TEJASVI NAVADHITAMASTU

"Let our (the teacher and the taught) learning be radiant"

Let our efforts at learning be luminous and filled with joy, and endowed with the force of purpose

Dr. Prabhakar Singh
Department of Biochemistry
Faculty of Science, V.B.S. Purvanchal University, Jaunpur
PIN-222 003, (U.P.), INDIA.
Mobile No.: +91-9454695363
E-Mail: pruebiochem@gmail.com

VEER BAHADUR SINGH PURVANCHAL UNIVERSITY JAUNPUR-222003

E –content

Course: M.Sc.
Subject: Biochemistry; Biotechnology, Microbiology, Environmental Science
Topic: Instrumentation and Analytical Techniques
S**ubtopic: ELECTROCHEMISTRY**
Prepared by: Dr. Prabhakar Singh
Department : Biochemistry
Faculty : Science
Email: pruebiochem@gmail.com
Contact: +91-9454695363

Note-This E-content has been prepared as a reading material for students without any commercial interest. Original contributors are acknowledged.

ELECTROCHEMISTRY

pH : Potential of Hydrogen

- In fact, the term pH was introduced in 1909 by Sörensen who defined it as:
- pH (short for “potential of hydrogen”) is a symbol and denotes the relative concentration of hydrogen ions in a solution.
- pH values extend from 0 to 14; the lower the value, the higher the acidity or the more hydrogen ions the solution contains.
- Water at 25 °C has a concentration of H ion of 10^{-7}; the pH, therefore, is 7.
- The pH scale was developed taking water as the standard. It is an experimental fact that only 1 mole in 5,50,000,000 moles of water ionizes into a H+ and OH−. This is the same proportion as 1 gram hydrogen ion in 10,000,000 litres of water. Hence, 1 litre of water contains 1/10,000,000 (or $1/10^7 = 10^{-7}$) of a gram of H+.

$$pH = \log \frac{1}{[H^+]} = -\log [H^+]$$

THE pH SCALE

- The ion product of water, Kw, is the basis for the pH scale. It is a convenient means of designating the actual concentration of H+ (and thus of OH−) in any aqueous solution in the range between 1.0 M H+ and 1.0 M OH−)
- Biochemical reactions are often defined in terms of hydrogen ion (H+) concentrations. In 1909, Soren Sörensen, a Danish biochemist, used a logarithmic scale for expressing the H+ concentration.
- This scale was called pH, where p stands for power and H for hydrogen ion concentration. He defined pH of a solution as the negative logarithm of the concentration (in moles/litre) of hydrogen ions.
- A solution of a strong acid, such as hydrochloric acid, at concentration 1 mol dm−3 has a pH of 0. ... Thus, measured pH values will lie mostly in the range 0 to 14, though negative pH values and values above 14 are entirely possible

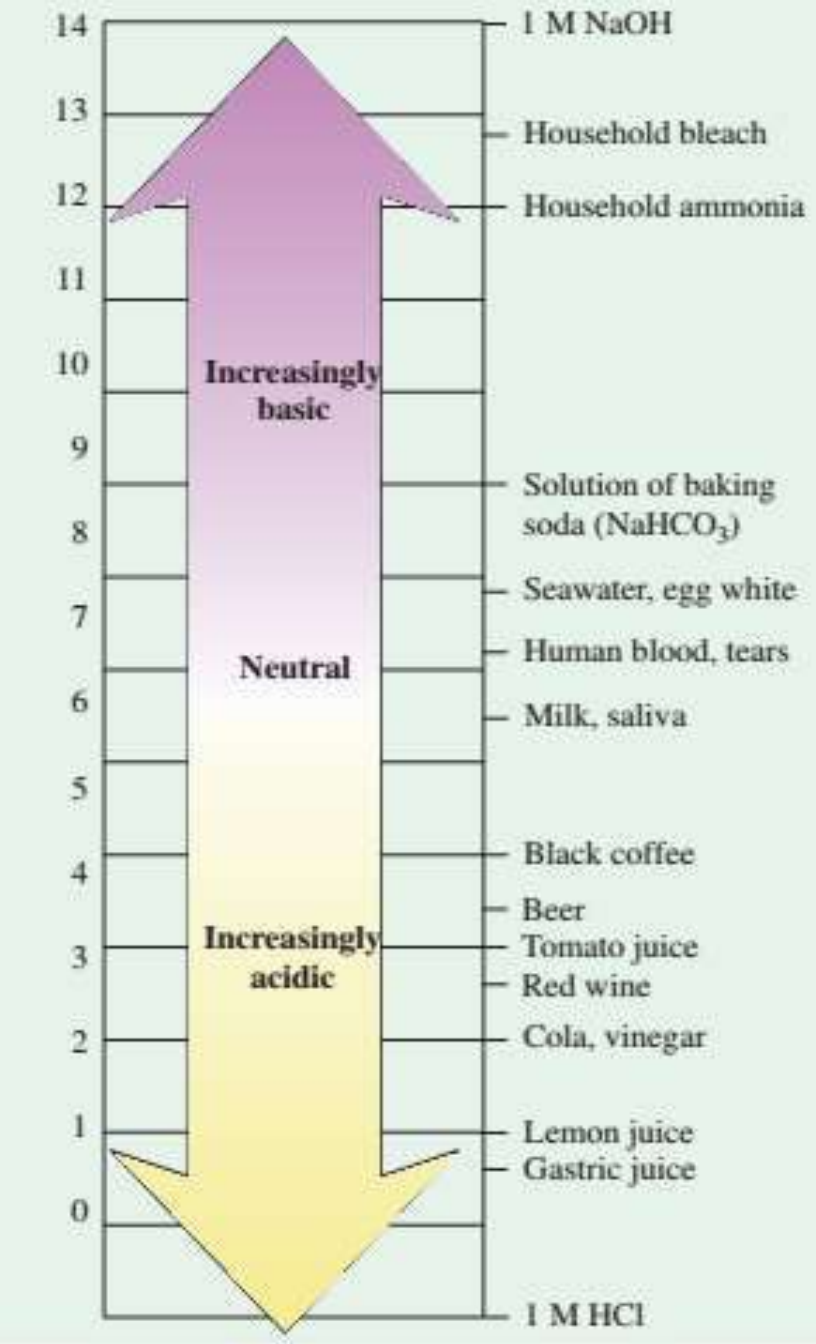


Fig. 3–2. The pH of some aqueous fluids

BRONSTED-LOWRY and G.N. Lewis Concept Of Acids and Bases

- A Brönsted−Lowry acid is defined as a substance that can donate a proton (H +); conversely, a Brönsted−Lowry base is a substance that can accept a proton. A proton donor (i.e., an acid) and its corresponding proton acceptor (i.e., a base) make up a conjugate (coniungere^{L} = to join together) acid−base pair

- G.N. Lewis, also in 1923, proposed yet another definition of acids and bases. According to him, an acid is a compound which can accept a pair of electrons from a base. Such compounds are also called electrophile (electron-loving). A base is defined as a compound which can donate an electron pair to an acid. Such compounds are also called nucleophile (nucleus-loving)

STRONG AND WEAK ACIDS

There are two general classes of acids — strong and weak.

