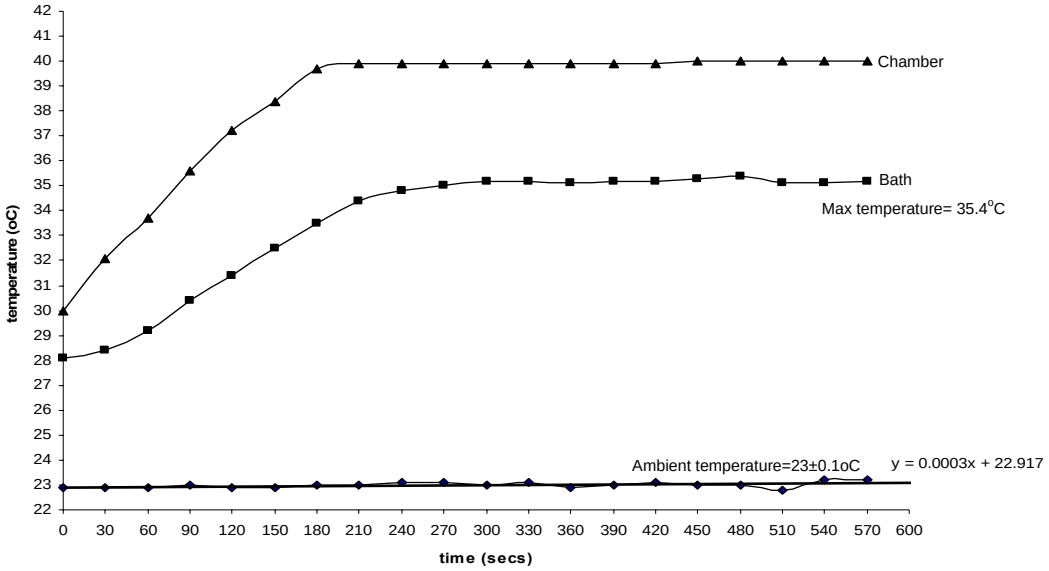
B

Heating 10°C + Immersion Objective. Lab traffic: moderately busy. Perfusion rate: 1ml/min. BV= 0.887ml



C

Heating 10oC + lid. Lab traffic: busy. Perfusion rate= 1ml/min. B.V= 0.55ml



4.1B. An IO increases the bath volume but does not affect response to a Δ10°C heating command.

The same 10 °C increase test was run in the presence of an IO, under the same lab conditions. The effect of the IO increased the bath volume to 0.89ml. The ambient temperature was 22.6±0.1 °C.

The results showed a similar chamber response to the previous exposed test, as would be expected. However, a more pronounced sigmoidal bath response to the increased temperature command was displayed. Similar to the previous test, the temperature also stabilised after 210s, with a temperature range of between 33.1 and 33.4 °C.

The difference in maximum bath temperature between the exposed test and the IO test is therefore shown to be approximately 0.7 °C, this temperature difference could be due to several possibilities such as the difference in bath size, or the IO operating as a heat sink. The difference is negligible, and the effect would be minimal and easily corrected for in a physiological experiment.

4.1C. Encapsulating the chamber with a lid facilitates a greater bath temperature but does not affect heating rate.

The effect of encapsulating the bath area was assessed after production of a lid. The same test protocol was applied as in the previous tests. The ambient temperature was 23±0.1 °C, in a busy lab. The bath volume was 0.55ml.

The chamber response was almost identical to the previous tests, as expected. The bath showed an increased temperature response, stabilising at a higher temperature within a range of 35-35.4 °C. The bath took longer to reach this maximum temperature stage (300s) compared to the previous tests, but was able to reach a higher temperature.

4.2A. A Δ10°C cooling command (40°C to 30°C) results in a total chamber cooling time of approximately 13 minutes.

Just as important as the response to a heating command is the response of the chamber to a cooling command. To further assess the speed and efficiency of commanded temperature changes, a 10°C decrease in temperature was commanded and sampled at 30 second intervals. Throughout the test the ambient temperature was 22.6±0.1 °C, and the lab was moderately busy. Perfusion rate was 1ml/min, and the bath volume was 0.43ml.

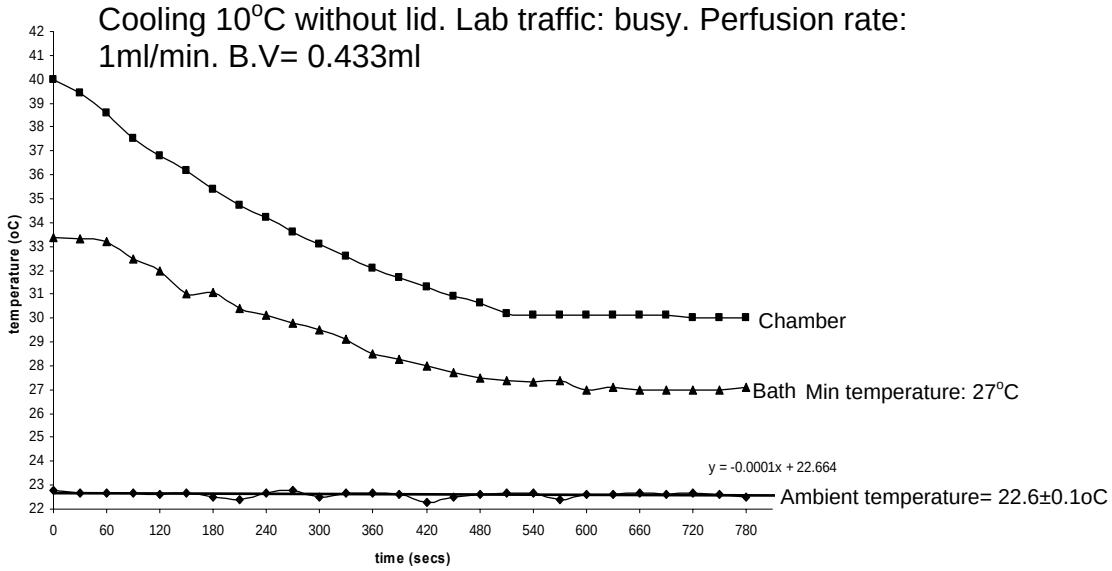
The chamber took over 510s to cool to a stable temperature of 30.1 °C, exemplified by the long shallow curve. This was until registering the commanded temperature of 30 °C, after 690s. The bath temperature decreased at a slower rate compared to the chamber, as would be expected, and stabilised at the minimum temperature of 27 °C after 630s.

