PRACTICAL 2: DETERMINATION OF SODIUM AND POTASSIUM ION CONCENTRATIONS

2.1 Introduction

The **aims** of this practical are:

- to demonstrate safe laboratory working procedures
- to demonstrate two simple but different techniques for making the clinically important measurements of sodium and potassium ion concentration
- to introduce the simple concept of quantitation using a calibration curve
- to highlight the difference between accuracy and precision and illustrate the importance of these factors in clinical assays
- to practice the correct use of automatic pipettes required for later practical classes

2.2 Theory and background

(**Note**: for a description of sodium, potassium and electrolyte balance see Marshall, W.J. "Clinical Chemistry". 3rd edition (1995), Mosby (RB 112.5.M36.3). In the spirit of PBL, only the briefest information is given here. You should attempt to find out the details for yourself).

Sodium and potassium

The major cation of the **extracellular fluid** is **sodium**. The typical daily diet contains 130-280 mmol (8-15 g) sodium chloride. The body requirement is for 1-2 mmol per day, so the excess is excreted by the kidneys in the urine.

Reference range (intervals) for sodium	
Serum	136-145 mM
Cerebrospinal fluid	130-150 mM
Sweat	10-40 mM
Urine (varies with intake)	40-220 mmol/day

Hyponatraemia (lowered plasma [Na^+]) and **hypernatraemia** (raised plasma [Na^+]) are associated with a variety of diseases and illnesses and the accurate measurement of [Na^+] in body fluids is an important diagnostic aid.

Potassium is the major cation found **intracellularly**. The average cell has 140 mM K^+ inside but only about 10 mM Na^+. K^+ slowly diffuses out of cells so a membrane pump (the Na^+/K^+-ATPase) continually transports K^+ into cells against a concentration gradient. The human body requires about 50-150 mmol/day.

Reference range (intervals) for potassium	
Serum	3.5-5.1 mM
Cerebrospinal fluid	about 70% of serum
Sweat	4.0-9.7 mM (men) 7.6-15.6 mM (women)
Urine (varies with intake)	25-125 mmol/day
Erythrocytes (intracellular)	105 mM

Hypokalaemia (lowered plasma [K^+]), **hyperkalaemia** (increased plasma [K^+]) and **hyperkaluria** (increased urinary excretion of K^+) are again indicative of a variety of conditions and the clinical measurement of [K^+] is also of great importance.

The flame photometer

A traditional and simple method for determining **sodium** and **potassium** in biological fluids involves the technique of emission **flame photometry**. This relies on the principle that an alkali metal salt drawn into a non-luminous flame will ionise, absorb energy from the flame and then emit light of a characteristic wavelength as the excited atoms decay to the unexcited ground state. The intensity of emission is proportional to the concentration of the element in the solution. You are probably familiar with the fact that if you sprinkle table salt (**NaCl**) into a gas flame then it glows bright orange (**KCl** gives a purple colour). This is the basic principle of flame photometry. A photocell detects the emitted light and converts it to a voltage, which can be recorded. Since Na^+ and K^+ emit light of different wavelengths (colours), by using appropriate coloured filters the emission due to Na^+ and K^+ (and hence their concentrations) can be specifically measured in the same sample. One drawback of flame photometers, however, is that they respond linearly to ion concentrations over a rather narrow concentration range so suitable dilutions usually have to be prepared. They are also rather complex and relatively expensive machines, as you will see.

A flame photometer can also be used to measure the element **lithium** in serum or plasma in order to determine the correct dosage of lithium carbonate, a drug used to treat certain mental disturbances, such as manic-depressive illness (bipolar disorder).

- *In this practical (Experiment A) you will calibrate a flame photometer using standard sodium and potassium solutions then measure the Na^+ and K^+ concentrations in a redissolved oral rehydration sachet.*

Ion-selective electrodes (ISEs)

You should already be familiar from A-level studies with the **pH electrode**, the most essential component of which is a sensitive glass membrane which permits the passage of **hydrogen** ions, but no other ionic species. A small potential difference develops across the membrane which is proportional to the logarithm of the H^+ concentration (pH) and which can be measured on a millivoltmeter. **Ion-selective electrodes (ISEs)** which respond relatively specifically to other ions (both **anions** and **cations**) operate on the same principle. In clinical laboratories they can be used to measure Ca^{2+}, K^+ and Cl^- in body fluids (blood, plasma, serum, sweat) and F^- in skeletal and dental studies. They are also used to measure a wide variety of other ions in, for example, environmental studies.