- A strong acid is defined as a substance that has a greater tendency to lose its proton and therefore completely dissociates (or ionizes) in water, such as HCl and H_2SO_4.

- A weak acid, on the other hand, is a molecule that has a lesser to lose its proton and they are diprotic (carbonic acid and glycine) or triprotic (phosphoric acid). Tendency to lose its proton (or, in other words, displays a high affinity for its proton) and, therefore, does not readily dissociate in water, such as CH_3COOH.

Dissociation constant and pKa of some common weak acids (proton donors) at 25°C

Acid	*Formula*	K_a *(M)*	pK_a
Formic acid	HCOOH	1.78×10^{-4}	3.75
Acetic acid	CH_3COOH	1.74×10^{-5}	4.76
Propionic acid	CH_3CH_2COOH	1.35×10^{-5}	4.87
Lactic acid	$CH_3CH(OH)COOH$	1.38×10^{-4}	3.86
Phosphoric acid	H_3PO_4	7.25×10^{-3}	2.14
Dihydrogen phosphate	$H_2PO_4^-$	1.38×10^{-7}	6.86
Monohydrogen phosphate	HPO_4^{2-}	3.98×10^{-13}	12.40
Carbonic acid	H_2CO_3	1.70×10^{-4}	3.77
Biocarbonate	HCO_3^-	6.31×10^{-11}	10.20
Ammonium	NH_4^+	5.62×10^{-10}	9.25

Henderson–Hasselbalch Equation

The quantitative relationship among pH, buffering action of a mixture of weak acid with its conjugate base, and the pKa of the weak acid is given by a simple expression called Henderson-Hasselbalch Equation.

For the dissociation of a weak acid HA into H^+ and A^-, the Henderson–Hasselbalch equation can be derived as follows :

The equation is expressed more generally as :

$$\text{pH} = pK_a + \log \frac{[\text{proton acceptor}]}{[\text{proton donor}]}$$

Consider the ionization of some weak acid, HA, occurring with an acid dissociation constant, K_a. Then,

$$HA \rightleftharpoons H^+ + A^-$$

and

$$K_a = \frac{[H^+][A^-]}{[HA]}$$

Rearranging this expression in terms of the parameter of interest, $[H^+]$, we have

$$[H^+] = \frac{[K_a][HA]}{[A^-]}$$

Taking the logarithm of both sides gives

$$\log [H^+] = \log K_a + \log_{10} \frac{[HA]}{[A^-]}$$

If we change the signs and define $pK_a = -\log K_a$, we have

$$pH = pK_a - \log_{10} \frac{[HA]}{[A^-]}$$

or

$$\mathbf{pH = pK_a + \log_{10} \frac{[A^-]}{[HA]}}$$

The equation is expressed more generally as :

$$pH = pK_a + \log \frac{[\text{proton acceptor}]}{[\text{proton donor}]}$$

A. When [A–] = [HA] or when an acid is exactly half neutralized : Under these conditions,

$$\text{pH} = pK_a + \log \frac{[A^-]}{[HA]} = pK_a + \log \frac{1}{1} = pK_a + 0 = pK_a.$$

Therefore, at half neutralization, pH = pK_a. The equation, thus, shows why the pK_a of a weak acid is equal to the pH of the solution at the midpoint of its titration.

B. When the ratio [A–]/[HA] = 100 to 1 :

$$\text{pH} = pK_a + \log \frac{[A^-]}{[HA]} = pK_a + \log \frac{100}{1} = pK_a + 2$$

C. When the ratio [A–]/[HA] = 1 to 10 :

$$\text{pH} = pK_a + \log \frac{[A^-]}{[HA]} = pK_a + \log \frac{1}{10} = pK_a + (-1)$$

If the equation is evaluated at several ratios of [A –] / [HA] between the limits 10^3 and 10^{-3}, and the calculated pH values plotted, the result obtained describes the titration curve for a weak acid as shown in Fig. 3–7