4.2B. The presence of an IO does not significantly affect the cooling curve.

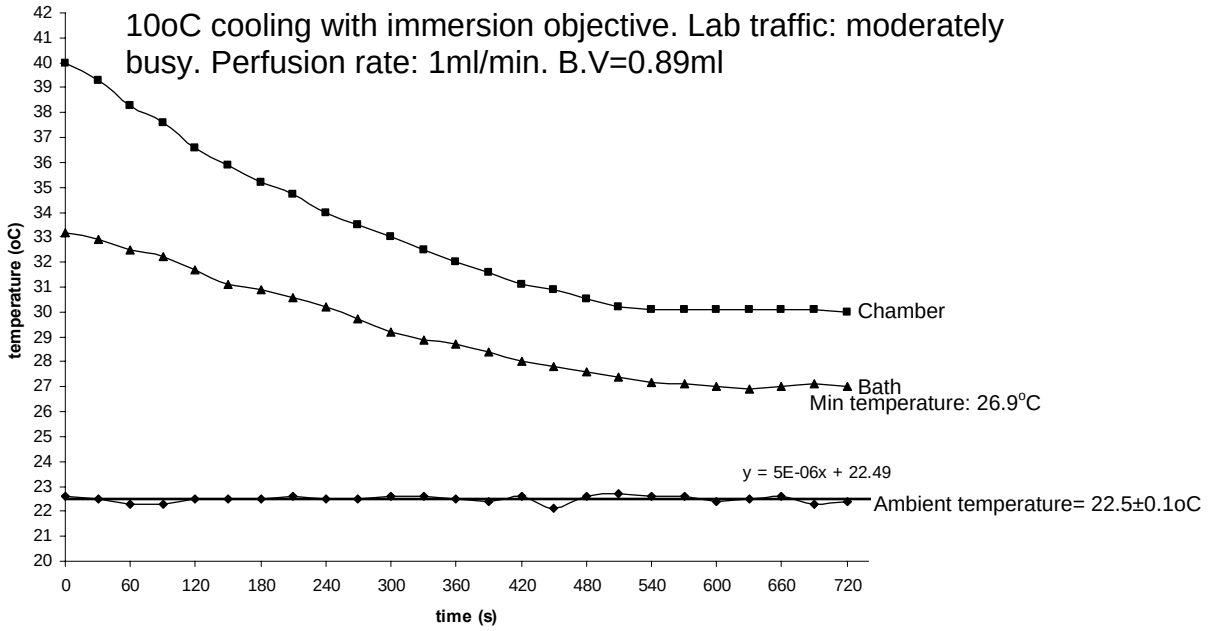
As previously described, the use of an immersion objective is a necessary part of an electrophysiological or imaging experiment. To observe the effect of the IO on

Figure 4.2.

A



B



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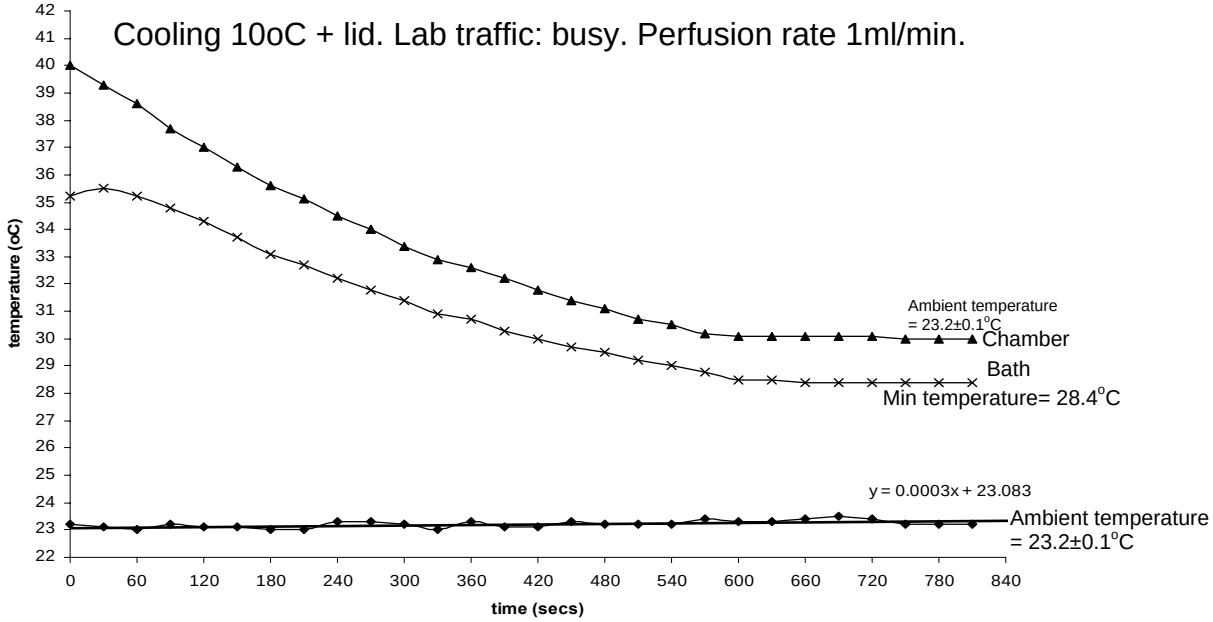


cooling a test was conducted using the same protocol as above, in the presence of an IO. The ambient temperature was 22.5±0.1 ^{o}C, in a moderately busy laboratory. The bath volume was 0.89ml.

