

AUTOMATED ANALYSERS

AIM:

To give theoretical background on different types, use, advantages and dis-advantages of automated analyzers used in a diagnostic laboratory. A brief account on future trends of automation will also be given.

What is automation and why do we need to know about it?

History

General advantages to automated procedures

Basic approaches to automated analysers

- 1)- Continuous flow analysers
- 2)- Centrifugal analysers
- 3)- Discrete auto analysers
- 4)- Dry chemical analysers

Advantages to automating procedures in a Medical Laboratory

- Increase the number of tests performed by one individual in a given time period (short turn around time)....speeds up the result
- Human factor is decreased during the mechanical and repetitive part of an assay. Labor is an expensive commodity in Medical laboratories.
- To minimize variation in results from one individual to another (for accuracy, coefficient of variation is reduced hence the reproducibility increases).
- The quality of patients test results is monitored continuously.
- Automation eliminates the potential errors of manual analyses such as volumetric pipetting, manual calculation of results, and interpretation of results (human error is reduced).
- Instruments can use very small amounts of samples and reagents subsequently allowing less blood to be drawn from each patient. In addition, the use of small amounts of reagents decreases the cost of consumable.

BASIC APPROACHES WITH AUTOMATED ANALYZERS:

1- Continuous flow analyzers (nearly out of date)

- Liquids (reagents, diluents and samples) are pumped through a system of continuous tubing.
- Samples are introduced in a sequential manner, following each other through the same network of tubes. Series of air bubbles at regular intervals serve as separating media. The internal diameter of the tubing and the rate of flow determine the volumes of sample prior to mixing with the reagents and the turn around time of the result.
- An incubator is used to promote color development or the completion of enzymatic reaction

Principle of detection:

- Detection is by measuring absorbency by spectrophotometer through a continuous flow cuvet (cell) at a certain wavelength.
- When there is no sample, the sampler probe is placed in distilled water to avoid blockages, clogging and precipitation.
- More sophisticated continuous flow analyzers use parallel single channels to run **multiple tests** on **one** sample.
- For single channel machines, results are plotted on a dot blot to check for possible systemic or random errors.

- For more sophisticated multi channel machines, computers are used to store and analyze data and results may be reported to appropriate units via intranet.

Uses:

- Multi channel machines are used for certain test profiles (e.g. liver function tests and lipid function tests).
- Single channel machines may be used for frequently requested independent analysis e.g. blood glucose.

Disadvantages:

- The machine does not allow test selection; all tests must be performed even if not requested.
- The machine must run continuously even when there are no tests.
- Because of the continuous flow, reagents must be drawn at all times even when there are no tests to perform; which results in reagent wasting. Therefore a good stock of reagents must be available to avoid system malfunction due to reagent depletion.
- The instrument must be closely monitored all the time for air bubbles uniformity; reagent availability and tubing integrity and most important of all carry over problems.
- Multi-channel machines are usually large in size and occupy large space.

2- Centrifugal Analyzer

- Samples and reagents are added in a specially designed centrifugal type cuvette that has three main compartments (see fig).
- Sample is added from the sample cup by auto-sampler into the sample compartment of the centrifugal cuvette.
- The reagent probe into the reagent compartment of the centrifugal cuvette adds Reagent.
- Both sample and reagents are allowed to equilibrate to the reaction temperature.
- Mixing of sample and reagent occurs when the rotor holding the cuvette is spun at high speed (4000 rpm) and then sudden stop. The spinning causes the sample to be added to the reagent while the turbulence caused by sudden stop results in mixing of sample and reagent.
- After mixing, the rotor is spun at 1000 rpm. The reaction mixture is pushed horizontally to the bottom of the cuvette.

