Chemistry Experiment 1: Pipetting

Quantitative techniques; Precision and Accuracy in Measurement, Analysis of the Propagation of Error and, the use of Significant Figures in laboratory Calculations.

Aim:

The aim of this simulated pipetting experiment is generally a requirement for the chemist in the laboratory to understand/or practise the correct quantitative techniques in order to use laboratory equipment. Consideration of calculated errors in measurement and sources of error contained in significant figures are practically an essential part of chemistry rationale and by the end of this introductory pipetting experiment, the analyst should be able to use the equipment with confidence and accuracy to obtain satisfactory results.

The learning outcomes of this practical include the following chemist's badge of honour code:

- Understand the correct techniques of the use of a volumetric pipette.
- To be familiar with the correct handling and use of a micropipette.
- Understand the correct techniques of the use of an analytical balance.
- Understand experimental errors (Quantitative) as it applies to chemistry and learn how to operate manufactured equipment in a basic general order of processes (qualitative) (Fudge 2014, p. 138).

Method:

As this was a simulated experiment, please refer to part A (Fudge 2014, pp.140-141) of the laboratory report book manual for volumetric pipette procedure. For part B please refer to part B (Fudge 2014, pp. 142-144) of the laboratory report book manual: systematic micropipette calibration techniques.

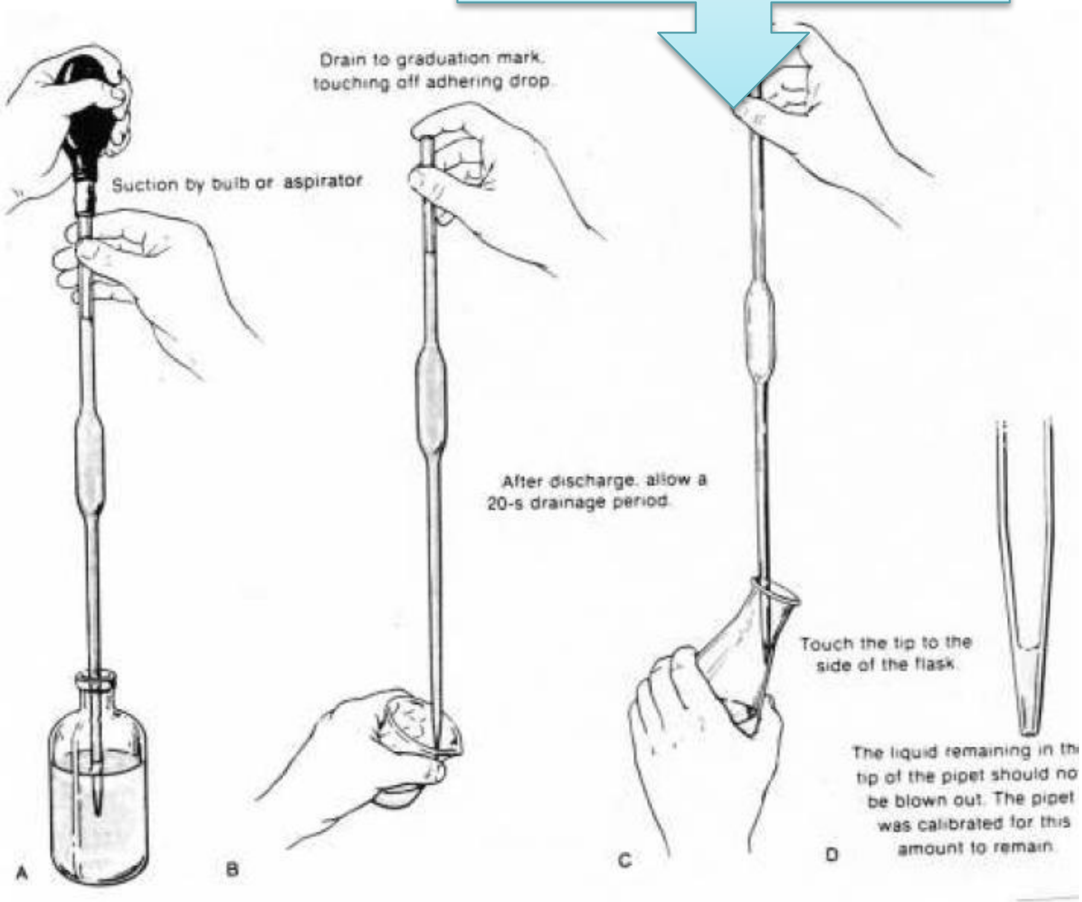
Observations:

In the lab the volumetric pipette is used to deliver a fixed volume under specific conditions with a level of precision and accuracy (NAIT 2008). To measure liquids with a pipette there are seven steps that outline the procedure:

1) Make sure the pipette is clean
2) Blow out any left over liquid
3) Rinse the pipette three times
4) Draw up aliquot and wipe the tip
5) Set the meniscus
6) Deliver the aliquot with the pipette at vertical level while the flask is at a 30˚ angle

Illustration outlining the general procedure of how to calibrate a volumetric pipette. Image source: (Docstoc 2012).