When compared to many other analytical techniques, ISEs are inexpensive and simple to use and, unlike the flame photometer, have a linear response over a wide concentration range. However, they have some disadvantages that require attention if good results are to be obtained:

1. First, despite their name, many of them are not entirely ion-specific. For example, the **sodium electrode** you will use also responds to **potassium ions** , although not with the same sensitivity. This means that Na^+ will be overestimated if a high concentration of K^+ is present. Mathematical techniques have been devised to compensate for this.

2. Secondly, they underestimate high concentrations because of "crowding" of the ions at the membrane – some just don't get "seen". The **activity coefficient** is a measure of this: **activity** equals **concentration** at low values, but is less than concentration at high values. ISEs measure activity.

    - *In this practical (Experiment B) you will use a sodium ISE to measure the apparent Na^+ concentration in a redissolved oral rehydration sachet **without** compensating for the presence of K^+ ions.*

Accuracy and precision

Accuracy (i.e. how close the measured value is to the true value) and **precision** (how close a series of measurements on the same sample are to each other) are two distinct factors in determining the usefulness of a clinical chemical assay. If the Na^+ concentration in a biological fluid is, say, **140 mM** and **method A** gives an averaged value of **137 mM** while **method B** gives a value of **125 mM**, then method A is more **accurate**. However, if repeated measurements by A gives values of 140, 147, 134, 127, 130 and 144 mM while B gives 124, 125, 125, 125, 126, and 125 mM, then B is clearly more **reproducible**, i.e. it is more **precise**. The ideal clinical assay should have high accuracy AND precision.

The concept of quality assurance

Quality assurance is the process that is performed to ensure that an analytical method produces reliable results. The performance of any assay can be monitored by measuring the concentration of the analyte (i.e. the substance being analysed) in samples that contain a known quantity of the analyte. These samples are called internal quality controls (IQC) and are analysed every time a batch of (patient) samples is processed. The actual values obtained are compared with the expected values and by cumulating these internal QC results the precision and accuracy of the method can be defined. As stated above, precision reflects the reproducibility of the method and accuracy reflects how close the measured value is to the actual value. Acceptable limits can be set for any analysis by determining the precision and accuracy using multiple measurements of the internal QC samples (n=20-30). The accuracy of any assay depends on the availability of standard material of the highest quality with an assigned concentration established by a gold standard analytical method.

- *At the end of the practical you will compare the results you obtained for Na^+ measurement by the two methods with the known value of Na^+ in the redissolved sachet and also compare the accuracy and precision of the two methods using the class data.*

2.3 Experiment A: Determination of Na^+ and K^+ in solution by flame photometry

Reagents: **oral rehydration sachet**
NaCl standards: 0.25, 0.5, 1.0, 2.0, 4.0 and 5.0 mM
KCl standards: 0.1, 0.2, 0.5, 1.0, 1.5, 2.0 mM

Procedure:

1. Carefully open an **oral rehydration sachet** and empty the contents into a **clean** 250 ml beaker. Add about 150 ml distilled water and gently swirl the contents until dissolved.
2. Pour the solution into a 200 ml volumetric flask and rinse out the beaker with small amounts of distilled water, adding the washings to the flask. Finally, make up the flask to exactly 200 ml and mix thoroughly.
3. Make a 1/50 dilution of the redissolved sachet solution by accurately pipetting 2 ml of the solution into a 100 ml volumetric flask and making up to 100 ml with distilled water**. Retain the undiluted solution for Experiment B**.

Instructions for use of the flame photometer:

4. Ensure that the photometer drain is leading into a sink and that the instrument is connected to gas, air and electricity supplies. Ensure the mains supply gas tap is **off**.
5. Turn the "**Sensitivity**" and instrument "**Gas**" controls control fully counterclockwise (towards you).
6. Insert the **sodium** optical filter.
7. Switch on the instrument and unclamp the galvanometer by turning counterclockwise.
8. Open the mica window, turn on the mains gas supply, light the gas and close the window.

(**CAUTION: DO NOT LEAN OVER THE INTRUMENT OR YOU WILL SET YOUR HAIR ALIGHT**)

9. Turn on the air supply control and adjust the air pressure to 10 lb/in^2. Leave for 1-2 minutes to stabilise.
10. Place a beaker of distilled water into position at the left hand side of the instrument and insert the narrow draw tube into it to allow water to pass through the photometer. (**NOTE: once set up, the photometer must have water running through it at all times when a salt solution is not being measured. The rate of uptake is fast, so make sure there is always enough water in the beaker**).
11. Adjust the gas control to give a flame with a large central blue cone then, with water passing through the instrument, **slowly** close the gas control until ten separate blue cones just form.
12. Set the galvanometer to zero using the "**Set zero**" control.

