## 2.1 PH MEASUREMENT

The chemical balance in the body can be determined by the ph value of blood and other body fluids.ph is defined as the hydrogen ion concentration of a fluid. It is the logarithm of the reciprocal value of h+ concentration. The ph equation is given as,

$Ph= - \log_{10} [H^+] = \log_{10} 1/[H^+]$

pH is the measure of acid- base balance in a fluid, A neutral solution has the ph value as 7. Solutions with pH value less than 7 are acidic and above 7 are basic. Most of the body fluids are slightly basic in nature.

**Construction and working**

The ph meter is made up of a thin glass membrane and it allows only the hydrogen ionsto pass through it. The glass electrode provides a membrane interface for H+ ions. The glass bulb at the lower end of the ph meter contains a highly acidic buffer solution. The glass tube consists of a sliver-sliver chloride (Ag/Agcl) electrode and the reference electrode which is made up of calomel sliver-sliver chloride(Ag/Agcl) is tan placed in the solution in which ph is being measured.

The potential is measured across the two electrodes. The electrochemical measurement, which should be obtained by each of the electrodes called half- cell. The electrode potential is called as half-cell potential. Here the glass electrode inside the tube constitutes one half –cell and the calomel or reference electrode is considered as the other half-cell. The figure 1 shows the pH electrode.

For easier ph measurement combination electrodes are used. In this type both the active glass electrode and reference electrode are present in the same meter. The glass electrodes are suitable only to measure ph values around 7. Since this type of glass electrodes produce considerable errors during the measurement of high Ph values, special type of Ph electrodes are used. After every measurement the pH meter is washed with 20% ammonium biflouride solution, for accurate results. The Ph meter with hydroscopic glass absorbs water readily and provides best pH value.

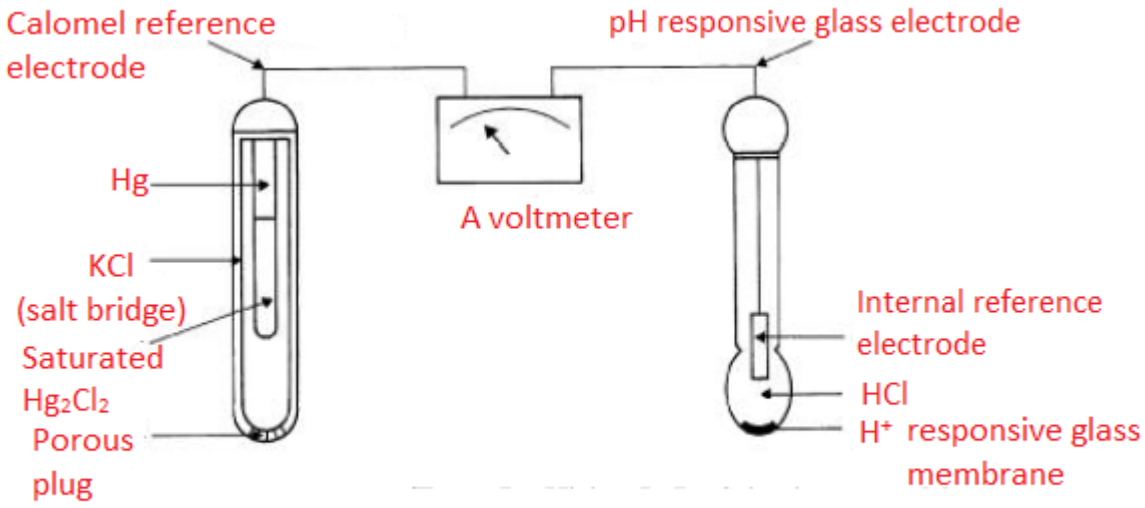


**Figure 1 pH Electrode**

***[Source : Leslie Cromwell, — "Biomedical Instrumentation and Measurement"]***

## $pO_2$ MEASUREMENT

The term po2 is defined as the partial pressure of oxygen respectively. The determination of po2 is one the most important physiological chemical measurement. The effective functioning of both respiratory and cardiovascular system can be by po2 measurement. The partial pressure of a gas is proportional to the quantity of that gas present in the blood.

The platinum wire, which is an active electrode, is embedded in glass for insulation and only its tip is exposed. It is kept in the electrolyte solution in which the oxygen is allowed to diffuse. The reference electrode is made up of silver-silver chloride (Ab/AgCl). A voltage of 0.7 is applied between the platinum wire and the reference electrode. The negative terminal is connected to the active electrode through a micro ammeter and the positive terminal is given to the reference electrode. The bellow figure 2 shows pO2 Electrode.