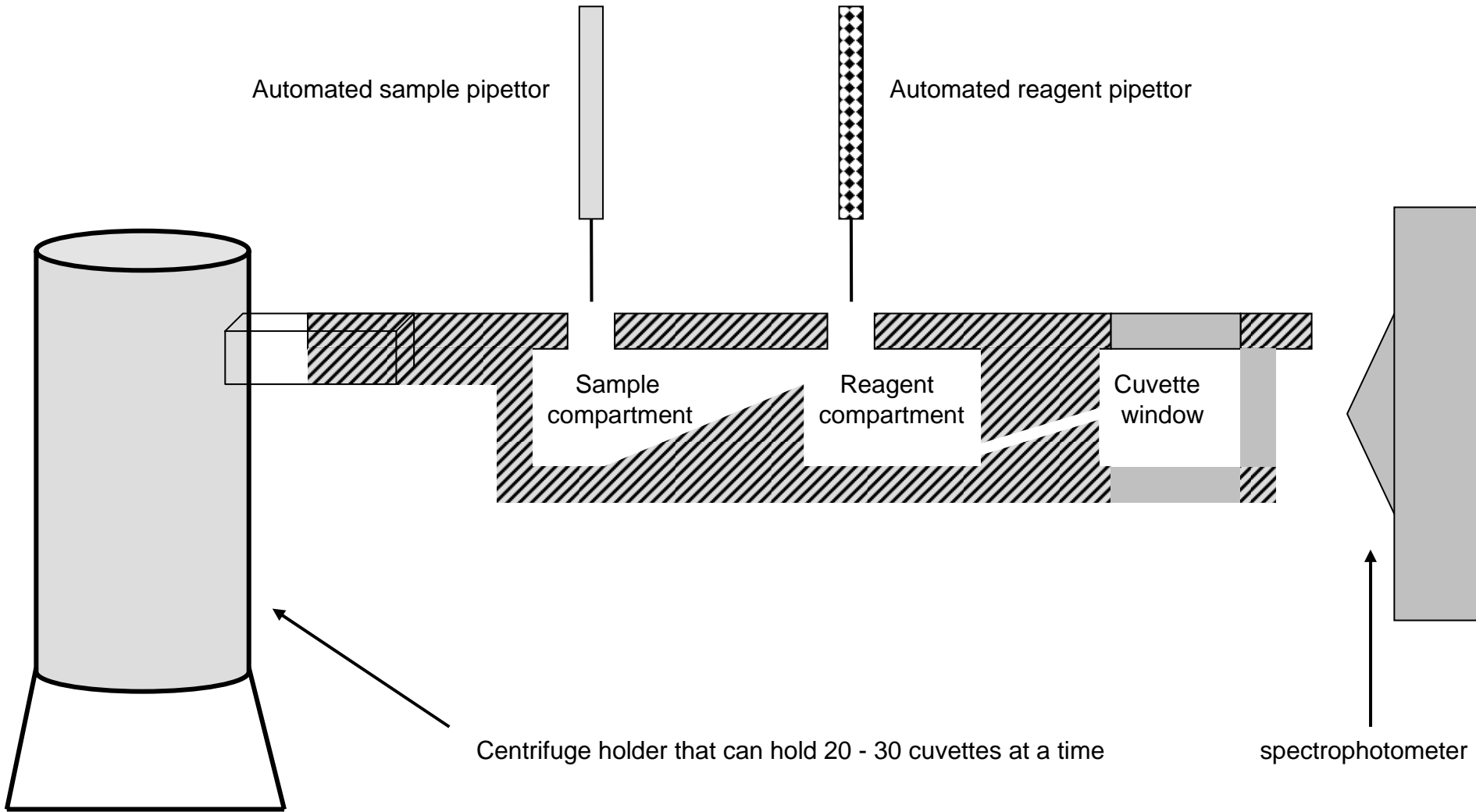
Principle of detection:

- It has clear transparent sides for spectrophotometric measurement.

Advantages:

- Rapid test performance analyzing multiple samples. Batch analysis is a major advantage because reactions in all cuvettes are read simultaneously

Special cuvette for CENTRIFUGAL ANALYZER



Cont....advantages of centrifugal analyzer

- Requires small sample (as little as 2 μ L of plasma, serum, urine or whole blood).
- Uses small reagent volumes (250 μ L).
- Can be programmed to carry out many different assay methods.

Disadvantages:

- Only one test type can be performed each time.
- The quality of cuvette and uniformity of detection window is crucial. Only reputable companies should be dealt with which adds to the cost of analysis.