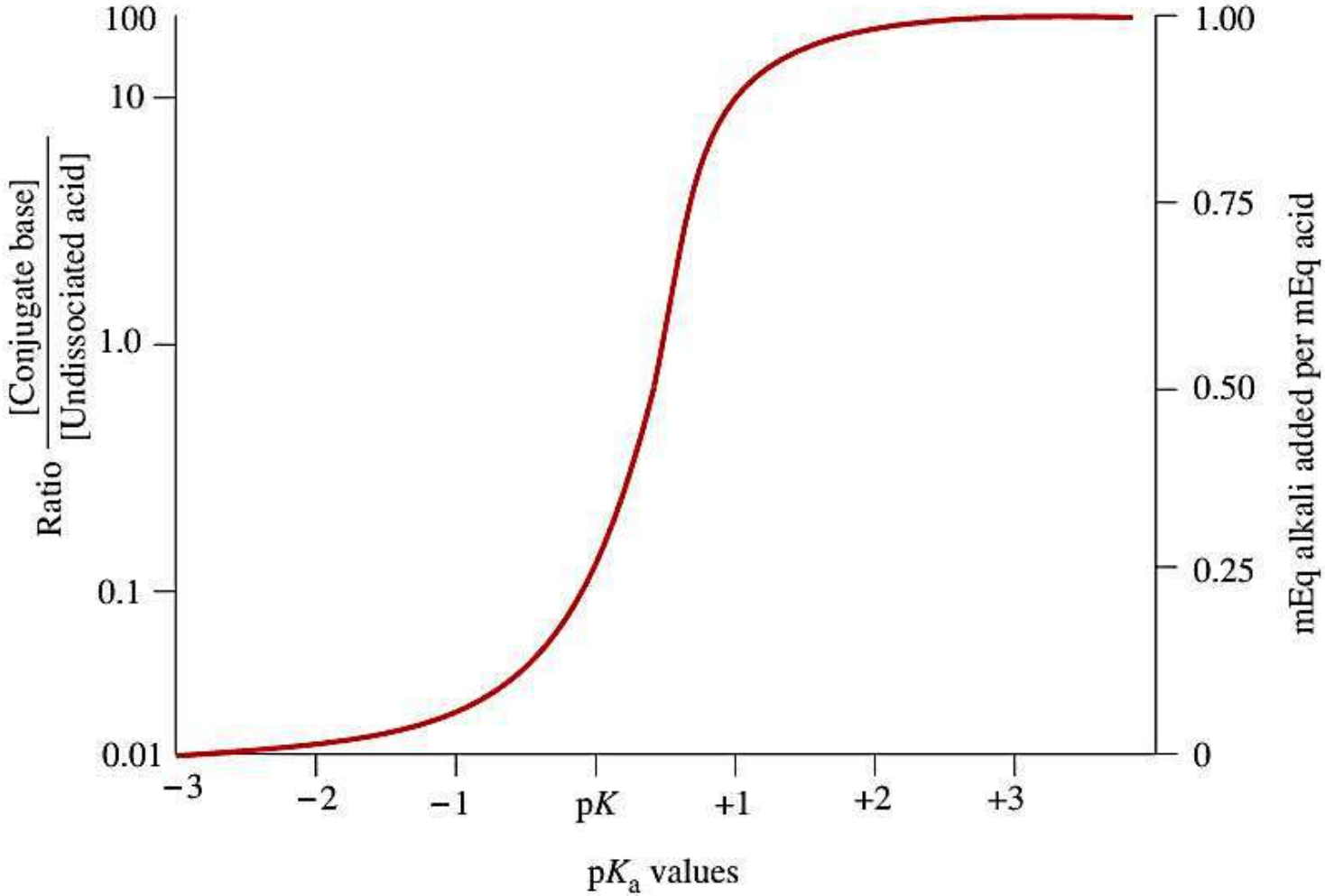


Fig. 3–7. General form of a titration curve calculated from the Henderson-Hasselbalch equation

BUFFERS

A buffer solution is one that resists a change in pH on the addition of acid (H+) or base (OH−), more effectively than an equal volume of water.

Most commonly, the buffer solution consists of a mixture of a weak Brönsted acid and its conjugate base;

Example, mixtures of acetic acid and sodium acetate or of ammonium hydroxide and ammonium chloride are buffer solutions.

A buffer system consists of a weak acid (the proton donor) and its conjugate base (the proton acceptor).

Buffer capacity

Buffer capacity, β, is a quantitative measure of the resistance of a buffer solution to pH change on addition of hydroxide ions or Hydrogen Ions.

BUFFERS ACTION/ CAPACITY

Two factors determine the effectiveness or capacity of buffer solution :

(a) Molar concentration of the buffer components. The buffer capacity is directly proportional to the concentration of the buffer components. The concentration of a buffer refers to the sum of the concentration of the weak acid and its conjugate base. Thus, a 0.1 M acetate buffer could contain 0.05 mole of acetic acid and 0.05 mole of sodium acetate in 1 litre of water. It could also contain 0.065 mole of acetic acid and 0.035 mole of sodium acetate in 1 litre of water.

(b) Relative concentrations of the conjugate base and the weak acid.
Most effective buffer would be one with equal concentrations of acidic and basic components, since such a mixture could furnish equal quantities of acidic and basic components to react, respectively with alkali or acid.

An inspection of the titration curve for acetic acid (Fig. 3−3) shows that the minimum change in pH resulting from the addition of a unit of base (or acid) occurs at the pKa for acetic acid. At this pH, the ratio CH_3COO^- to CH_3COOH is 1. On the contrary, at values of pH far removed from the pKa (and therefore at ratios of conjugate base to acid greatly differing from unity), the change in pH for unit of acid or alkali added is much larger.