13. Replace the distilled water with the 5 mM NaCl standard and adjust the "**Sensitivity**" control till the galvanometer reads 100.
14. Quickly but carefully, replace the 5 mM NaCl standard with standards of decreasing concentration from 4 mM to 0.25 mM and note the readings in the Table below.
15. Run water through the instrument again for 1-2 min then place the draw tube into a beaker containing the **1 in 50 diluted** rehydration sachet solution and note the galvanometer reading.
16. Run water through the instrument again and replace the sodium with the **potassium** filter.
17. Repeat the above procedure with the KCl standards, setting to 100 with 2.0 mM KCl, then reading the others in reverse order. Then read the **1 in 50 diluted** rehydration sachet solution.
18. Finally, run water through the instrument until the flame appears free of colour again.
19. <u>When the instrument is no longer required</u>, switch off in the following sequence:

    i. Turn off the gas control and the mains gas supply
    ii. Wait for the flame to die out.
    iii. Turn off the air supply.
    iv. Switch off the electricity
    v. Clamp the galvanometer.

$[Na^+]$ (mM)	**5.0**	**4.0**	**2.0**	**1.0**	**0.5**	**0.25**	**0**
Galvo reading.	100						0
$[K^+]$ (mM)	**2.0**	**1.5**	**1.0**	**0.5**	**0.2**	**0.1**	**0**
Galvo. reading	100						0

20. Plot the galvanometer readings against Na^+ and K^+ concentrations on the graph paper provided (separate graph for each ion) and from these **calibration curves** determine the Na^+ and K^+ concentrations in the diluted sachet solution. Finally, calculate the Na^+ and K^+ concentrations in the **undiluted** sachet solution.

	Galvanometer reading	Diluted concentration (mM)	**Undiluted concentration (mM)**
Sodium ion			
Potassium ion			

2.4 Experiment B: Determination of Na^+ in solution with an ion-selective electrode

Reagents: **oral rehydration sachet**
NaCl standards: 10, 20, 40, and 80 mM

Technical note: In order to measure the change in potential difference across the ion-selective membrane as the ionic concentration changes, it is necessary to include in the circuit a **reference electrode** which acts as a half-cell from which to measure the relative deviations.

Procedure:

NOTE: the bottom surfaces of the sodium ISE and reference electrode are very fragile. Do not let them drop on to the bottom of a beaker or bottle.

1. Ensure that the multimeter (yellow box) and electrode amplifier (grey box) are connected properly with the **black** cable in the lowest socket (**COM**) and the **red** cable in the middle socket (**VΩmA**) and that the silver BNC connector from the electrode head is plugged into the back of the amplifier.

2. With the electrodes immersed to a depth of about 3-4 cm in NaCl storage solution, switch on the multimeter to the **2000 mV** position (4 clicks counterclockwise) and the amplifier to position **I** (down). Wait until the reading has stabilised (up to 5 min.) then remove the electrodes from the storage solution, wipe the bodies (but **NOT** the bottoms) with a tissue, transfer to a beaker of distilled water and rinse with swirling for about 30 sec.

3. Remove the electrodes from the water, wipe as before, then transfer to the 10 mM NaCl standard. Swirl the solution for 15 sec to help the ISE membrane to equilibrate with the solution, then leave undisturbed for a further minute. Note the multimeter reading (this is millivolts, mV) (the meter may fluctuate if you wave your hand over it so ensure that the environment is undisturbed when taking the reading).

4. Remove, wipe and transfer the electrodes to the 20 mM standard and measure as before, waiting one minute before taking the reading.

5. Repeat the procedure for the 40 mM and 80 mM standards.

$[Na^+]$ (mM)	**10**	**20**	**40**	**80**
Log_{10} $[Na^+]$				
Meter reading (mV)				

6. Finally, take a reading in the same way from the **undiluted** redissolved sachet from Experiment A.

7. Transfer the electrodes back to distilled water and, <u>if nobody else is going to use the system</u>, switch off the multimeter and amplifier (position O).

8. Plot mV (*y*-axis) against $\log_{10}$ [Na^+] (*x*-axis) and from this graph determine the apparent [Na^+] in the redissolved sachet solution.