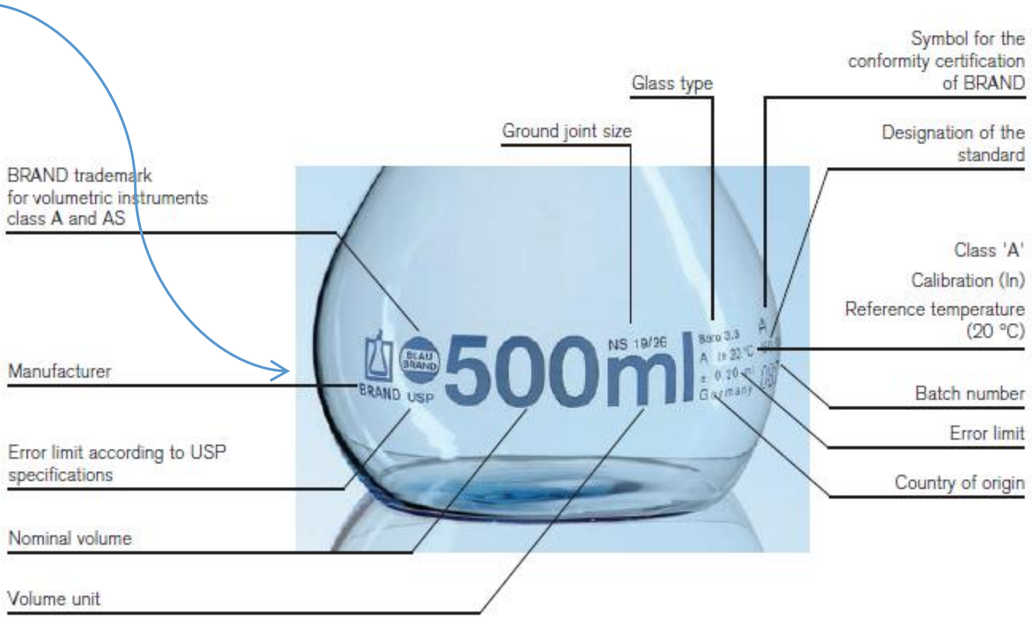
7) Wash the sides of the flask (NAIT 2008)



Identification of BLAUBRAND® USP volumetric instruments

Example: BLAUBRAND® volumetric flask, USP, class A

Illustration showing an example BLAUBRAND volumetric flask. Image source: (BRAND 2013, p. 3).



Here is the 'fake data' sheet supplied by UniSA lecturer Anthea Fudge.

Results:

UniSA

For externals or those who missed it

'Fake' data to use for Chemistry Practical 2: Quantitative Techniques

• Pipetting results: (using 20mL volumetric pipette)

Tube	Initial (no water) (g)	Final (water) (g)	Weight (g)
1	10.8765	30.9850	?
2	11.1002	31.2599	?
3	10.9215	30.0091	?

?

∴ • You will need to calculate the weight of water in (g) grams.

The temperature: was found to be 15.0°C

Use the density of water graph to find the density of water at that temperature.

⇒ Then use the equation

$$d = \frac{m}{V}$$

to calculate the volume of water that you pipetted. Note you will need to re-arrange the equation to make volume the subject.

(The density should be read off the graph to 4 decimal places ie. 0.9989)

& $cm^3 = mL$ ∴ density is g/mL.

Here is the 'fake data' sheet supplied by UniSA lecturer Anthea Fudge.