The chamber cooling curve is almost identical to the exposed test, as expected, reaching 30.1 ^{o}C after 510s and reaching 30 ^{o}C after 690s. The bath cooling curve measured by the temperature probe shows a slightly faster rate of cooling, reaching a plateau stabilisation of between 27.1 and 26.9 ^{o}C after 510s. A 2-tailed, equal variance t-test shows the bath temperatures measured are not significantly different from those measured in the exposed test (p=0.385).

4.2C. A lid significantly affects the cooling curve by slowing cooling and stabilising the bath environment.

The use of a lid to shelter the set-up is a useful way of ensuring stability during testing and for maintaining optimum conditions for the slice. The same protocol was used in the presence of a lid to investigate its effect on the cooling of the bath. The ambient temperature was 23.2±0.1 ^{o}C, in a busy lab. Perfusion rate was 1ml/min, the bath volume was 0.55ml.

The chamber cooling has a very similar shape to the previous two tests although it reaches a temperature 30.1 ^{o}C after 570s, and stabilises at 30 ^{o}C after 720s. The bath temperature has a slower decay than in the previous tests and maintains higher temperatures per 30s sample in comparison with the previous tests. The bath temperature stabilises after 570s at 28.4-28.5 ^{o}C. A 2-tailed equal variance t-test confirms the observed temperatures in the lid test are significantly different to those in the exposed test (p=0.004).

5. The perfusion of gas over the bath reduces the temperature gradient.

The 7800 allows gas to perfuse over the heat exchanger and over the bath. Perfusion of warm gas over the bath will, in theory, reduce the temperature gradient between the bath and the environment. This would allow greater temperature control.

5.1A. Calibration analysis reveals that greater bath temperatures at set command temperatures can be reached in the presence of gas perfusion.

At 54^{o}C chamber temperature a bath temperature of 41.5^{o}C is obtained, which is 19.2^{o}C above ambient temperature. This can be compared to 40.3^{o}C without gas, which is 16.2 ^{o}C above ambient. This test was achieved in a moderately busy lab. Perfusion rate was 1ml.min, gas flow rate was 10ml/min and bath volume was 0.75ml.

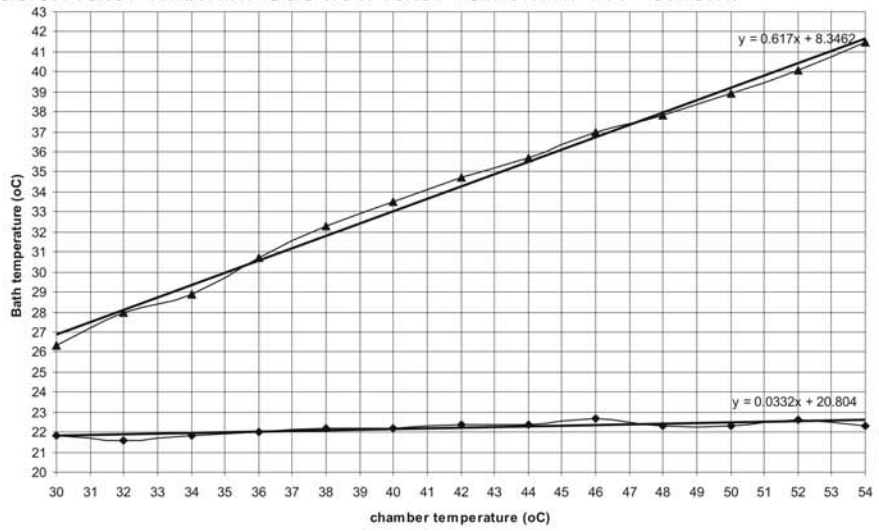
5.1B Gas does not speed up the rate of bath heating following a 10^{o}C increase chamber command temperature.

To test if gas perfusion has any effect during a command to change temperature, tests were conducted with an identical protocol to those mentioned above. The 10^{o}C increases in temperature were commanded with the addition of gas perfusion.

Figure 5.1

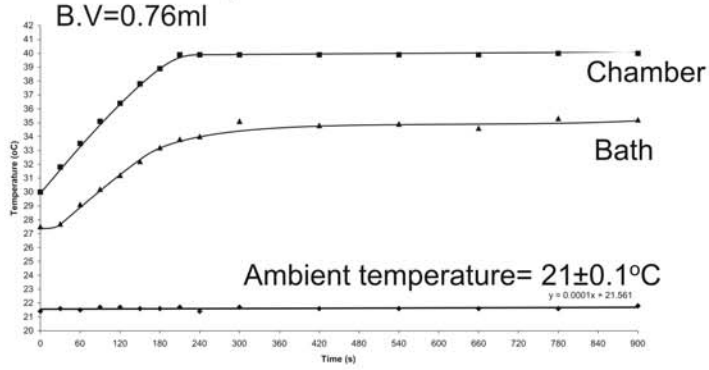
Calibration curve in the presence of gas perfusion. Lab traffic: moderately busy. Perfusion rate: 1ml/min. Gas flow rate: 10ml/min. B.V=0.75ml