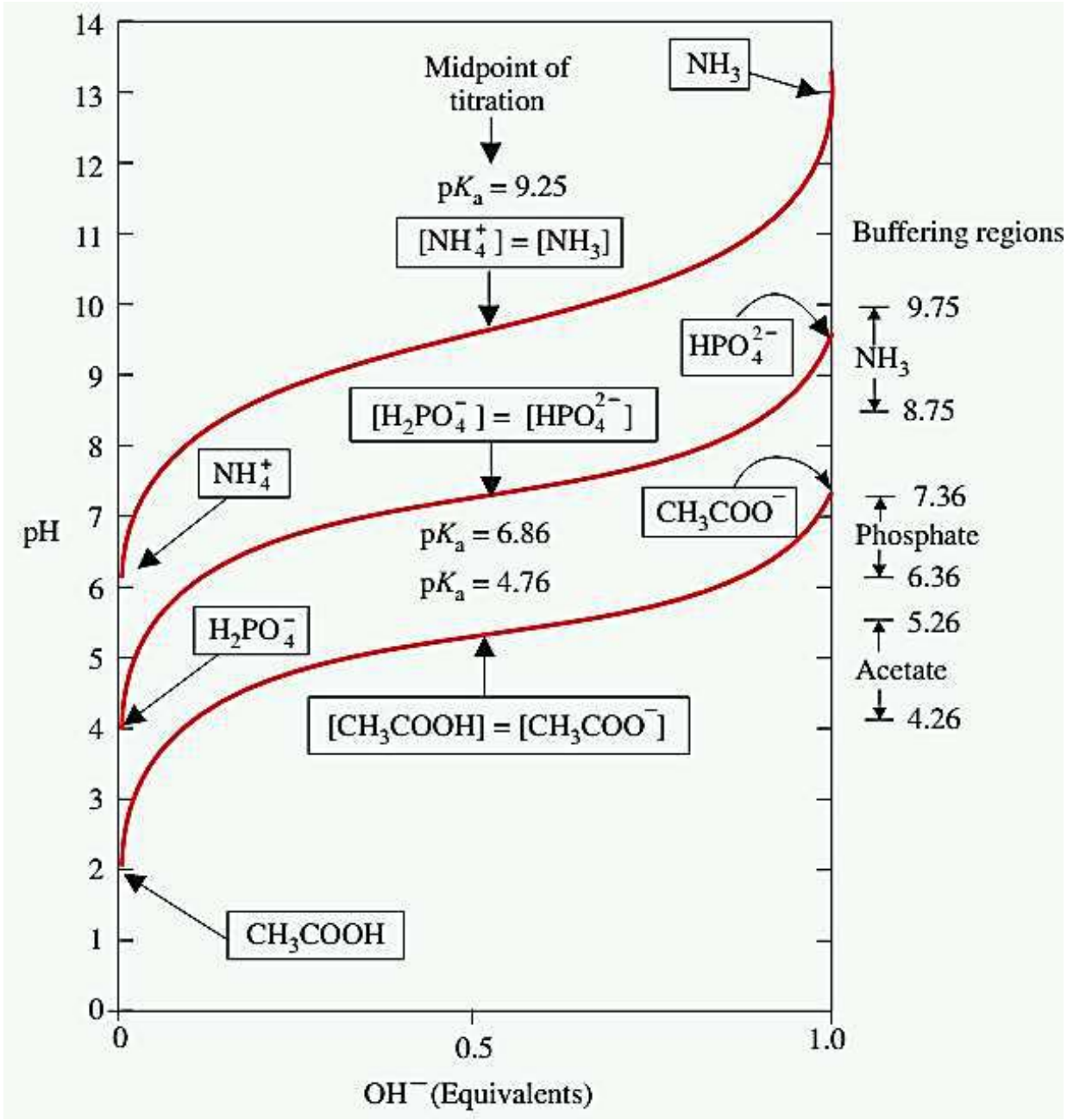


Fig. 3–6. Comparison of the titration curves of 3 weak acids, CH_3COOH, $H_2PO_4^-$ and NH_4^+

APPLICATIONS OF BUFFER

1. A buffer solution is an aqueous solution consisting of a mixture of a weak acid and its conjugate base, or vice versa.

2. Its pH changes very little when a small amount of strong acid or base is added to it.

3. Buffer solutions are necessary to keep the correct pH for enzymes in many organisms to work. Many enzymes work only under very precise conditions; if the pH moves outside of a narrow range, the enzymes slow or stop working and can denature. In many cases denaturation can permanently disable their catalytic activity.

4. A buffer of carbonic acid (H_2CO_3) and bicarbonate (HCO_3) is present in blood plasma, to maintain a pH between 7.35 and 7.45.

5. Industrially, buffer solutions are used in fermentation processes and in setting the correct conditions for dyes used in colouring fabrics.

6. They are also used in chemical analysis and calibration of pH meters.

7. The majority of biological samples that are used in research are made in buffers, especially phosphate buffered saline (PBS) at pH 7.4.

BIOANALYZER

The Bioanalyzer is a chip-based capillary electrophoresis machine to analyse RNA, DNA, and protein.

It is produced by Agilent and widely used, among other things, in RNA quality control measurements before downstream experiments like microarrays

Importance of Bio-analyzer

Increasing quality and speed of gel electrophoresis

Sample volumes 1 - 5 µl

10 -12 samples depending on Assay

Separation, staining, detection of samples

Results in 5-30 minutes available

No extra waste removal needed

Disposable Chip, no crosscontamination

ELECTROPHRESIS SEPERATION BY BIOANALYZER

DNA Assays:
1000, 7500, 12000

- Sizing
- Quantitation
- PCR products, digests, larger DNA fragments
- 12 samples in 30 min.

RNA Assays:
nano, pico, Small RNA

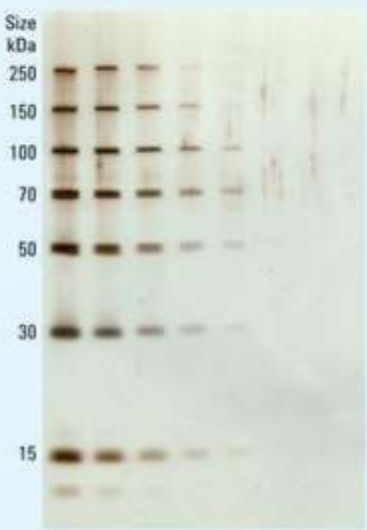
- Quantitation (Sizing in Small RNA)
- total RNA, mRNA
- purity & integrity determination
- 10 samples in 30 min.

Protein Assays:
P80, P230, HSP-250

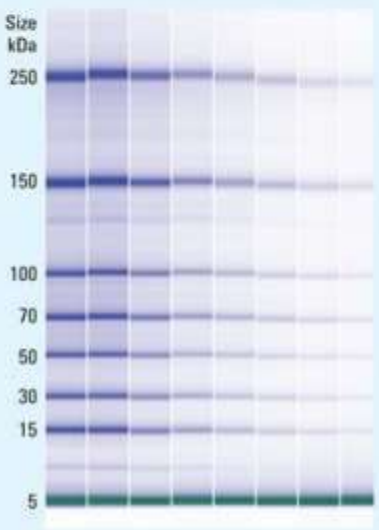
- Sizing
- Quantitation
- cell lysates, column fractions, purified proteins, antibodies etc.
- 10 samples in 40 min.

Protein Applications

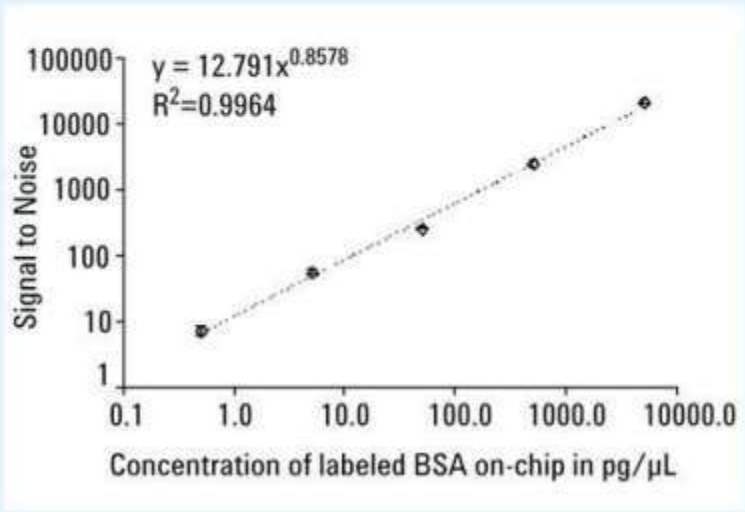
Protein Purification | Protein Expression | Protein Production | Food Analysis | Purity and QA/QC