Meter reading for undiluted sachet solution	Log_{10} [Na^+] from graph	[Na^+] in redissolved sachet (mM)

2.5 Results:

1. From the information provided on the sachet packaging, calculate the expected concentrations of Na^+ and K^+ ions in the solution you made.

[Na^+] (mM)	**[K^+] (mM)**

2. How do your own values for Na^+ and K^+ determined by flame photometry and for Na^+ determined with the ISE compare to the expected values?

3. The class data for **sodium determination only** by both methods will be collected and tabulated on the blackboard in the class. Collect these data in the table below before you leave (12 sets maximum). Using a calculator, calculate the mean and standard deviation (S.D.) for the two data sets and compare these for **accuracy** and **precision**. What do you conclude?

	1	2	3	4	5	6	7	8	9	10	11	12
$[Na^+]$(flame)												
$[Na^+]$ (ISE)												

Sodium concentration (mean ± S.D, *n* =) by flame photometry = …… (mM)

Sodium concentration (mean ± S.D, *n* =) by ISE = …… (mM)

4. **Conclusions:**

NOTE: If you do not have a nifty calculator that automatically calculates S.D.'s, or if you do not have time to collect the class data, an alternative method is provided for you for calculating the mean values ± S.D. for the class data and, if you are especially keen, for the whole year data (all 5 classes). The data sets from all 5 classes will be posted after each class on the Practical 3 Web pages: (http://www.liv.ac.uk/~agmclen/Medpracs/Practical_3/practical_3.html). An Excel spreadsheet can be downloaded and the data entered following the simple instructions provided. The spreadsheet will automatically calculate and display the mean and S.D. for the data sets you enter. If you are not used to using a spreadsheet like this, this is a good opportunity to try a simple exercise. You will find such things very useful in the future.

2.6 Experiment C: Correct use of automatic pipettes

As there is sufficient free time during the course of this practical, you will take the opportunity to practice the correct use of the automatic pipettes that will be used extensively in later practical classes. It is **essential** that you learn how to use these properly if you are going to achieve reliable results in these classes. This exercise also provides another demonstration of ***accuracy*** and ***precision***.

Accuracy: Assuming the pipette you use has been accurately calibrated (unfortunately not guaranteed), the closeness of the values you measure to the expected values will show how accurate your technique is. Any significant inaccuracy with a low standard deviation would indicate a **consistent** error in your technique, e.g. always (wrongly) pushing the pipette button fully down **before** drawing liquid into the pipette (or an improperly calibrated pipette).

Precision: Even though your mean value may appear accurate, if your individual values vary all over the place (high standard deviation), then your technique obviously suffers from a variety of errors (i.e. **general carelessness**!).

Procedure:

1. Read the instructions on the use of pipettes in **Appendix 2** and watch and listen carefully to the demonstration that will be given.

2. Practice pipetting water out of and into a beaker, watching all the time what you are doing and checking the water in the tip for air spaces, drops of water left at the top of the tip after expelling etc. Show the demonstrator your technique before proceeding to the next step.

3. Place a plastic weighing boat on the balance and tare the balance so that it reads zero (this will be demonstrated).

4. Using a "blue button" pipette, pipette 1.0 ml (1000 µl) distilled water into the weighing boat and record the weight. **If the pipette has been accurately calibrated, what should the reading be?**

5. Pipette another 1.0 ml water into the boat and record the weight again.

6. Repeat this until you have taken 10 readings (10 ml total pipetted) then calculate the weight of each individual 1.0 ml sample.

7. Calculate the mean and standard deviation of your 10 measurements.

1 ml samples

Number	1	2	3	4	5	6	7	8	9	10
Weight (g)										

Mean weight = _____ g

Standard deviation = ± _____ g

Conclusions:

APPENDIX 1:

Undergraduate Safety Regulations, School of Biological Sciences

1. Smoking, eating and drinking in the laboratory are strictly forbidden.

2. Students should purchase a laboratory coat and keep it clean and in good repair. Students without an adequate laboratory coat will not be allowed into the laboratory. The coat should be worn at all times in working areas and kept buttoned-up. Soiled laboratory coats are a potential hazard and should not be worn in areas where food and drink are consumed or outside the building. It is recommended that they should be wrapped in a plastic container when not in use and should be laundered regularly.

3. Safety spectacles are provided, and you are urged most strongly to wear them at all times in the laboratory. You must wear them when doing anything hazardous or potentially hazardous. Protective gloves and face masks are also available, and will be recommended for use when necessary in specific experiments.