A



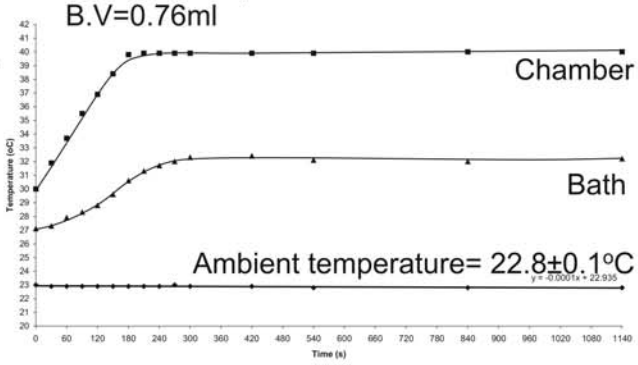
B

10 degree heat. Gas only. Lab traffic: moderately busy. Perfusion Rate: 1ml/min.Gas perfusion: 10ml/min B.V=0.76ml



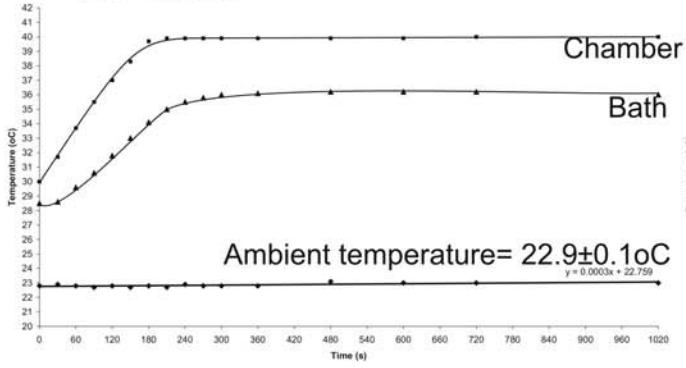
C

10 degree heat. Gas +IO. Lab traffic: moderately busy. Perfusion Rate: 1ml/min.Gas perfusion: 10ml/min B.V=0.76ml



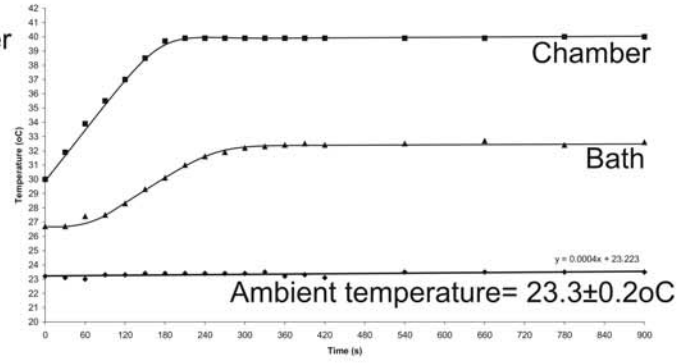
D

10 degree heat. Gas + lid. Lab traffic: moderately busy. Perfusion Rate: 1ml/min.Gas perfusion: 10ml/min B.V=0.76ml



E

10 degree heat. Gas +lid + IO. Lab traffic: moderately busy. Perfusion Rate: 1ml/min.Gas perfusion: 10ml/min B.V=1.29ml



The rate of bath heating is dependent on the chamber heating. This reaches 39.9^{o}C at the same rate in every test due to the constraints of the P.I.D algorithm. Gas perfusion does not affect chamber warming, but does increase the final bath temperature reached, which in this test was 35.3±0.1^{o}C. This test was undertaken in a moderately busy laboratory, with an ambient temperature of 21±0.1^{o}C. Perfusion rate was 1ml/min, gas flow was 10ml/min and bath volume was 0.76ml.

5.1C. The effect of an IO as a heat sink is greater in the presence of gas.

A 10^{o}C increase command was applied in the presence of gas and an IO. Chamber heating rate was unaffected in this test. The rate of bath heating seemed similar when compared to the previous test, taking 300s to reach plateau level. However, the maximum temperature reached was lower: 32.5^{o}C compared to 35.3^{o}C. The lab was moderately busy. Perfusion rate was 1ml/min, gas flow rate was 10ml/min and bath volume was 1.1ml, this larger volume could be explained by the presence of the IO.

5.1D. Gas + lid ensures bath temperatures in the physiological range at 40^{o}C chamber temperature.

The effect of encapsulating the chamber in the presence of gas does not affect bath heating rate, as this is dependent on the rate of chamber heating. However the maximum temperature reached by the bath is improved, reaching a maximum of 36.2^{o}C. The lab environment was moderately busy with an ambient temperature of 22.85±0.1 ^{o}C. Perfusion rate was 1ml/min, gas flow rate was 10ml/min and bath volume was 0.76ml.

5.1E. Encapsulating the gas + IO test with a lid does not improve the bath temperature reached.

The chamber heating rate was again un-affected as this is dependent on the P.I.D algorithm. The bath heating rate was also similar to the previous tests, reaching a plateau level after 300s. The maximum bath temperature attained in this test however was 32.7^{o}C. This is similar to that obtained in the gas + IO test. This test was carried out in a busy lab environment, ambient temperature was 23.3±0.2^{o}C. Perfusion rate was 1ml/min, gas flow rate was 10ml/min and bath volume was 1.29ml.