Silver stained PA-gel



2100 Pseudo gel view



DNA Applications

PCR product purity

Multiplex PCR Applications

Gene expression analysis via RT-PCR (target validation)

GMO testing

Pathogen detection (homeland defense, hospitals, environmental)

Genotyping applications

- **Duplications/ deletions**
- **Allele frequency**
- **Bacterial sub-typing**
- **Forensics**

Cancer diagnostics

mPCR validation, impurity check | Gene Expression | Restriction Digest Analysis | Food Analysis | Forensic Testing

RNA Applications

Analysis of totalRNA, mRNA and small RNA samples in ng and pg concentration range

Standardized RNA integrity assessment with **RIN*** algorithm

Multi-analysis capabilities: DNA, RNA, Proteins and Flow Cytometry

RIN = RNA Integrity Number, an Agilent patented algorithm to Determine RNA quality in a normalized way

RNA QA/QC for Microarrays | Gene Expression | RNA QA/QC for qPCR | RNA QA/QC for mPCR | smallRNA QA/QC

Features of the RNA 6000 Assays

total RNA

determine integrity and quality of total RNA

determination of RNA concentration

identify ribosomal peaks

calculate the ratio of ribosomal peaks (18S/28S or 16S/23S)

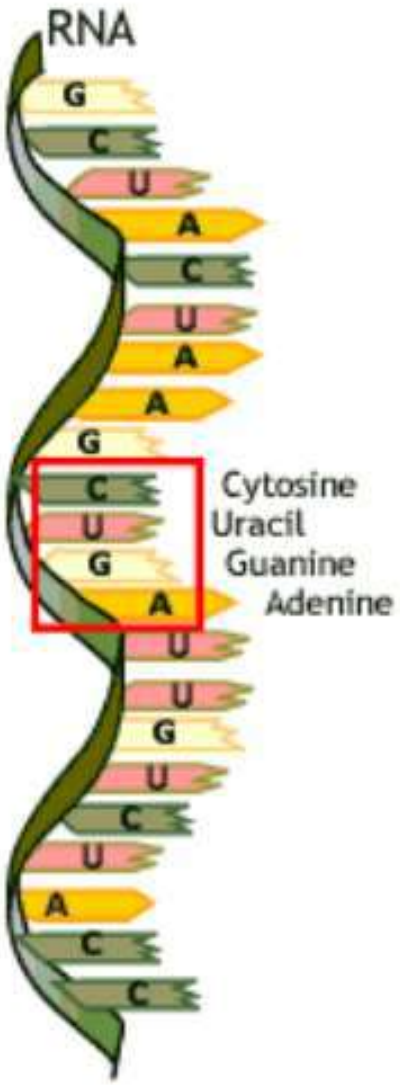
RNA integrity number (RIN)

mRNA

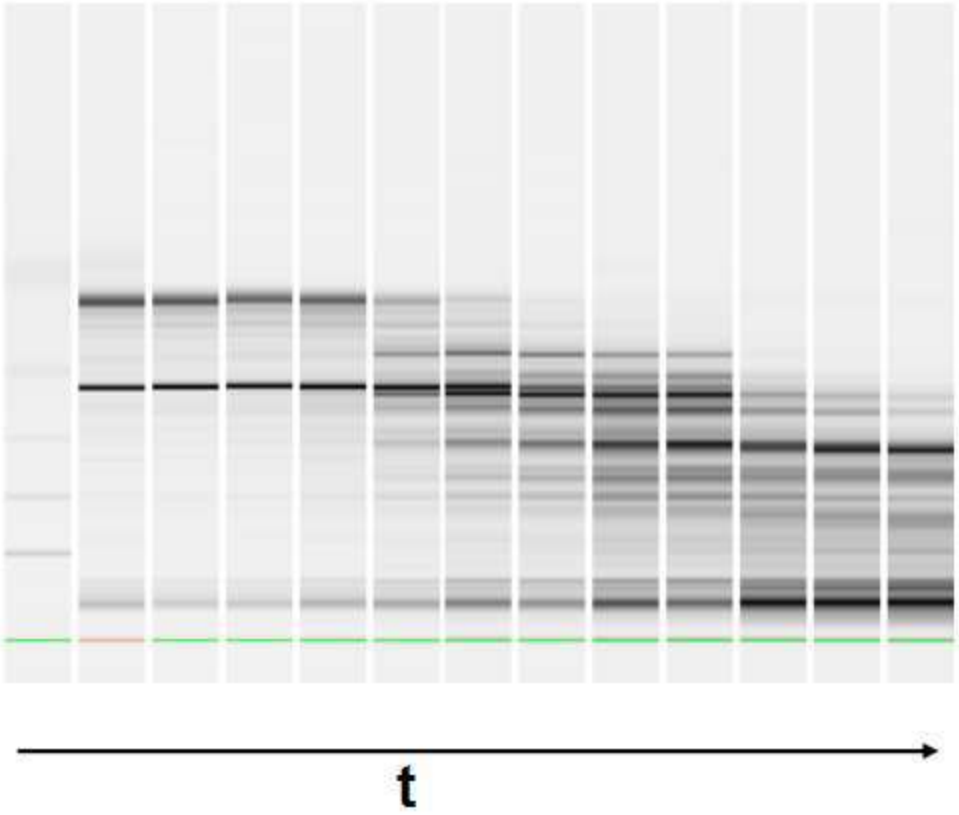
determine integrity and quality of mRNA samples

Determination of mRNA concentration

calculate % ribosomal RNA in mRNA samples



Problem Description



The ratio of ribosomal bands is not sufficient to describe RNA integrity!

RNA degradation is a gradual process.

Results have to be interpreted by visual inspection.

Overlay of electropherograms only works well for samples with the same concentration.

Instrument dependency in **signal height**

- **Labeled cRNA from different levels of RNA degradation results in low Cy Dye incorporation and low yield of cRNA**
- **RNA integrity levels of starting material had serious impact on downstream gene expression microarray results**
- **The RIN is an effective tool that can be used to evaluate RNA integrity objectively**

RNA INTEGRITY NUMBER (RIN)

- The **RNA integrity number** (RIN) is an algorithm for assigning integrity values to RNA measurements. The integrity of RNA is a major concern for gene expression studies and traditionally has been evaluated using the 28S to 18S rRNA ratio, a method that has been shown to be inconsistent. This inconsistency arises because subjective, human interpretation is necessary to compare the 28S and 18S gel images.