3- Discrete auto analyzers

Principle:

Non-continuous flow using random access fluid which is a hydrofluorocarbon liquid to reduce surface tension between samples/reagents and their tubing and therefore reduce carry over.

Discrete analyzers have the capability to run multiple tests one sample at a time or multiple samples one test at a time. They are the most versatile analyzers.

Each sample is treated differently according to the tests requested and programmed by the operator:

- E.g. sample 1 glucose, urea, creatinine and electrolytes
- sample 2 total protein, albumin, calcium
- sample 3 triglycerides, cholesterol
- sample 4 bilirubin, ALT, AST, ALP

These instruments are heavily dependent on electronic control.

Sample is aspirated by the auto sampler from the sample cup and placed in the reaction cuvet. Samples are programmed or adjusted to reach a prescribed depth in those cups to maximize use of available sample.

Mixing of sample and reagents may be achieved by several methods such as:

- a)-Spinning of the cuvet at high speed followed by sudden stop.
- b)-Introducing the reagent into the cuvet by jet action.
- c)-Introducing air bubbles into the cuvet.

The reaction chamber temperature is controlled for colour development or enzyme assay to proceed.

The absorbency of the reaction in the reaction cuvet is read by a spectrophotometer, which is housed in the reaction chamber.

Computer then calculates the results and produces it in printed format.

Many of these machines have a Q.C system built in and automatically checks on the results of the Q.C samples to determine whether to accept or reject the results of the run.

Kinetic rather than endpoint methodologies are used (minimize protein error and give more accurate results)

Some of these machines have the ability to store or file patient results.

Uses:

Analytes that can be measured include; glucose, BUN, ammonia, bilirubin, uric acid, cholesterol, triglycerides, total calcium, total protein, albumin, creatinine, phosphorus, and serum enzymes e.g. Kodak Ektachem.

Advantages:

These machines are robust and produce reliable results with minimum problems.

These are high throughput machines that can analyze up to 75 samples in one go for single or multiple testing

Require little volume of sample and reagents.

Results are directly sent to the clinics via compatible computer system

Printers may be attached for printing data and error charts for the control samples

Dis-advantages:

Are expensive to purchase

Produce a lot of waste

Are expensive to maintain

Since each sample is in a separate reaction container, uniformity of quality must be maintained in each cuvet so that a particular sample quality is not affected by the cuvet it is placed in.

4-Dry Chemical Analyzers

Dry chemical methods utilize reagent slides that are composed of several layers which may include:

- 1-spreading layer
- 2-scavenger layer
- 3-reagent layer(s)
- 4-plastic or support layer

Principle:

Spreading layer is for sample or control or standard.

Scavenger layer allows selected components to filter through and penetrate to the reaction layer(s), which in turn activate the dehydrated reagents.

Reagent layer(s) contains; enzymes, and buffers necessary for the analysis of a specific analyte.

A chemical reaction is initiated to produce a colour.

Light is passed from beneath the support or plastic layer and is directed through the reagent layer (s).

As the light hits the white spreading layer, some of the light reflects back through the reagent layer(s) to a photocell.

The amount of reflected light, which is in proportion to colour intensity, is used to determine the concentration of the analyte.