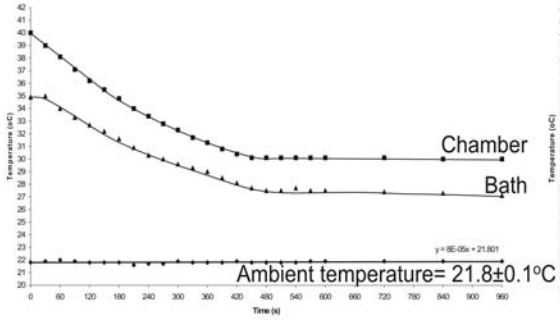
5.2A. The presence of gas perfusion increases the rate of bath cooling.

In the following tests a 10^{o}C cooling command was applied in the presence of gas perfusion.

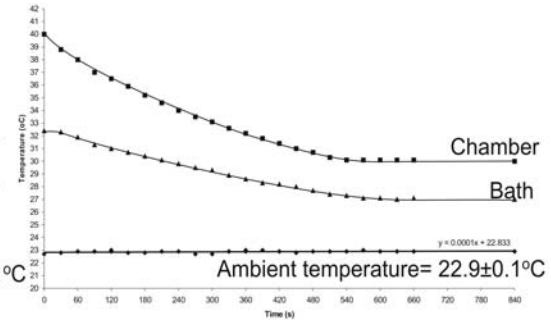
In the gas only test (figure 6.2A), the rate of chamber cooling was increased, taking 450s to reach the plateau level of 30.1^{o}C. The bath took 480s to reach a plateau temperature of 27.5. The chamber finally reached its target 30^{o}C after 840s, and the bath followed this by dropping to 27.1 ^{o}C after 960s. In the test without gas the chamber takes 540s to reach the plateau level of 30.1^{o}C, but drops to the target 30^{o}C after 720s, this is quicker than in the presence of gas. The test was undertaken in a moderately busy laboratory, ambient temperature was 21.8±0.1^{o}C. Perfusion rate was 1ml/min, gas flow rate was 10ml/min and bath volume was 0.69ml.

Figure 5.2

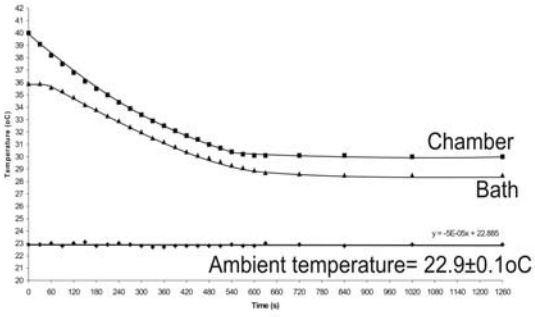
A 10 degree cool. Gas only. Lab traffic: moderately busy. Perfusion rate: 1ml/min. Gas perfusion: 10ml/min. B.V= 0.69ml



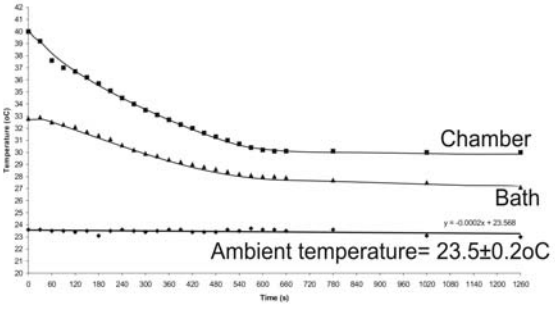
B 10 degree cool. Gas + IO. Lab traffic: moderately busy. Perfusion rate: 1ml/min. Gas perfusion: 10ml/min. B.V=1.1ml



C 10 degree cool. Gas +lid. Lab traffic: moderately busy. Perfusion rate: 1ml/min. Gas perfusion: 10ml/min. B.V= 0.76ml



D 10 degree cool. Gas + lid+ IO. Lab traffic: busy. Perfusion rate: 1ml/min. Gas perfusion: 10ml/min. B.V= 1.29ml



5.2B. Gas does not affect the rate of cooling in the presence of an IO.

The chamber cools to its near target level of 30.1^{o}C in 540s; this is the same as the test without gas. The bath also cools similarly to the test without gas, reaching the same minimum temperature, 26.9^{o}C, after 600s. The test was carried out in a moderately busy laboratory, ambient temperature was 22.9±0.1^{o}C. Perfusion rate was 1ml/min, gas flow rate was 10ml/min and bath volume was 1.1ml.

5.2C. Gas perfusion slows the rate of cooling in the presence of a lid.

The chamber reaches its near target level of 30.1^{o}C after 540s in the gas + lid test. This is 30s faster than in the test without gas perfusion. The bath temperature takes over 720s to reach its coolest temperature of 28.5^{o}C; this is 0.1^{o}C higher than the test without gas which took 660s to reach 28.4^{o}C. The lab was moderately busy with an ambient temperature of 22.9±0.1^{o}C. Perfusion rate was 1ml/min, gas flow rate was 10ml/min and bath volume was 0.76ml.

5.2D. Gas perfusion slows the rate of cooling in the gas + lid + IO test.

The chamber reaches its near target temperature of 30.1^{o}C after 600s, this is slower than in the previous gas + IO test, but similar to the previous gas + lid test. The bath cooling rate is the slowest of all the tests with gas perfusion, reaching its coolest temperature of 27.1^{o}C after 1200s. This test took place in a busy laboratory with an ambient temperature of 23.5±0.2^{o}C. Perfusion rate was 1ml/min, gas flow rate was 10ml/min and bath volume was 1.29ml.

6. Testing the effect of perfusion rate

Having assessed the different conditions that can affect temperature control and manipulation, the effect of the rate of perfusion was investigated (figure 7). The same protocol for a 10^{o}C change in temperature was used, and three different perfusion rates were tested.