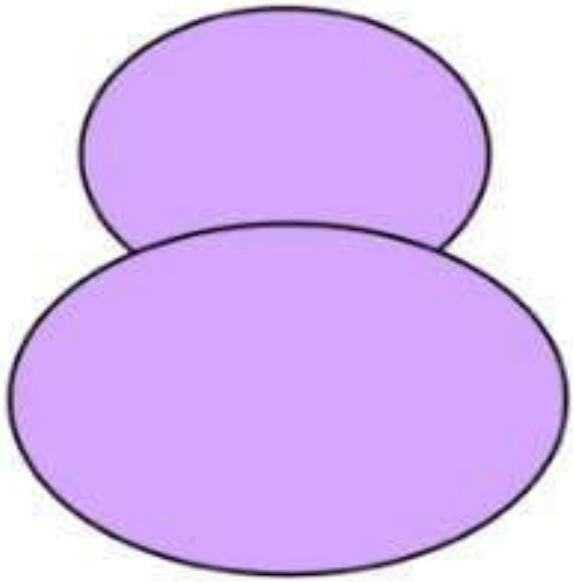
- The RIN algorithm was devised to overcome this issue. The RIN algorithm is applied to electrophoretic RNA measurements, obtained using capillary gel electrophoresis, and based on a combination of different features that contribute information about the RNA integrity to provide a more universal measure.

- RIN has been demonstrated to be robust and reproducible in studies comparing it to other RNA integrity calculation algorithms, cementing its position as a preferred method of determining the quality of RNA to be analyzed

- The RNA Integrity Database (RINdb) is a freely accessible repository holding hundreds of user submitted total RNA traces. By searching the database scientists can now see what is a "normal" profile for different tissue types as well as the effects of using different RNA extraction methods and kits.

Eukaryotic ribosomes

- Eukaryotic ribosomes are called 80S ribosomes.
- The 80S ribosome is composed of two subunits:
  - Large subunit (60S):
    - 28S rRNA
    - 5.8S rRNA
    - 5S rRNA
  - Small subunit (40S):
    - 18S rRNA



80 S

Principle: RIN

The RNA integrity number (RIN) is a software tool designed to help scientists estimate the integrity of total RNA samples.

The expert software automatically assigns an integrity number to an eukaryote total RNA sample.

Using this tool, sample integrity is no longer determined by the ratio of the ribosomal bands, but by the entire electrophoretic trace of the RNA sample.

This includes the presence or absence of degradation products. In this way, interpretation of an electropherogram is facilitated, comparison of samples is enabled and repeatability of experiments is ensured.

The assigned RIN is independent of sample concentration, instrument and analyst therefore becoming a de facto standard for RNA integrity.

RIN Computation

RIN for a sample is computed using several characteristics of an RNA electropherogram trace, with the first two being most significant. All the following descriptions apply to mammalian RNA:

- **Total RNA ratio**
  - Calculated by taking the ratio of the area under the 18S and 28S rRNA peaks to the total area under the graph, a large number here is desired, indicating much of the rRNA is still intact.
- **Height of 28S peak**
  - Again, a large value is desired. 28S is used in RIN calculation as it is typically degraded more quickly than 18S, and so measuring its peak height allows for detection of the early stages of degradation.
- **Fast area ratio**
  - The fast region is the area between the 18S and 5S peaks on an electropherogram. Initially, as this value increases, it indicates degradation of 18S and 28S rRNA to an intermediate size, though the ratio subsequently decreases as RNA degrades further.
- **Marker height**
  - A small number is desired here, indicating only small amounts of RNA have been degraded and proceeded to the smallest lengths, indicated by the short marker.

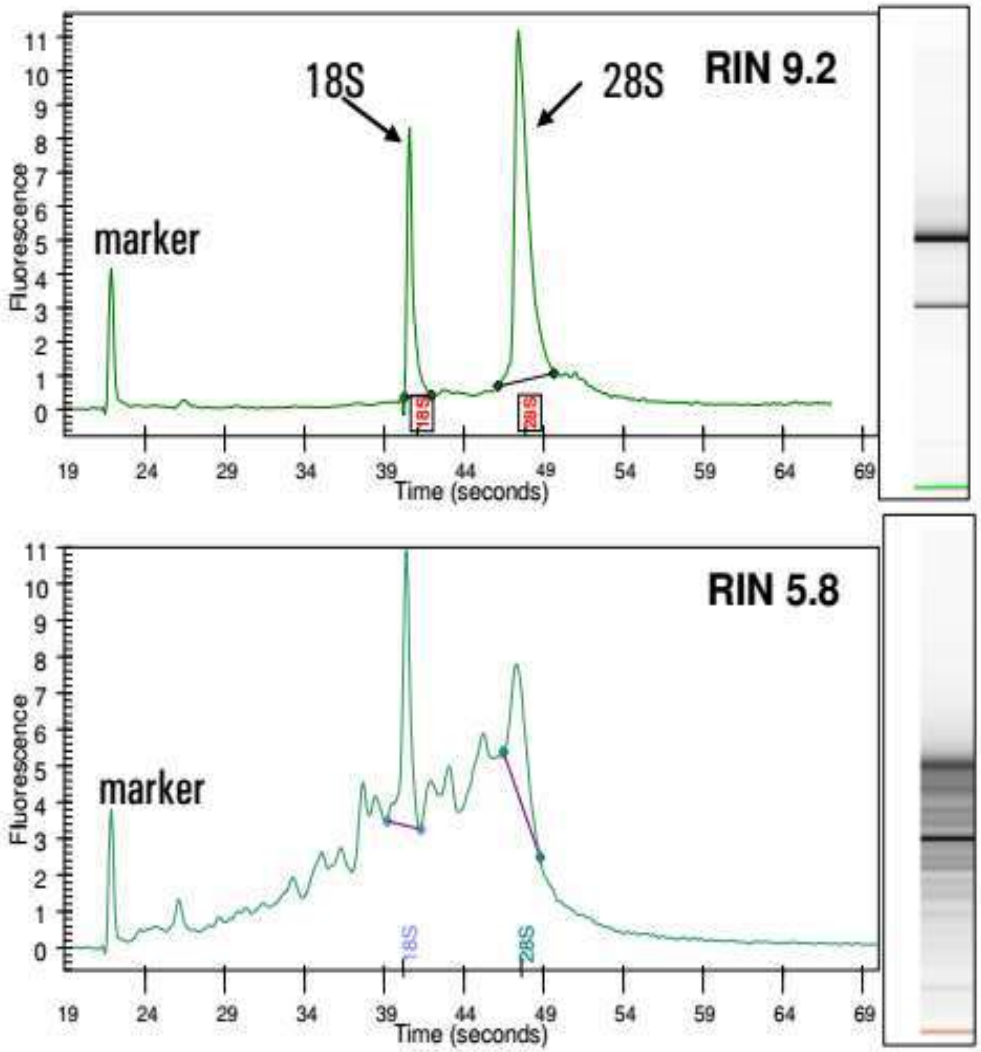
What is the meaning of the 28S/18S ribosomal ratios?