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Pipetting results: (using 10mL volumetric flask)

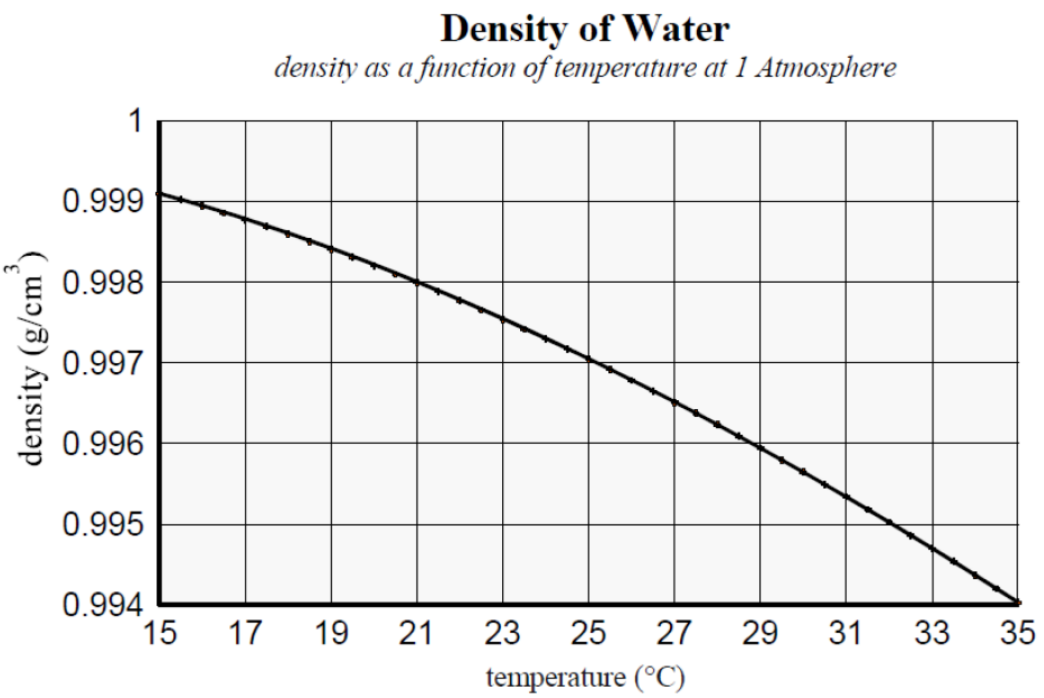
Tube	Initial (no water) g	Final (water) g	Weight (g)
1	10.9001	20.1002	?
2	11.2765	21.5662	?
3	10.0897	20.3427	?

?: You will again need to calculate the weight of water in (g) grams.

The temperature of this water was still also 15.0°C.

Therefore, do the calculations the same way as before - and calculate the volume of water again (you are obviously hoping for ~ 10mL)

(Image source on previous pages 3-4: Fudge, A 2014, *'Fake data' to use for chemistry practical 2: Quantitative techniques*, NASC18, University of South Australia, Adelaide).



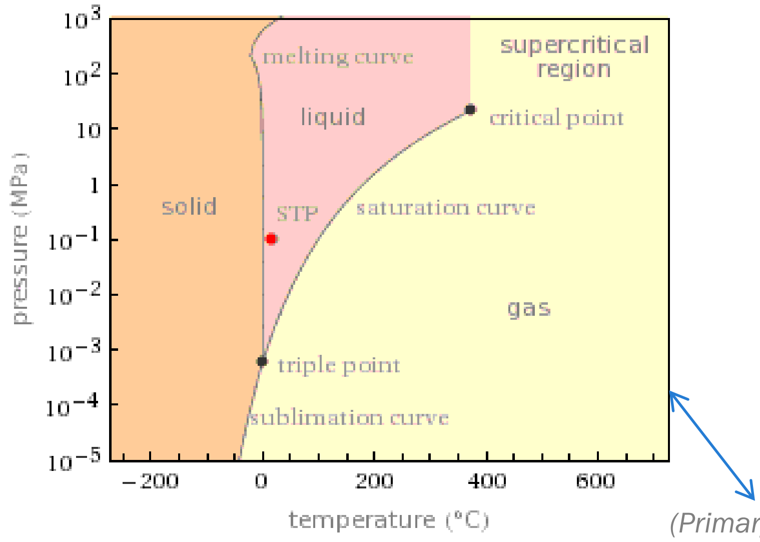
(Image source: Fudge 2014, p.145)

Enthalpy of liquid water at the temperature of 15.0° Celsius.