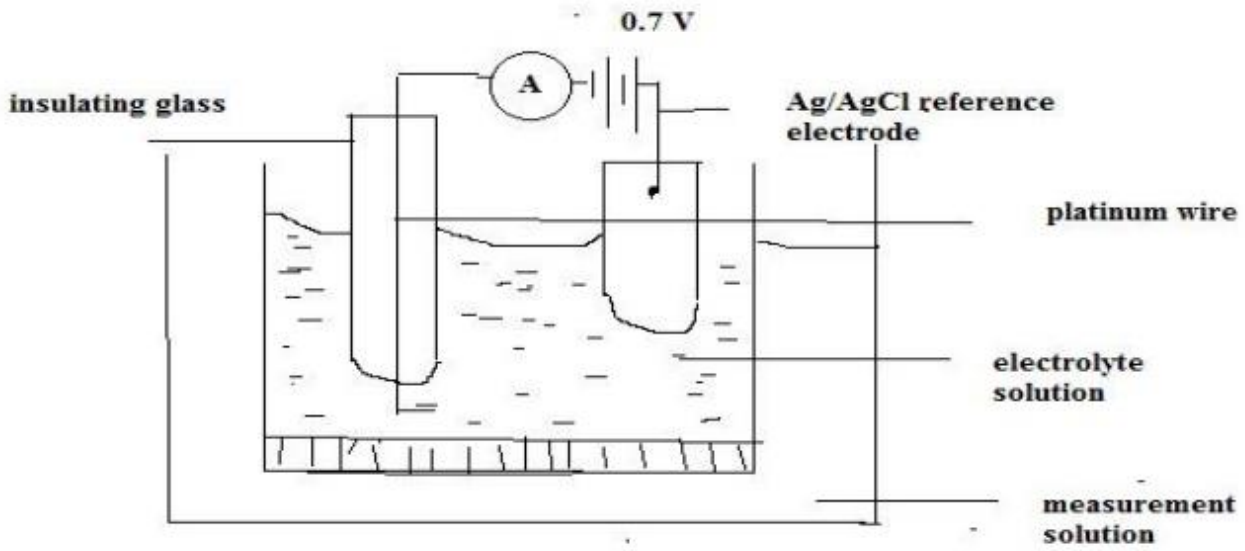


**Figure 2 $pO_2$ Electrode**

***[Source : Leslie Cromwell, — "Biomedical Instrumentation and Measurement"]***

Due to the negative terminal, the oxygen reduction takes place at the platinum cathode. Finally the oxidation reduction current proportional to the partial pressure of oxygen diffused into the electrolyte can be measured in the micro ammeter. The electrolyte is generally scaled in the electrode chamber by means of a membrane through which the oxygen can diffuse from the blood or sample solution.

There are two types of $pO_2$ measurement. They are

I) Vitro measurement

II) Vivo measurement

In case of dark electrode the platinum cathode and the reference electrode is present in a singleunit. This electrode is used for vitro and vivo measurements.

**In Vitro Measurements**

In this method the blood sample is taken and the measurement for oxygen saturation is made in the laboratory. The electrode is placed in the sample blood solution and the $pO_2$ value is determined.

**In Vivo Measurements**

In this method the oxygen saturation is determined while the blood is flowing in the circulatory system. A micro version of the $pO_2$ electrode is placed at the tip of the

catheter so thatit can be inserted into various parts of the heart or circulatory system.

The $pO_2$ measurement also has some disadvantages in it. The reduction process in the platinum cathode removes a finite amount of the oxygen from the cathode. And there is a gradualreduction of current with respect to time. However careful design and proper procedures in modern $pO_2$ electrodes reduce the errors.

**$pCO_2$ MEASUREMENT**

The term pco2 is defined as the partial pressure of carbon dioxide respectively. The determination of pco2 is one the most important physiological chemical measurement. The effective functioning of both respiratory and cardiovascular system can be by pco2 measurement. The partial pressure of a gas is proportional to the quantity of that gas present in the blood.

The partial pressure of carbon dioxide can be measured with the help of $pCO_2$ electrodes. Since there is a linear relationship between the logarithm of $pCO_2$ and pH of a solution. The $pCO_2$ measurement is made by surrounding a pH electrode with a membrane selectively permeable to $CO_2$.

The modern improved $pCO_2$ electrode is called as severinghous electrode. In this electrode the membrane permeable to $CO_2$ is made up of Teflon which is not permeable to other ions which affects the pH value. The space between the Teflon and glass contains a matrix layer which allows only the $CO_2$ gas molecules to diffuse through it.
One of the demerits in older $CO_2$ electrode is, it requires a length of time for the $CO_2$ molecules to diffuse through the membrane. The modern $CO_2$ electrode is designed in such a way to overcome this demerit. Here the $CO_2$ molecules diffuse rapidly through the membrane and the measurement can be done easily.