In all 10^{o}C heating tests the rate of chamber heating was unaffected, its hyperbolic trend reaching 39.9^{o}C after 180s, and then taking a long time to reach the target temperature of 40^{o}C.

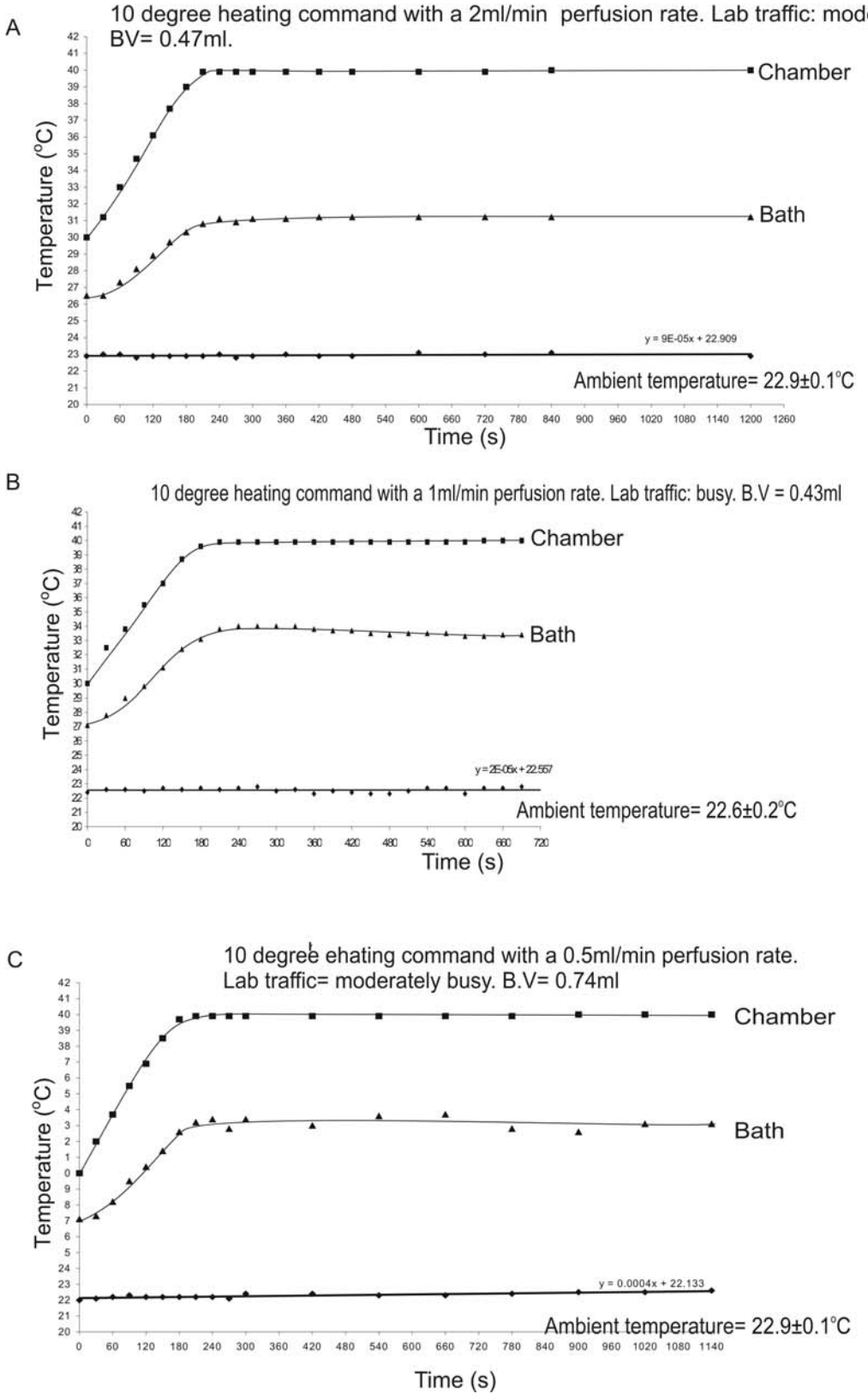
6A. A perfusion rate of 2ml/min confers a lower bath temperature, but greater stability.

After 210s the sigmoidal shape of the bath temperature curve reaches its plateau level and stabilises at 31.2 ^{o}C after 360s, the temperature range of the plateau was 30.8-31.2^{o}C. The test was carried out in a moderately busy laboratory, ambient temperature was 22.9±0.1^{o}C. Perfusion rate was 2ml/min and bath volume was 0.47ml.

6B. A perfusion rate of 1ml/min enables greater bath temperatures.

The bath took over 210s to reach its plateau temperature of 34.0^{o}C; the temperature was maintained in a range of between 33.3 and 34.0^{o}C for the remainder of the test.

Figure 6.1 The effect of perfusion rate on bath temperature following a 10 degree heating command.



Laboratory environment was moderately busy with an ambient temperature of 22.6±0.2°C. Perfusion rate was 1ml/min and bath volume was 0.43ml.

6C. A perfusion rate of 0.5ml/min shows decreased bath temperature stability.

The bath temperature plateaus after 210s, and after 900s stabilises at 33.1°C. The range in temperature over the plateau is 32.6-33.7°C. The test was carried out in a moderately busy laboratory with an ambient temperature of 22.3±0.2°C. The rate of perfusion was 0.5ml/min and the bath volume was 0.74ml.

6D. A perfusion rate of 2ml/min enhances the rate of a 10°C cool.

The chamber takes over 480s to cool to its near target temperature of 30.1°C. The bath also cools swiftly reaching its minimum temperature of 26.5°C after 750s. This test was performed in a moderately busy laboratory, the ambient temperature was 23.0±0.2 °C. The rate of perfusion was 2ml/min and the bath volume was 0.47ml.