- The 28S/18S ribosomal RNA ratio is frequently used to assess the quality of total RNA purified from any given sample. The 28S and 18S rRNAs are produced by the cleavage of a single RNA transcript.

- Since they are produced from a single transcript, the ratio of the number of 28S rRNA molecules to that of 18S molecules present in a cell equals 1.

- However, if you want to detect these rRNAs on an agarose gel, the intensity of the bands produced by these molecules depends on the number of nucleotides present in each molecule.

- In humans, 28S rRNA has ~5070 nucleotides, and 18S has 1869 nucleotides, which gives a 28S/18S ratio of ~2.7. A high 28S/18S ratio is an indication that the purified RNA is intact and hasn't degraded. Usually, a 28S/18S ratio of >2 is taken to mean that the purified total RNA is of high quality.

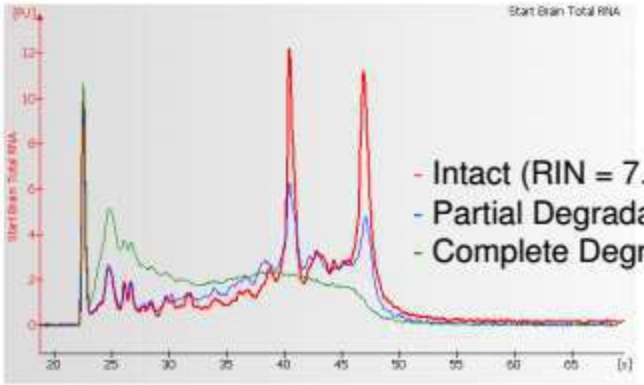


Typical first QC step after RNA sample prep prior to microarrays or real-time PCR

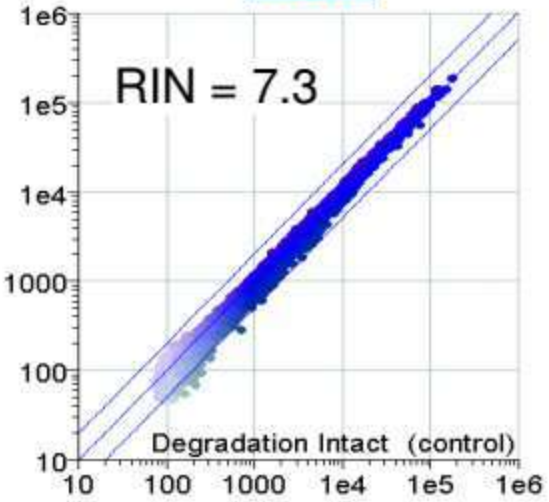
High quality total RNA

Partially degraded total RNA

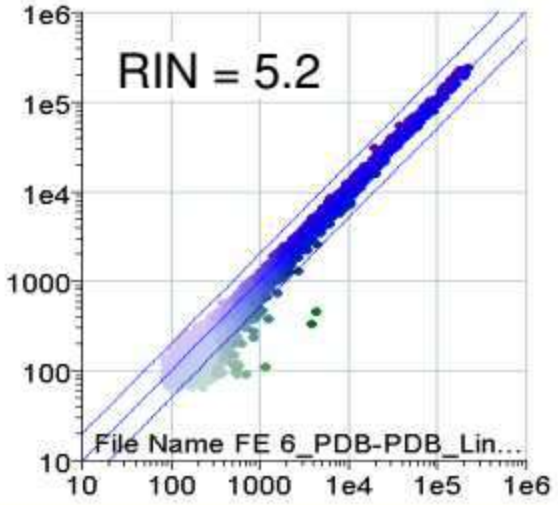
Scatter Plots : Self vs. Self



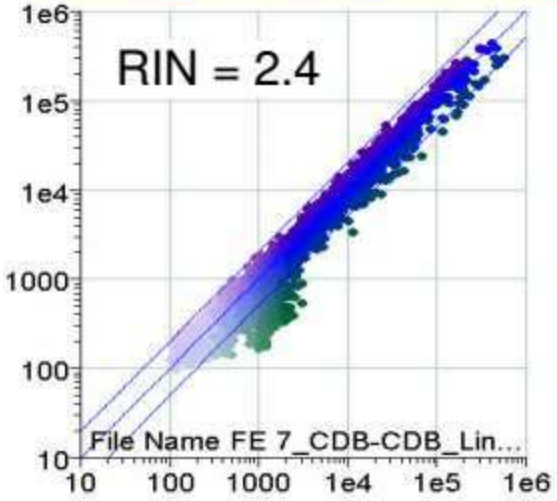
Intact



Partial Degradation



Complete Degradation



Drawback of RIN

A major criticism to RIN is when using with plants or in studies of eukaryotic-prokaryotic cells interactions. The RIN algorithm is unable to differentiate eukaryotic/ prokaryotic/ chloroplastic ribosomal RNA, creating serious quality index underestimation in such situations

Applications

- RNA integrity is critical for proper results in gene expression studies, such as microarray analysis, Northern blots, or quantitative Real-Time PCR (qPCR).
- qPCR and similar techniques are very expensive, taking a good deal of both time and money, so continuing research being undertaken to decrease the cost while maintaining qPCR's accuracy and reproducibility for gene expression and other applications.
- RIN assessment allows a scientist to evaluate an experiment’s trustworthiness and reproducibility before incurring substantial costs in performing the gene expression studies.