**MEASUREMENT OF $PHCO_3$**

- Blood gas analyzers are used to measure the content of pH, pCO and $PO_2$ from theblood.

Two gases of accurately known $O_2$ and $CO_2$ percentages are required for calibrating the analyzer in $pO_2$ and $pCO_2$ modes. These gases are used with precision regulators for flow and pressure control.

- Two standard buffers of known pH are required for calibration of the analyzer inthe pH mode.
- Input signal to the calculator is obtained from the outputs of the pH and $pCO_2$amplifiers
- The outputs are adjusted by multiplying with a constant and are given to an adder circuit. The bellow Figure 3 represents the circuit diagram of computation of bicarbonate.
- The output of adder is passed to antilog generators circuit. Then it is passed toA/D converter for display. Resistance R is used to adjust zero at the output.
- Total $CO_2$ is calculated by summing the output signals of the calculators and the output of the $pCO_2$ amplifier

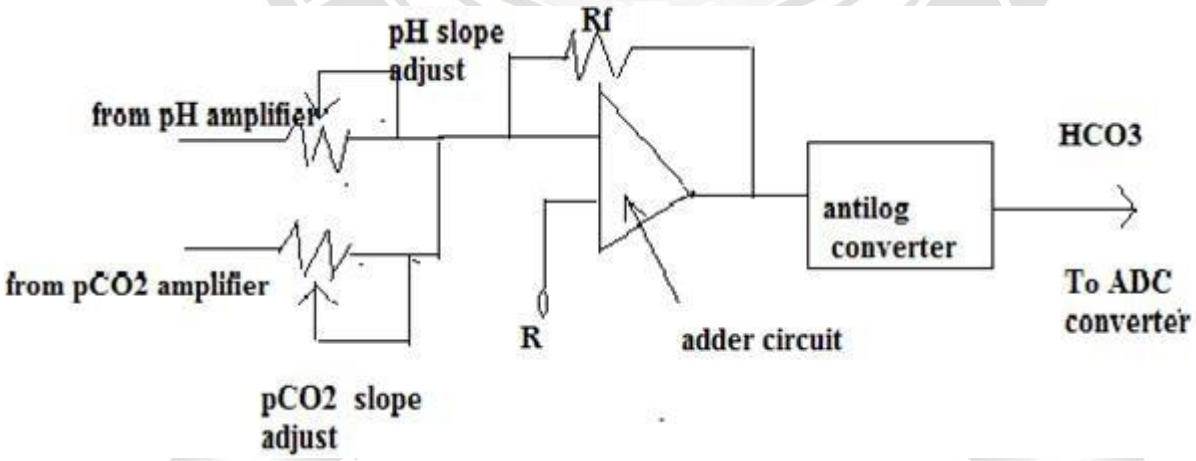


**Figure 3 circuit diagram of computation of bicarbonate**

***[Source : Leslie Cromwell, — "Biomedical Instrumentation and Measurement"]***

**The base excess calculator consists of three stages.**

In the first stage, the output of pH amplifier is inverted in an operational amplifier, whose gain is controlled by a potentiometer. The bellow figure 4 shows the circuit diagram for computation of base excess.