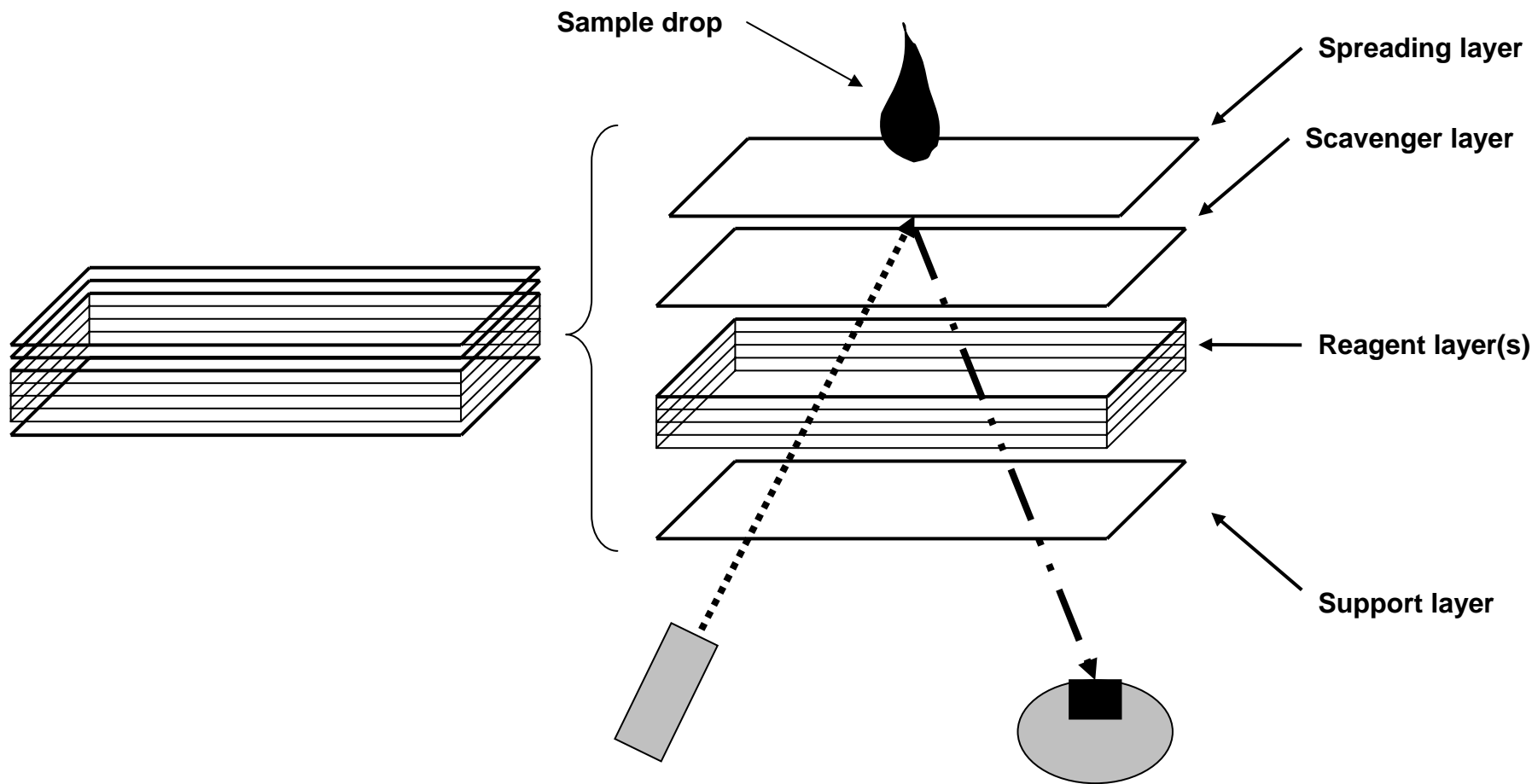


Figure showing various layers in a dry chemical analyzer and principle of detection

- **Advantages:**

The storage requirements for reagents are minimal since no wet reagents are required.

No pipetting steps are needed as the manufacturing company prepares the slides.

No sample dilution is required and 10 or 11 μl of sample per test is used.

ELECTROCHEMISTRY

- It's a branch of science that deals with chemical reactions (oxidation / reduction), that involve liberation, transfer and movement of charges in solid and liquid media.
- Applications of electrochemistry in clinical laboratory include:
Potentiometry (*most common*), amperometry, coulometry, conductivity, polarography
- **ISE** (*prime example of potentiometry*)
Used for measurement of many analytes in various biological fluids
Na⁺, Ca²⁺, K⁺, Cl⁻, pH, pO₂, pCO₂, NH₄⁺ can be measured either in blood, plasma, serum, CSF, urine or sweat.