6E. Rate of cooling is slowed in a 1ml.min test.

The chamber takes over 510s to cool to its near target temperature of 30.1°C, and over 690s to reach 30°C. The bath takes 660s to cool to a temperature of 27°C. The lab environment was moderately busy and the ambient temperature was 22.6±0.1 °C. The rate of perfusion was 1ml/min and the bath volume was 0.43ml.

6F. A perfusion rate of 0.5ml.min slows the rate of chamber cooling.

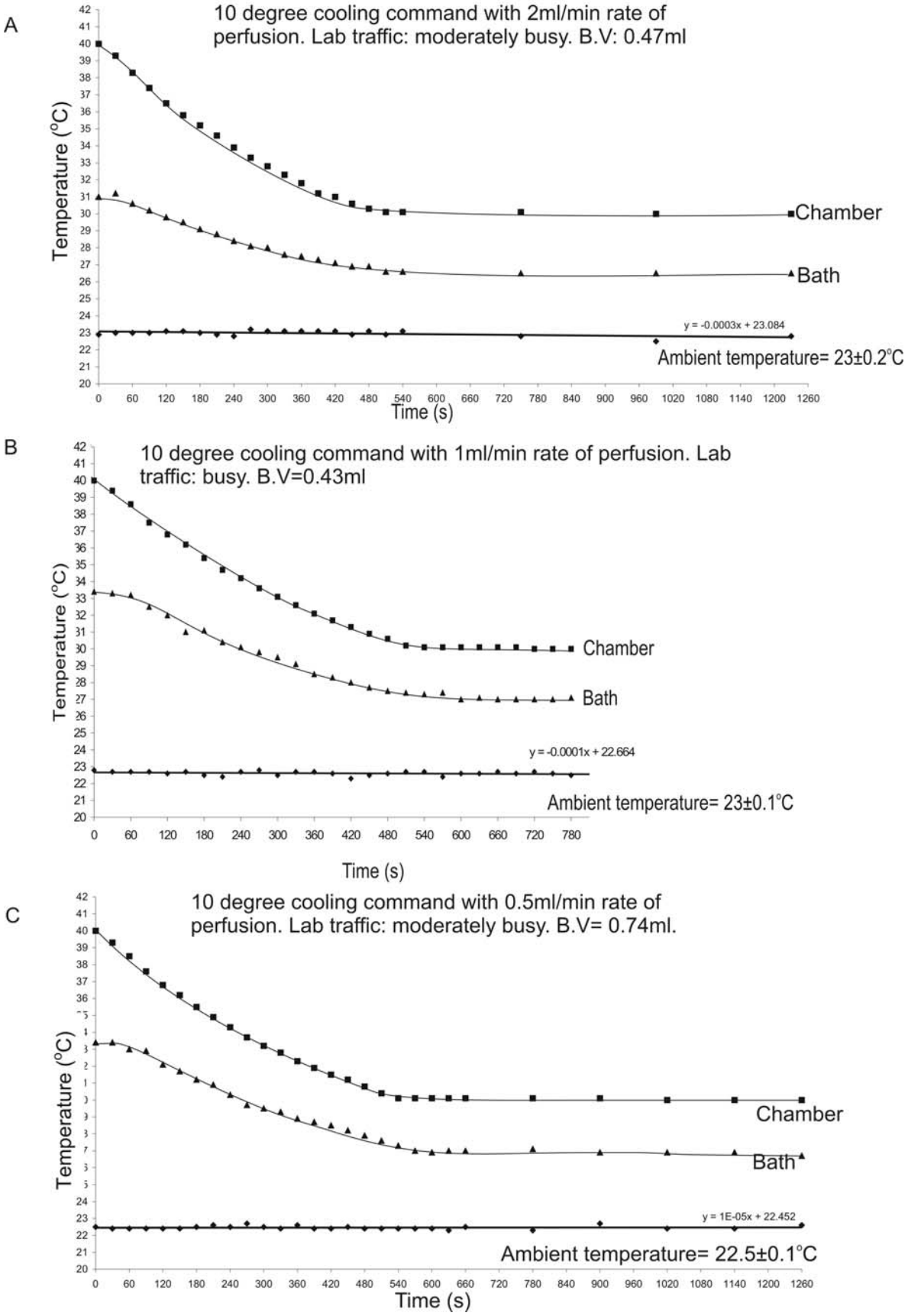
The chamber takes over 510s to cool to its near target temperature of 30.1°C, and over 900s to drop to 30°C. The bath temperature reaches 27°C after 540s and stays in a range of between 26.9-27.1°C until dropping to a final temperature of 26.7°C. This test was carried out in a moderately busy laboratory, ambient temperature was 22.5±0.1°C. The rate of perfusion was 0.5ml/min and the bath volume was 0.74ml.

7.Investigation of heat transfer properties.

Tubing enters the heat exchanger unit and moves, in one of two tracks, around the outside of the bath before perfusing into the bath. The heat exchanger heats the tubing via the aluminium body in which the tracks are situated. The journey of the tubing around the outside of the bath maximises the time for heat transfer to the perfusate before entering the bath. This design enables up to two tubes to fill each track, benefiting experiments in which pharmacological manipulation is needed.

The aim of this experiment was to investigate the size of tubing required to obtain optimal heat transfer, and perfusate delivery. Two different sizes of tubing were used, and tested at different rates of perfusion before and after the addition of heat sink compound (RS components, UK). The chamber command was set to 10°C above ambient. Perfusate temperature was measured before entering the perfusion system (labelled ‘solution’), and the perfusate temperature was measured as it entered the bath (labelled ‘bath’). The results are displayed in the following tables.