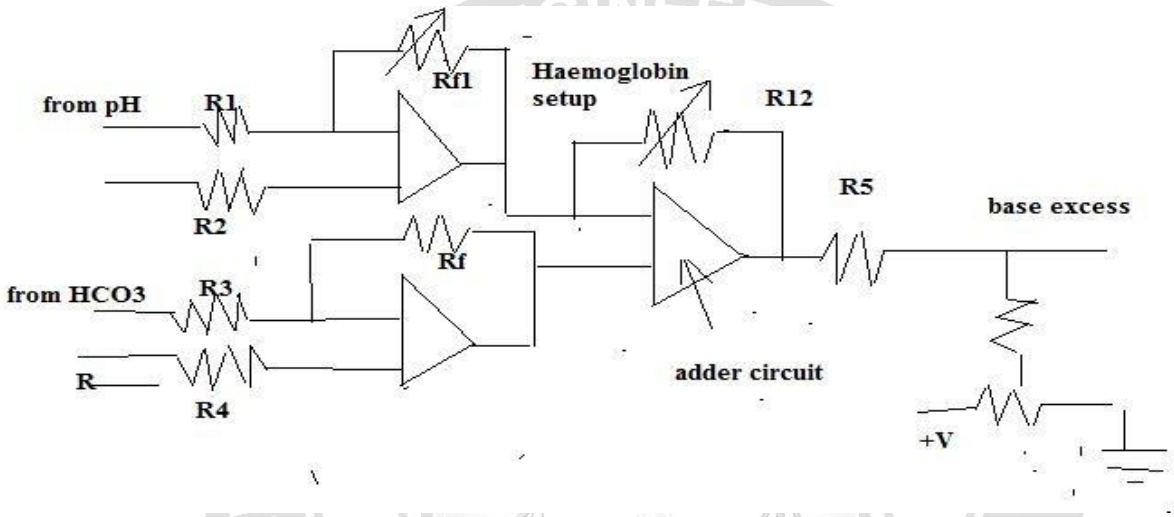


**Figure 4 Circuit diagram for computation of base excess**

***[Source : Leslie Cromwell, — "Biomedical Instrumentation and Measurement"]***

The output of $HCO_3^-$ calculator is inverted in the second stage.

The third stage is a summing amplifier $A_3$ whose output is given to A/D converter, that gives adigital read out.

**ELECTROPHORESIS**

In clinical laboratories, various devices are used based on the electrophoretic principle.

These devices are used for the following applications.

- To measure the quantity of protein in plasma, urine, etc.
- To separate enzymes into their components is enzymes.
- To identify antibodies.

**Basic principle**

Electrophoresis is defined as the movement of a solid phase with respect to a

liquid. The buffer solution is used to carry the current and to maintain the pH value of the solution as a constant one during the migration.

In this title, zone electrophoresis is explained. In this technique, the sample is applied to the medium and under the effect of the electric field, group of particles that are similar in charge, size, and shape migrate at the same rate. So the particles are separated into zones.

**Factors Affect the Speed of MigrationMagnitude of charge:**

The mobility of a given particle is directly related to the net magnitude of the particles charge. Mobility is defined as, the distance in cm, a particle moves in unit time per unit field strength.

**Ionic Strength of Buffer**

If the buffer is more concentrated then the migration of the particles is slow. Because, if greater the proportion of buffer ions present, then greater the proportion of the current they carry.

**Temperature:**

Mobility is directly related to temperature. Heat is produced when the current flows through the resistance of the medium. So, the temperature of the medium is increased and resistance is decreased. Finally, the rate of migration is increased.

The water is evaporated from the surface of the medium due to heat. So, the concentration of particle is increased. Finally the rate of migration is increased. When the gel is used as a medium; this heat will create a problem. So, for this medium, constant current sources are used to minimize the heat production.

**Time:** The distance of migration is related to the time period during which electrophoresis takesplace.

**Types of Support Media:**

Cellulose acetate, starch gel and sucrose are used as support media in various electrophoretic applications. We can see the cellulose acetate electrophoresis in the following sections**.**

**Cellulose Acetate Electrophoresis**

Cellulose acetate strip is saturated with the buffer solution and placed in the membrane holder. It is otherwise known as bridge. The two ends of the bridge are placed in the cuvette in which buffer solution is available.

The sample for each test is placed on the strip at a marked location. Then, the constant electric potential(250 V) is applied across the strip 4 – 6 mA of initial current is obtained .After 15-20mins, the electric voltage is removed, then, migrated protein band is stained with buffer and it is dried in preparation for densitometry.

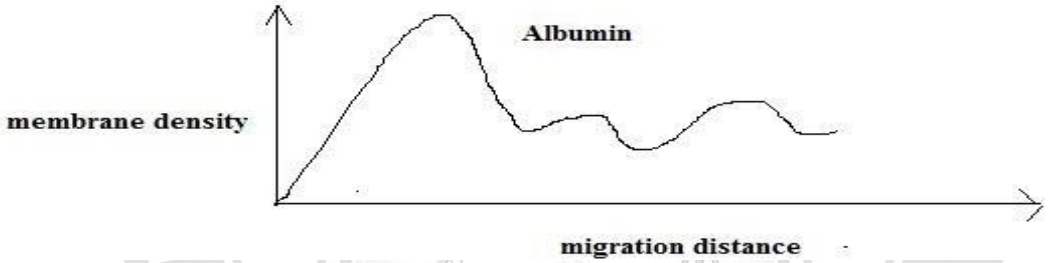


**Figure 5 Pattern**

***[Source : Leslie Cromwell, — "Biomedical Instrumentation and Measurement"]***

The membrane is placed in the holder of densitometer. The path of the migration ofone of the specimen is scanned. The low voltage output is amplified and recorded using x-y recorder. The bellow figure 6 shows the cellulose acetate electrophoresis