Advantages:

- ISE is relatively less expensive and simple to use
- Can be used rapidly without prior handling
- Can be used for continuous monitoring
- They are unaffected by sample colour or turbidity
- Can measure both positive and negative ions
- Can be used in aqueous solutions over a wide range of temperatures

BASIC THEORY OF ISE MEASUREMENT:

ISEs are relatively simple & inexpensive sensors e.g. pH electrode.

ISE measures the activity of ions across a liquid junction and **not** the concentration.

pH Electrode:

A device used to measure activity of hydrogen ions and hence the degree of acidity of a solution. pH=7 is same as 1×10^{-7} moles per liter (negative logarithm of hydrogen ion concentration)

Principle

The electrode has a special, sensitive glass membrane which permits the passage of hydrogen ions but no other ionic species. When the electrode is immersed in a test solution containing H^+ ions, the external ions diffuse through the membrane until an equilibrium is reached between the external and internal H^+ activity. Thus there is a build up of charge on the inside of the membrane which is proportional to the number of H^+ in external solution

However the potential difference has to be measured relative to stable reference electrode. The reference electrode takes the measurement of standard solution of known H^+ concentration in which it's immersed (*see figure-1*).

Immersion of reference electrode in standard solution before measuring H^+ ions in test solution is known as **calibration** of reference electrode.

A voltmeter or a digital system is used to read the potential difference between the reference and test solutions.