Figure 6.2 The effect of perfusion rate on bath temperature following a 10oC cooling



Tubing sizes:

- Original tubing (Campden Instruments, UK) Outer diameter (OD) = 0.75mm. Inner diameter (ID) = 0.3mm.
- Larger tubing (Cole Parmer, US) Outer diameter (OD) = 1.1mm. Inner diameter (ID) = 0.6mm

Without heat sink compound.

Original tubing: OD= 0.75mm ID= 0.3mm						
Perfusion rate	Ambient (^{o}C)	Solution (^{o}C)	bath volume (ml)	chamber (^{o}C)	bath (^{o}C)	chamber - bath (^{o}C)
0.5mil/min	21.4	21.5	0.69	31.4	28.4	3
1mil/min	22	21.9	0.63	32	29.4	2.6
2mil/min	22	22	0.68	32	26.2	5.8
Larger Tubing: OD= 1.1mm ID=0.6mm						
perfusion rate	Ambient (^{o}C)	Solution (^{o}C)	bath volume (ml)	chamber (^{o}C)	bath (^{o}C)	chamber - bath (^{o}C)
0.5mil/min	21.9	21.9	0.77	32	30.5	1.5
1mil/min	22	21.9	0.64	32	29.3	2.7
2mil/min	22.2	22.1	0.9	32	27.4	4.6

In both tubing sizes the biggest temperature difference is shown at the fastest flow rate. The best level of heat transfer is observed in the largest tubing at the slowest flow rate, a 1.5^{o}C difference between chamber temperature and bath temperature. Interestingly a flow rate of 1ml/min produces a similar temperature difference between the tubing sizes.

With heat sink compound.

original tubing + heating jelly						
Perfusion rate	Ambient (^{o}C)	Solution (^{o}C)	Bath volume (ml)	Chamber (^{o}C)	Bath (^{o}C)	Chamber - bath (^{o}C)
0.5mil/min	21.9	21.8	0.64	32	30.4	1.6
1mil/min	21.9	21.8	0.66	32	29.7	2.3
2mil/min	21.9	21.8	0.38	32	27.8	4.2
Larger Tubing + heating jelly						
Perfusion rate	Ambient (^{o}C)	Solution (^{o}C)	Bath volume (ml)	Chamber (^{o}C)	Bath (^{o}C)	Chamber - bath (^{o}C)
0.5mil/min	22.2	21.7	0.46	32	30.8	1.2
1mil/min	21.9	21.7	0.52	32	30.5	1.5
2mil/min	21.9	21.7	0.68	32	29	3

The heat sink jelly improved heat transfer to both sets of tubing, reducing the difference between the chamber temperature and the bath temperature for every test. The larger tubing size and 0.5ml/min perfusion rate produce the lowest temperature difference between the chamber and bath. Accordingly the fastest perfusion rate and smaller tubing produced the greatest temperature difference.

8. The CI7800 contributes a small amount of noise to the rig set-up.

Electrophysiology is used to measure small ionic currents elicited by cells. Signal interference or electrical noise that interferes with these measurements can severely impair data acquisition. It is of great importance to eliminate or dampen all sources of electrical noise from within an electrophysiological rig, and this can be achieved by correct grounding.
The CI7800 was installed as part of a working electrophysiology rig, and its contribution to noise signals within the rig was assessed using the I_{RMS} function on the Axopatch 200B (Axon instruments, US) rig amplifier.

The test was carried out under quiet lab conditions, with an ambient temperature of 20.3^{o}C. Carbogen bubbled aCSF was perfused into the bath at a rate of 1ml/min. The set temperature was 47.1^{o}C, and the bath temperature was 37.8^{o}C. Bath volume was 0.76ml.

Condition	I_{RMS} value (pA)
Pre –CI7800 power-on	0.89
CI7800 Power on	0.89
CI7800 at final chamber temperature	0.96
CI7800 + temperature probe	0.98

The results show that the noise contribution of the CI7800 increased as the chamber temperature increased. When the chamber had reached its set temperature of 47.1^{o}C the I_{RMS} was 0.96pA, compared to 0.89pA at the power-on stage. The signal to noise value increased to 0.98pA when the CI7800 temperature probe was added to the bath.

Discussion

The results have described the magnitude of the influence the external environment has on temperature control and stability. The environment is a huge thermal mass compared to that of the bath and so there is understandably a massive temperature gradient present. This is neatly described by the tests in which the chamber is encapsulated. The encapsulation enables control of the bath environment and so facilitates manipulation of the temperature gradient, thus aiding thermal stability. Other features also contribute to better thermal performance, such as optimum perfusion rates and tubing diameters.

The PID algorithmic control of the chamber heater restrained the heating rate of the unit. An adjustment of the algorithm control could enable a faster heating rate time-to-half-rise.

An Immersion Objective produced an offset in the bath temperature, but stabilised. This could be compensated for in an experiment. Rates of heating and cooling in the presence of an IO were not significantly different to those in IO absence.

The signal contribution of the unit to set-up noise was dependent on heating temperature. A higher set temperature required more power usage and so generated

more noise. The noise level generated was marginal, less than 0.1pA RMS. Addition of the temperature probe to the bath increased the I_{RMS} by 0.02pA.

In summary, the CI7800 is a sensitive temperature control device that allows a temperature resolution to 2dp. The device ensures a bath temperature stability that is within the acceptable range for *in vitro* electrophysiological experiments. The rate of bath heating needs assessment, but the stability of the chamber temperature given the exposed conditions displays the high level of performance ensured by the CI7800.