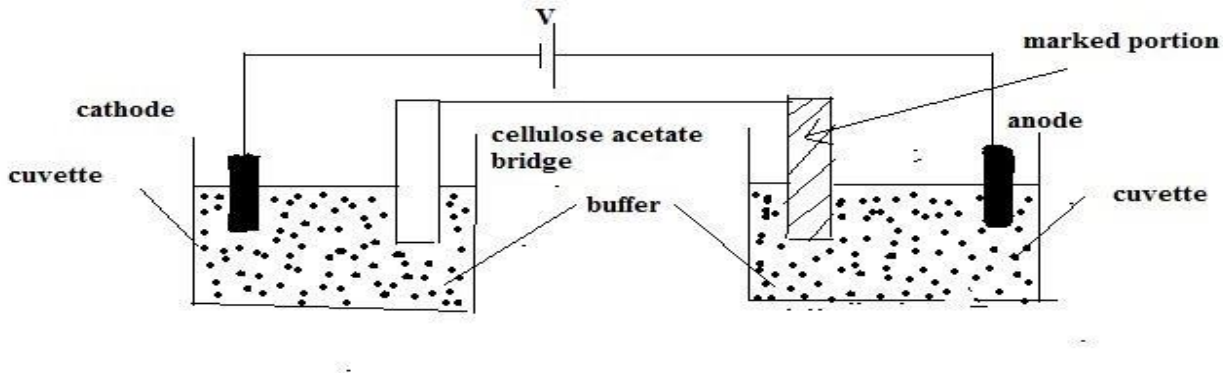


**Figure 6 Cellulose acetate electrophoresis**

***[Source : Leslie Cromwell, — "Biomedical Instrumentation and Measurement"]***

### 2.1.1 COLORIMETER

- Measures the color concentration of a substance in a solution by detecting the color lightintensity passing through a sample containing the substance and a reagent
- Optical color filters are used to detect the color wavelength of interest. E.g., urine passes yellow light and absorbs blue and green. The figure 7 shows the colorimeter.
- Laser LEDs are preferred if their wavelength is suitable due to purity of the monochromatic color.

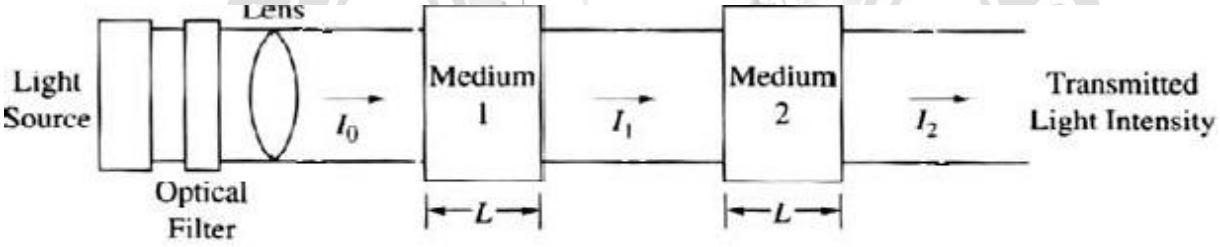


**Figure 7 Colorimeter**

***[Source : Leslie Cromwell, — "Biomedical Instrumentation and Measurement"]***

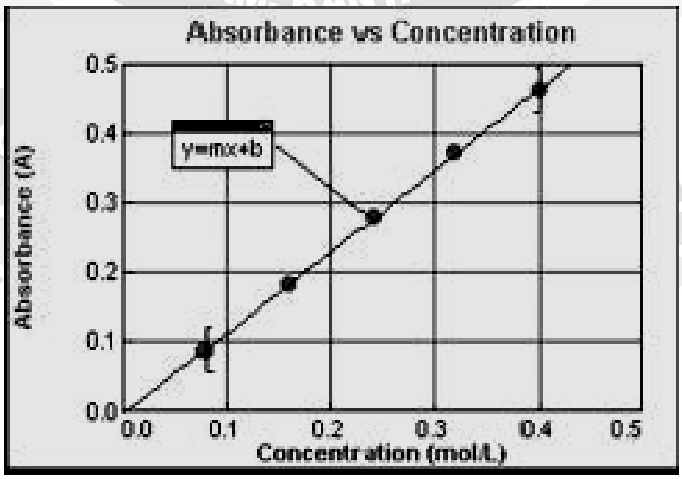


**Figure 8 Concentration vs Absorbance**

***[Source : Leslie Cromwell, — "Biomedical Instrumentation and Measurement"]***

**Transmittance**

$T= I_1/I_0 * 100\%$

**Absorbance**

$A= - \log I_1/ I_0$

$A=\log 1/T$

If the path length or concentration increases, the transmittance decreases and absorbance increases, a phenomenon expressed by Beer's Law. Absorbtivity related to the nature of the $A=aCL$ absorbing substance and optical wavelength (known for a standard solution concentration). C: Concentration, L: Cuvette path length