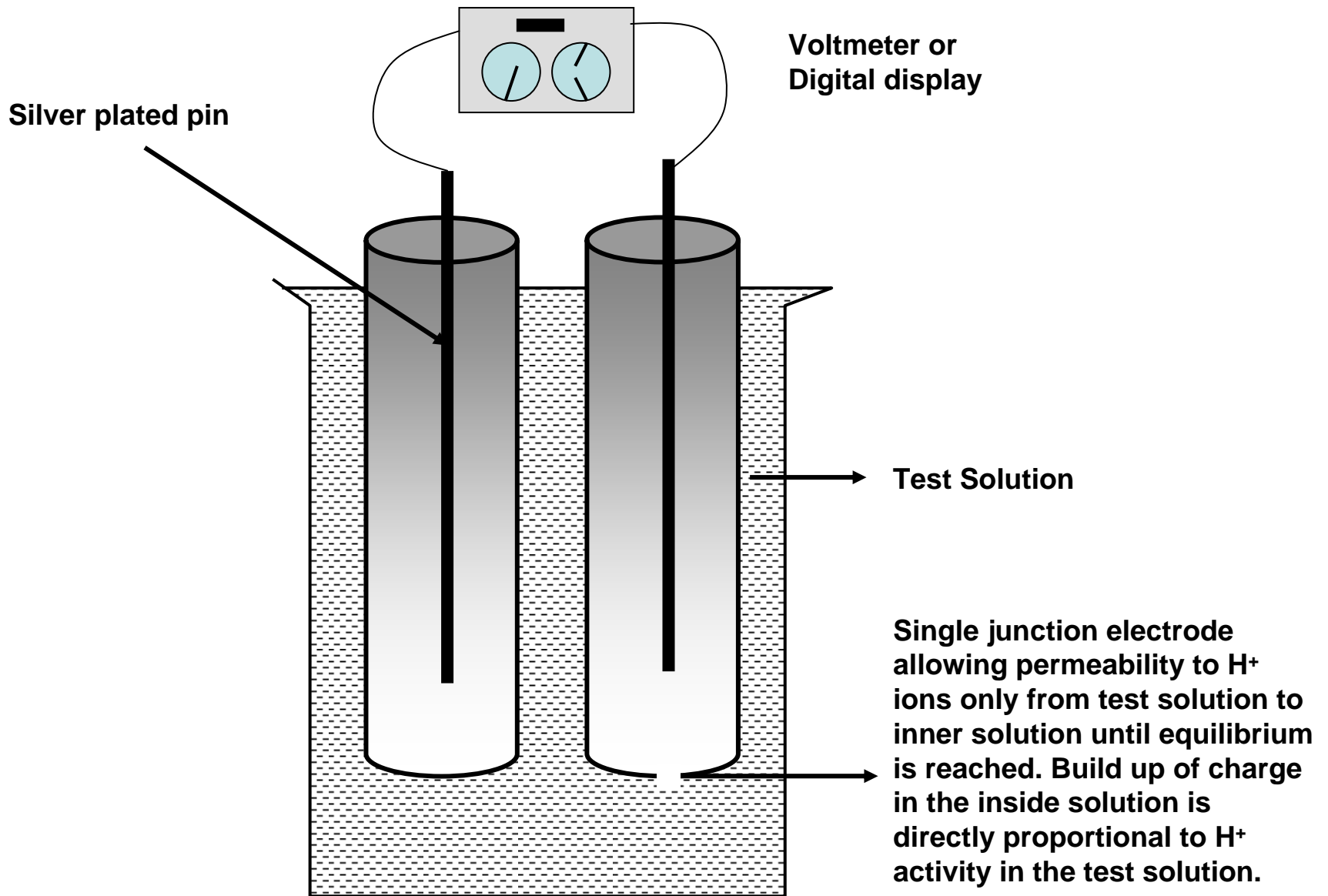


Fig-1; A simple pH electrode with corresponding single junction pH reference electrode

OTHER TYPES OF ISEs

In contrast to pH meters other ISEs are not entirely H^+ ion-specific and can permit the passage of other ions which may be present in the test solution using selective membranes.

ISE structural components:

ISEs come in various shapes and sizes, however all consist of a cylindrical tube made up of chemical resistant plastic material. An ion-selective membrane is fixed at one end so that the test solution can only come in contact with the external outer-surface of this membrane. The inner surface of the membrane is fitted with a low-noise gold (Au) or silver (Ag) plated pin or cable for connection to the multi-volt meter or digital system.

Most common ion selective membranes available in the market selectively measure:

cations: NH_4^+ , Ba^{2+} , Ca^{2+} , Cd^{2+} , Cu^{2+} , Pb^{2+} , Hg^{2+} , K^+ , Na^+ , Ag^+

Anions: Br^- , CO_3^{3-} , Cl^- , CN^- , F^- , I^- , NO_3^- , NO_2^- , S^- , SCN^-

*The manner how the ion selective membranes selectively allow transport of one type of ion is a variable and sometimes rather complex mechanism (*explained later on*).

Types of ion selective membranes:

- solid crystal matrix (e.g. Fluoride electrode containing lanthanum crystal)
- plastic or rubber film impregnated with a complex organic material which acts as a ion carrier (e.g. Potassium electrode impregnated with macrocyclic antibiotic valinomycin membrane). This membrane has hexagonal rings, exactly the size of K^+ ion allowing it's selective transmembrane passage while inhibiting other ions in the solution.

Principle of ion-selectivity:

Apart from the type of ion selective membrane the ability of ISE to distinguish between different ions (e.g. Na^+ , K^+ & Cl^-) in the same test solution e.g. a plasma sample is expressed as selectivity coefficient (SC).

If Na^+ is the test ion for which the electrode is selective and the interfering ions are K^+ & Cl^- then the SC of 0.1 would mean that the electrode is 10x more sensitive to Na^+ than K^+ & Cl^- and will give the most accurate reading but if the SC is 1 then the electrode is equally sensitive to all ions, hence can't distinguish the ions and will give the most unreliable reading.

Unfortunately SC is not constant from one test to the other due to variation in test solution's turbidity, concentrations, total ionic strength & temperature. Therefore the BSS (British Standard Specifications) for ISE has set standards for manufacturers to provide details of SC together with conditions under which they were tested.

REFERENCE ELECTRODES in ISE

Reference electrode is an integral part of the ISE. Without reference electrodes ionic measurements are meaningless. As mentioned earlier for pH meters where reference electrodes are relatively simple to calibrate prior to taking measurement of test solution, reference electrodes for other ISE may be quite complex as their applications are far more selective than simple H^+ ion measurement.

There are various types of reference electrode systems. They have advantages and disadvantages according to their function.