Thermodynamic properties chart:

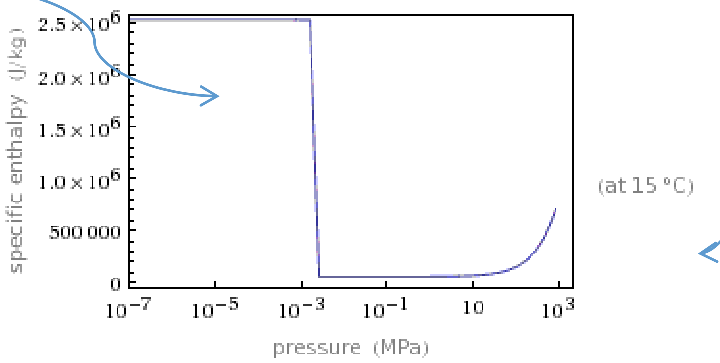
phase	liquid (liquid water)
temperature	288.2 K 15 °C
pressure	101325 Pa 1 atm
density	999.1 kg/m³ 0.9991 g/cm³
speed of sound	5277 km/h 1466 m/s

(Image source: Wolfram Alpha 2014)



(Primary image source: Wolfram Alpha 2014). This computational data illustrates the phase diagram of temperature and atmospheric pressure.

Primary source image: taken from Wolfram Alpha 2014 computational data that illustrates the variation of temperature (at 15.0 degrees Celsius) with constant



pressure.

Calculations:

<u>Calculations for the 20ml volumetric pipette:</u>

<u>Tube 1:</u> Firstly, to calculate the weight of the water for tube 1, some pure mathematics is applied to the task. 30.9850-10.8765 is put into the pocket calculator that gives a reading that equals=20.1085ml. The next order of operations is to re-arrange the equation to make volume the subject using the standard equation V (volume) =M (mass)/D (density). But before calculations begin it is important to find what the density off the graph given for 15˚Celsius is. The answer is of the temperature reading is 999.1. For tube 1, next is to divide this density value by 1000

to obtain it in g/ml units. This gives a reading of 0.9991. Then it is necessary to find the volume. To do this simple calculations for volume say V=20.1085g/ (0.9991g/ml) which gives the final answer 20.12661395ml.

Tube 2: Firstly, to calculate the weight of the water for tube 2, the same pure mathematics task is applied again as previously with tube 1. (31.2599-11.1002)=20.1597. Then it is time to re-arrange the equation to make volume the subject. V=20.1597g/ (0.9991g/ml) which gives an answer of V=20.17786007ml.

Tube 3: (30-0091-10.9215)=19.0876. Then the density=M/V formula is used. To make volume the subject for tube 3 it is important to again re-arrange the equation to make V=19.0876/ (0.9991g/ml) that gives 19.10479431ml.

Pipetting calculation results: using 10ml volumetric flask

Tube 1: (20.1002-10.9001) =9.2001. Next it is useful to use D=m/v to find the volume. Re-arrange the equation to make volume central V=m/d. V=9.2001/ (0.9991g/ml). This gives the volume reading 9.208387549ml.

Tube 2: (21.5662-11.2765) =10.2897. Next the density which equals the mass divided by the volume is applied. So, the equation is re-arranged to give volume the subject matter (V=m/d). Say this volume V=10.2897/ (0.9991g/ml) gives the final volume of 10.29896907ml.

Tube 3: (20.3427-10.0897g) =10.253. Next it is time to apply the equation D=m/v. So, the equation re-arranges to V=m/d. Because volume is the subject the volume equals= V=10.253/ (0.9991g/ml). The final reading of volume is 10.26223601ml.

Discussion:

Report book questions

Pipette calibration verification by the operator is done to verify if error limits for the 20ml volumetric pipette and the 10ml volumetric flask actually equals around the calibrated mark error limit printed in the individual BRAND certificate (BRAND 2013, p. 1).

The factors that could attribute to possible sources of error in the experiment can include changes in temperature, air displacement between the liquid and the piston, and either the reduction or increasement of surface area liquid being filled into the tip (ThermoScientific 2010, p. 10).

The mean volume for the BLAUBRAND volumetric pipette(20ml) which equals a sum of 46.68 after rounding off the digits from 46.6727379 is not within the measured range of 50(±0.05). This leaves for a margin of error of 3.32%. Fudge confirmed this by email on 1 June 2014 that for example; if the mean (average) were 49.95 or 50.05 then the average would sit within the manufactures claim.