**PHOTOMETER**

**FLAME PHOTOMETER**

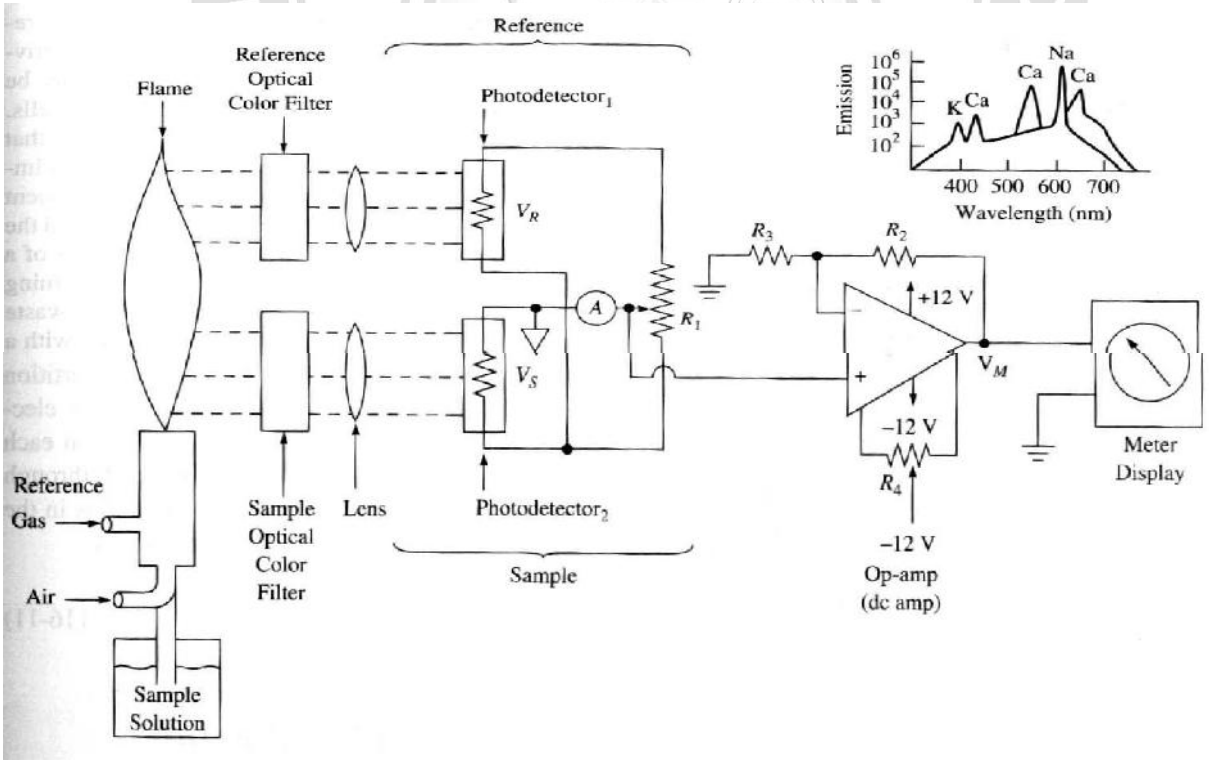


**Figure 9 Flame Photometer**

***[Source : Leslie Cromwell, — "Biomedical Instrumentation and Measurement"]***

Measures the color intensity of a flame supported by O2 and a specific substance. Sample's emission of light is measured (rather than the absorbance of light).Typically used to determine the conc. of pure metals and/or Na+, K+, Li+ and Ca++. The above figure 9 shows the flame photometer.

In this method, fine droplets of the sample is aspirated into gas flame that burns in a chimney. A known amount of lithium salt is added to the sample, as a reference. As a result, red light is emitted by the lithium and yellow and violet beam are emitted due to sodium and potassium respectively. These diffracted colours are made to incident on photodiodes. The photo detector circuits consists of a reverse biased diode in which the current flow increases as intensity of incident light increases. A calibration potentiometer is used in every channel. Since the lithiumis used as a standard reference, the output of sodium and potassium channel are calibrated in terms of differences with the known lithium. The output can be compared with the spectral illustration.

**SPECTROPHOTOMETER**

The general name given to the group of instruments whose principle of operation is based on the fact that substances of clinical interest selectively absorb or emit EM energy (light) at different wavelengths. The bellow figure 10 shows the spectrophotometer.