4. Personal belongings are not to be brought into laboratories or placed where they can be contaminated. An area near the laboratory entrance will be reserved for bags, etc., and top-coats are to be left outside the laboratory.

5. Toxic and/or corrosive chemicals will normally be dispensed from burettes or automatic dispensers. Use rubber bulbs or automatic pipettes for the transfer of all other toxic solutions and solvents

6. The most common cause of laboratory accidents is broken glass. Do not use broken, jagged or cracked glassware at any time. Return damaged apparatus, and dispose of broken glass in the special bins provided. Do not attempt to pick up broken glass with your bare hands.

7. The second most common cause of accidents in the teaching laboratory is slipping on wet surfaces. Mop up any spillages of water, etc., on the laboratory floor as soon as you make them and report any other observed wet areas.

8. Keep benches clean and put away all apparatus not in use; carelessness and untidiness result in poor experimentation and can lead to accidents.

9. Never use a bunsen burner unless the experimental protocol specifically instructs you to do so. Never light a bunsen burner until you have checked that inflammable solvents are not nearby. If you have long hair it must be secured before a bunsen burner can be used.

10. Most solvents are inflammable and/or toxic. Whenever possible use them only in a fume

hood. Waste solvents must be disposed of in special containers in the fume hoods, and not down the sinks.

11. Do not operate any instrument with which you are unfamiliar; ask a demonstrator to show you how to use it.

12. Do not rush or act hastily in a crowded laboratory. Think before you act, and if in doubt ask the help of a demonstrator.

13. Students who feel unwell during a practical should report to the lecturer in charge. It is unsafe to work when ill.

14. Each teaching laboratory has a first aid box containing bandages etc. for minor accidents. Always consult a demonstrator if you cut yourself. If you accidentally ingest any reagent or spill it on yourself, immediately rinse your mouth or the affected area and report the incident to a demonstrator.

15. In the event of fire, raise the alarm, retire to a safe distance allowing the demonstrators and technicians to deal with the incident. In the case of major fire, the alarms will sound continuously, and you should leave the building by the nearest exit in an orderly manner. Do not congregate around exit doors and stairways.

16. When using bench centrifuges, ensure that the correct types of tubes are used, that they are correctly balanced, and are placed diametrically opposite each other in the rotor. If the centrifuge becomes unbalanced whilst it is running it will become noisy or start to vibrate. Switch it off immediately. A demonstrator or a technician will check it before it is used again. Do not open the lid of any centrifuge until it has stopped spinning completely

17. Immediately report any malfunction in electrical apparatus. Do not attempt to repair it yourself, however minor the fault appears to be. Report worn leads or loose connections.

APPENDIX 2:

Use of automatic pipettes

You will make extensive use of automatic pipettes with disposable tips in these practicals. Please treat these with care. Do not drop them. There are three different pipettes which are used for different volume ranges. Make sure you select the correct pipette and tip (**yellow** tips for orange and yellow button pipettes; **blue** tips for blue button pipettes)

	Volume range (µl)	Volume range (ml)
Orange button	5 – 40	0.005 – 0.04
Yellow button	40 – 200	0.04 – 0.2
Blue button	200 – 1000	0.2 – 1.0

1. Firmly push the correct tip on to the end of the pipette.

2. Dial up the required volume (in **microlitres**) by twisting the coloured button. **N.B. Do not try to dial in a figure above or below the stated volume range or you will jam the pipette. If you need a volume outside the stated range, choose a different pipette.**

3. Holding the pipette by the barrel, push down the button with your thumb to the **first** position of resistance and hold it there.

4. Submerge the disposable tip just below the surface of the liquid to be pipetted and **gently** allow the plunger to rise again by **slowly** releasing the pressure from your thumb (do **not** just take your thumb off the end and allow the plunger to shoot up otherwise you will get liquid into the barrel of the pipette). The correct volume of liquid should now be in the tip. Once the plunger has risen fully (and **NOT** before), remove the pipette tip from the liquid. Check that the volume looks right (this becomes easier with experience) and that there are no air bubbles or air gaps.

5. Place the tip in the receiving tube (near the bottom and touching against the side) and slowly push down on the plunger with your thumb through the first position of resistance to the second. Remove the pipette from the tube and check that all the liquid is out of the tip.

6. You can continue to use the same tip if (a) you are pipetting more of the same solution or (b) if you are pipetting dilutions of the same solution as long as you are working from the most dilute to the most concentrated (but **NOT** the other way round). Otherwise eject the tip into a waste bin by pushing down on the eject slider with your thumb.