The mean deviation for the BLAUBRAND® volumetric flask (10ml) was calculated at 22.9280899 on the pocket calculator. The digits are then re-calculated and rounded off to 22.93. For the 10ml volumetric BLAUBRAND®amber glass, class A, USP batch certificate states that the error limit for the flask is ±0.02, so to specify if whether the reading is within range of error printed on the certificate, a simple calculation was done (BRAND 2013, p. 4). 22.93-20(range)= 2.93% margin of error %. This informs and advises the analyst that the standard deviation of this flask is about .93%. Interestingly, BLAUBRAND volumetric instruments are calibrated with and accuracy and precision level of up to 99.6 %(BRAND 2013, p. 1).

The manufacturer claims that the calibrated error limit of up to 0.03 is expected for a BLAUBRAND volumetric pipette (BRAND 2013, p. 4). In the BLAUBRAND glass Instruments auditing of current DIN EN ISO Standards, USP Certificates outlining Class A specifications for individual error limits are audited by the FDA (Food & Drug Administration Authority) under the current United States pharmaceutical regulations body (BRAND 2013, p. 2). The same regulations apply to the 10ml capacity of the Class A, amber glass volumetric flask.

This practical also involved the correct measurement and weighing of the mass of water delivered by both types of pipettors. In this report log book errors have shown to vary with the different sampling sizes being weighed. Kaur (2008, p. 48) states that in the physical world errors in measurement that could affect the outcome of the experiment include anything from a '…faulty construction of balances', to the 'use of improperly calibrated weights', to also errors in 'graduated glassware and other instruments'.

The density=mass/volume equation has been used for the purpose of this experiment using the density of water graph provided in the lab manual, on page 145 of Fudge (2014). The accuracy and precision of the pipetting results are to be examined. It is interesting to note that to remain constant with accuracy in general; the thermodynamic; heat transfer specific enthalpy of liquid water at the temperature 15 ˚ Celsius is given to show how thermodynamics of a given temperature is closely related to the amount of air pressure and/or displacement being delivered by the pipettor (Wolfram Alpha 2014).

BLAUBRAND's reference temperature of all the volumetric instruments in the catalogue is calibrated at 20˚ C (BRAND 2013, p. 2). Heat variations at additional temperature can result in different error composition levels as they tend to change. However, the manufacturer claims that their volumetric instruments do have a smaller probability of making significant errors (BRAND 2013, p. 2). Therefore, if significant errors are made then the possibility of making propagation of errors in lab work lies not within the volumetric instruments which is made by the manufacturer.

Chemist's commonly use the standard deviation formula to work out how accurate the pipetting trials were. The formula is given in the mathematical expression below:

$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(x_i - \mu\right)^2}$$

(Image source: (Truman University 2013)

In statistical measurements this equation below is of a higher precise indicative marker:

$$S = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}\left(x_i - \bar{x}\right)^2}$$

(Image source: (Truman University 2013)

Typically, chemists also manually work out the percentage error of the pipetting trials in the following manner below (Truman University 2013):

$$\% \text{ error} = \frac{(\text{accepted value} - \text{measured value})}{(\text{accepted value})} \times 100$$

For the accuracy of the analytical balance used in the experiment, please consult Fudge (2014, p. 140) that states the balances accuracy of ± 0.0001g per weighing of quantities.

Conclusion:

This experiment showed the correct way to pipette in a lab setting. The study conducted indicates that while it is assumed that systematic error is tested in the relevant department of chemistry, still an amount of uncertainty (represented as a percentage %) can remain under the parameters of standard deviation. This is why bio-clinical standards are used to test the precision and accuracy of the quantitative methods used so that by means of ruling out any reference sample error, absolute and relative error, and any indeterminate error are not found to be reproducible (Fudge 2014, p. 244-246). With further statistical measurements it can serve a higher precise, indicative marker to gain general chemistry understanding.

Questions:

Four important things to remember for correct pipetting technique are described in the following objectives. According to a ThermoScientific Finn pipette good laboratory guide there are four there are four pipetting techniques to be used for general micro-pipettors. These are noted in forward pipetting, repetitive pipetting, reverse pipetting, and lastly pipetting of heterogeneous sample material (ThermoScientific 2010, pp. 6-7). The forward pipetting technique is standard measure for aqueous solutions (ThermoScientific 2010, p. 6). Repetitive pipetting technique is used for repeated pipetting of the same sample volume (ThermoScientific 2010, p. 7). Reverse pipetting is used for high viscosity solutions and is recommended for pipetting small volumes (ThermoScientific 2010, p. 7). While pipetting of heterogeneous samples is only intended for blood or serum.

References

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