- Depending on the substance being measured, the wavelength used is typically in theultraviolet (200-400 nm), visible (400-700nm) or infrared (700 to 800 nm) range.
- Spectrophotometer can be used to determine the entity of an unknown substance, or theconcentration of a number of known substances.
- The type of source / filters used typically determines the type of the spectrophotometer.
- Rays of light bend around sharp corners, where the amount of bending depends on the wavelength! This results in separation of light into a spectrum at each line.
- In spectrophotometer, selection filter of colorimeter is replaced by a monochromator. Monochromatic uses a diffraction grating G to disperse light from the

lamp. Light falls through the slit $S_0$ into its spectral components.

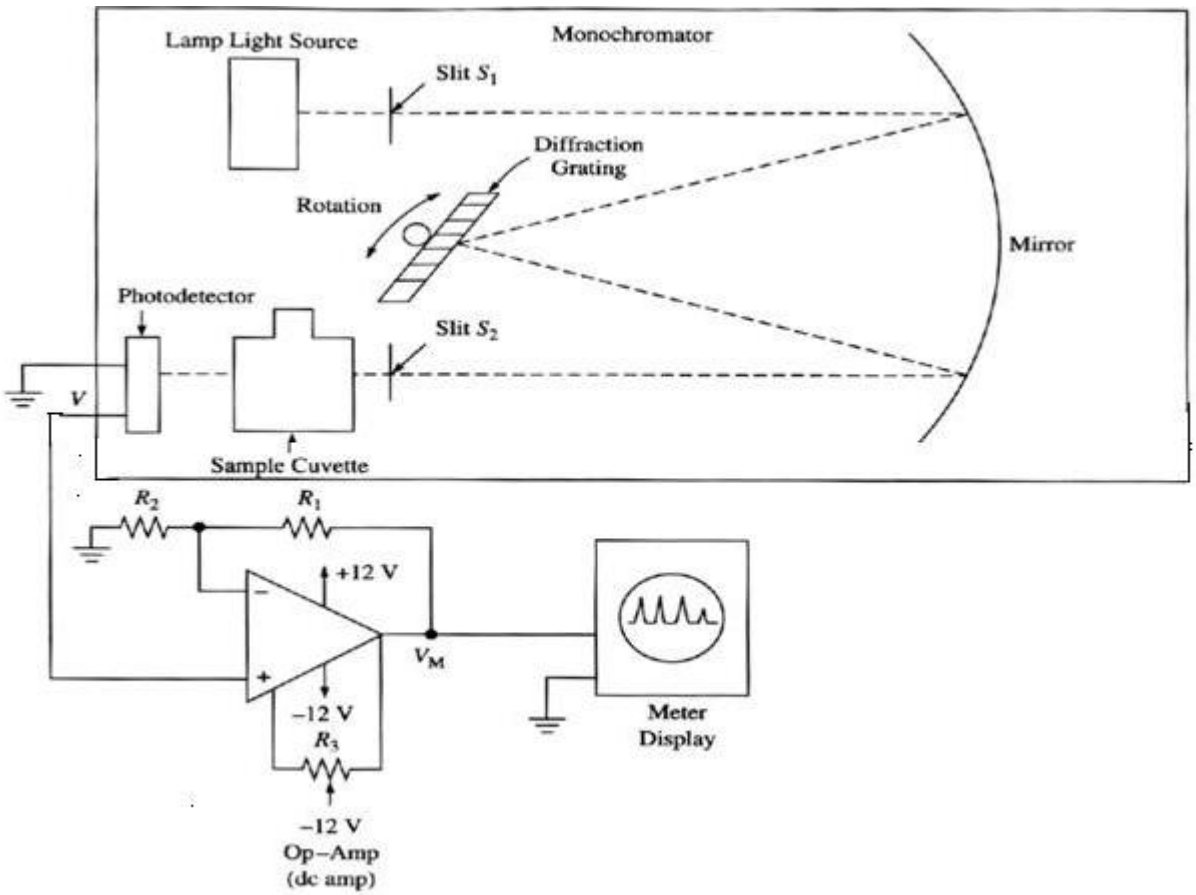


**Figure 10 Spectrophotometer**

***[Source : Leslie Cromwell, — "Biomedical Instrumentation and Measurement"]***

**AUTOANALYZER**

An auto analyzer sequentially measures blood chemistry through a series of steps of mixing, reagent reaction and colorimetric measurements.