Types of reference electrode systems:

- a)- Silver / silver chloride Single Junction Reference Electrode
- b)- Double Junction Reference Electrode (*see figure-2*)

Commonly used outer filling solutions:

Sodium Chloride for K^+ analysis

Ammonium Sulfate for NO_3^- analysis

Magnesium Sulfate for NH_4^+ analysis

Potassium Nitrate for Cl^- analysis

Lithium Acetate can be used for any other ISE analysis (general purpose)

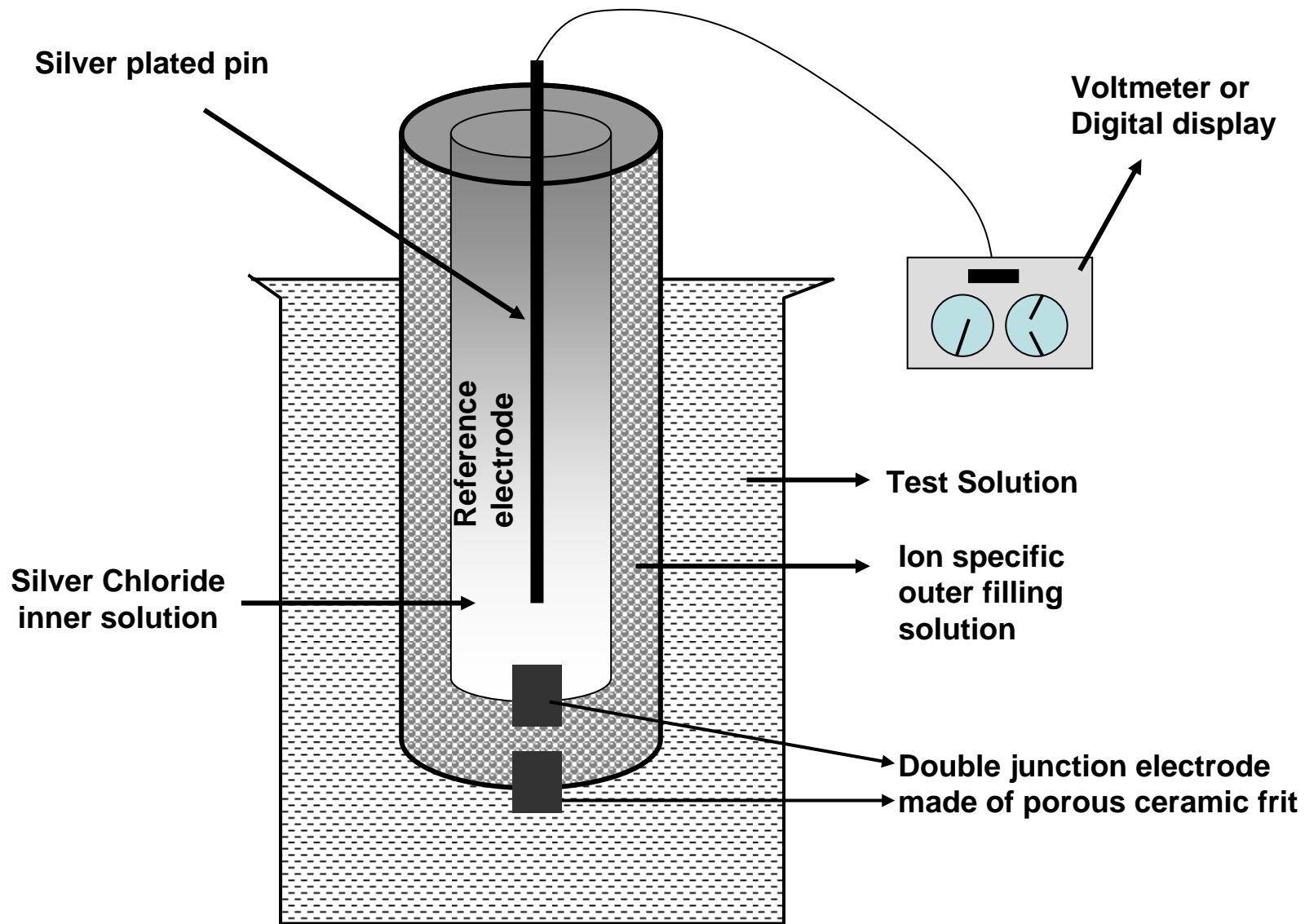


Fig-2; An ISE with integrated double junction reference electrode

c)- Combination Electrode

d)- Ion Multiple Electrode heads (IMT are latest ISEs installed in latest Multi-channel discrete auto analyzers for rapid electrolyte measurements).

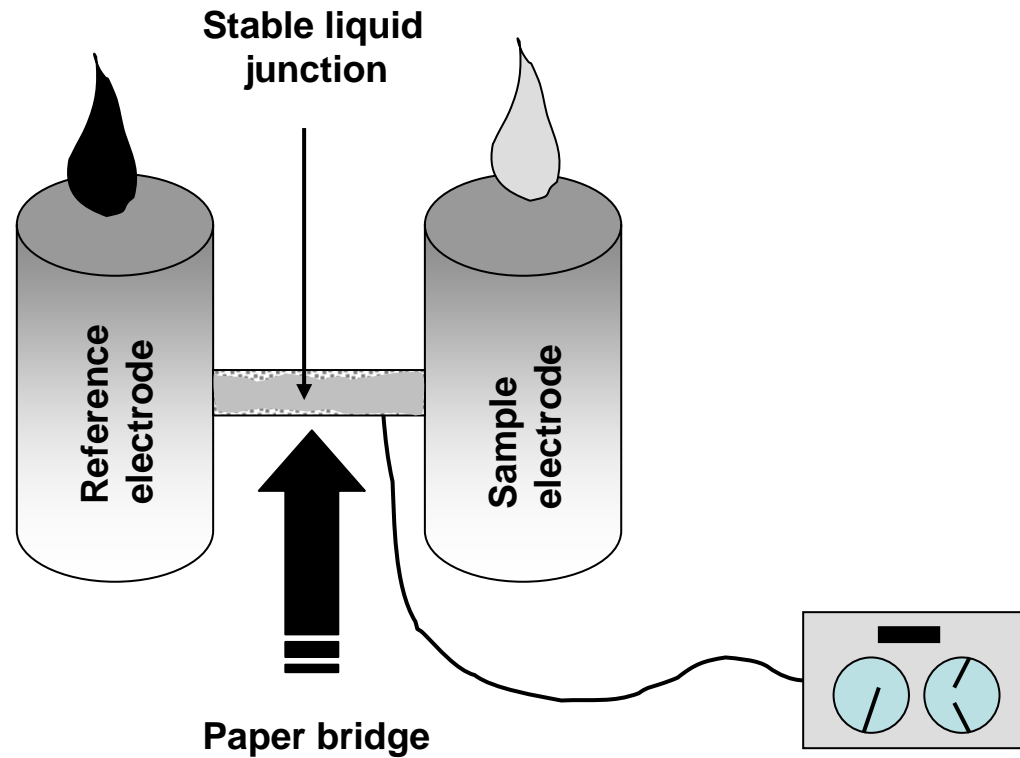
Calibration of ISE & validation

- Standard solutions

For most ISEs only a two point calibration is necessary. The new ISE based analyzers draw the linear curve in order to check std dilution errors. Three or four point calibrations are recommended to improve linearity.

- Validation

Running control samples is very important to ensure reliable readings from ISE. Ideally once calibration is over, controls must be tested immediately and acceptable readings are within $\pm 2SD$.



The samples interact with coated reagent layers to create a pair of electrochemical half-cells.

The drops spread toward one another across the paper bridge, meeting at the center and forming a stable liquid junction.

A voltmeter measures the potential difference of the two half-cells, which is then used to determine the concentration values as the value for the reference is already known.

Uses

- Widely used in many clinical laboratories.
- Many offer the ability for the operator to include his own test procedures (open system).
- Examples; The Hitachi group of analyzers (Hitachi 717, Hitachi 917), The Technicon RA 1000.

Advantages

- Uses dry chemistry hence incurring minimum storage costs.
- ISE has a major advantage which allows a sample to be analysed for electrolytes separately even when the analyser is analysing a batch of other samples for various other tests.

Disadvantages

- Samples with abnormal high protein may introduce significant errors (sample dilution may be necessary).