It consists of

- **Sampler:** Aspirates samples, standards, wash solutions into the system
- **Proportioning pump:** Mixes samples with the reagents so that proper chemical color reactions can take place, which are then read by the colorimeter
- **Dialyzer:** separates interfacing substances from the sample by permitting selective passage of sample components through a semi permeable membrane

- **Heating bath:** Controls temperature (typically at 37 °C), as temp is critical in color development
- **Colorimeter:** monitors the changes in optical density of the fluid stream flowing through a tubular flow cell. Color intensities proportional to the substance concentrations are converted to equivalent electrical voltages.
- **Recorder:** Displays the output information in a graphical form.
- lit $S_1$ is used for selecting a narrow band of the spectrum which is used to measure the absorption of a sample in the cuvette. The bellow figure 11 shows the block diagram of autoanalyzer. The light from the cuvette is given to photo detector. It converts light into a electrical signal. This electrical signal is amplified by using an amplifier. The output from the amplifier is given to meter which shows absorbance.
- Light absorption is varied when the wavelength is varied. Mirror M is used to reduce the size of the instruments.

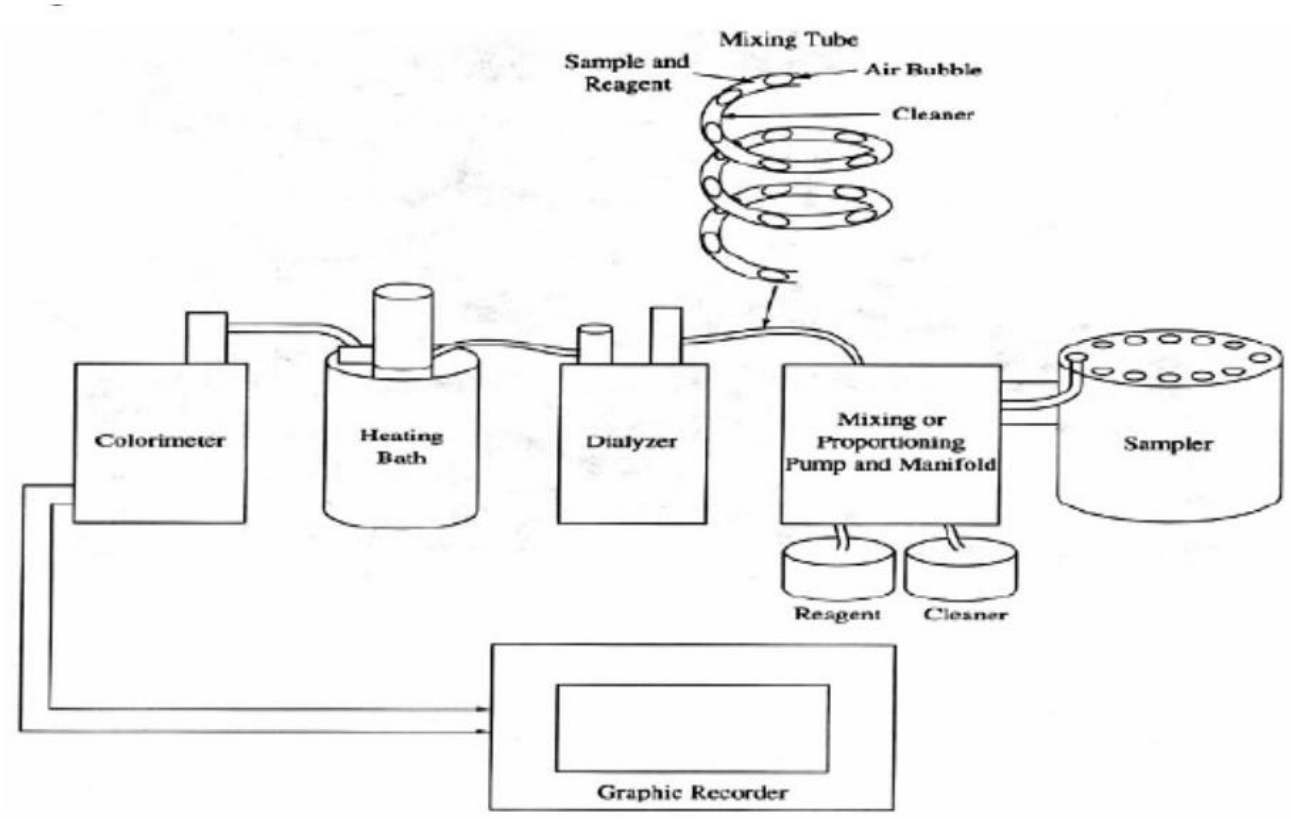


**Figure 11 Block diagram of Autoanalyzer**

***[Source : Leslie Cromwell, — "Biomedical Instrumentation and Measurement"]***