What the RIN ***can*** do:

1. Obtain a numerical assessment of the integrity of RNA.

2. Directly compare RNA samples, e.g. before and after archival, compare integrity of same tissue across different labs.

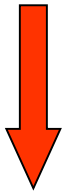
3. Ensure repeatability of experiments, e.g. if RIN shows a given value and is suitable for microarray experiments, then the RIN of the same value can always be used for similar experiments given that the same organism/tissue/extraction method is used.

What the RIN ***cannot*** do:

1. Tell a scientist ahead of time whether an experiment will work or not if no prior validation was done (e.g. RIN of 5 might not work for microarray experiments, but might work well for an appropriate RT-PCR experiment. Also, a RIN that might be good for a 3' amplification might not work for a 5' amplification).

BIOSENSORS

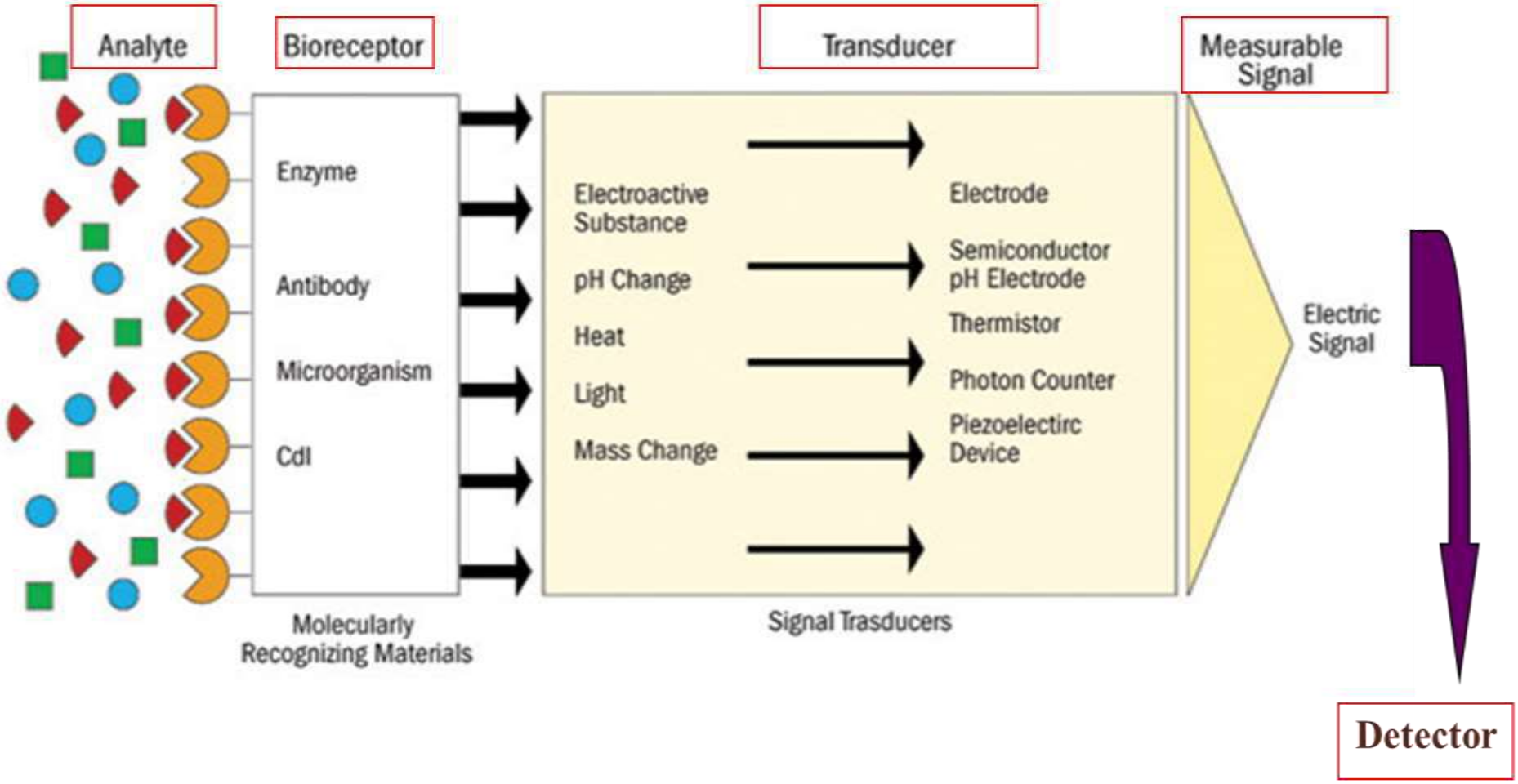
Self-contained integrated device that is capable of providing specific qualitative or semi-quantitative analytical information using a biological recognition element which is in direct-spatial contact with a transduction element. (IUPAC,1998)



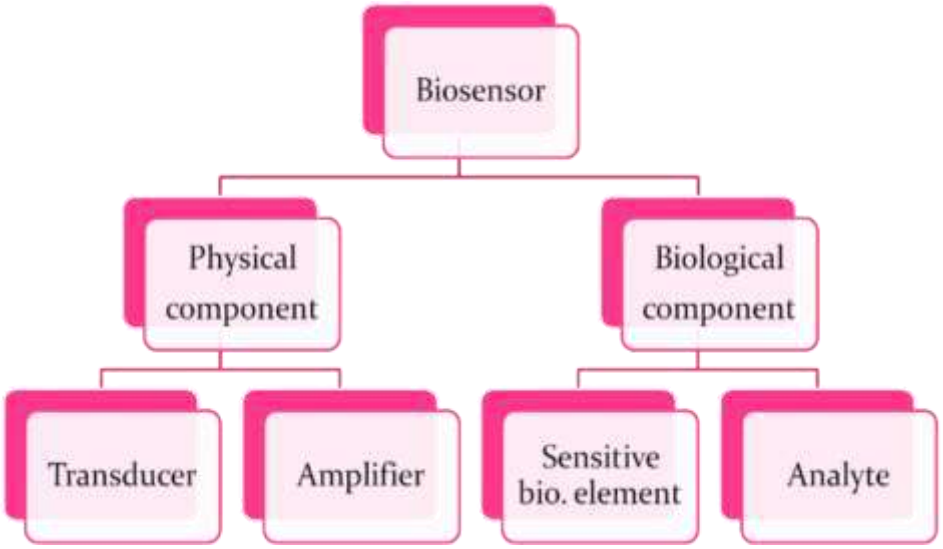
1) Biosensor ≠ Bioanalytical system

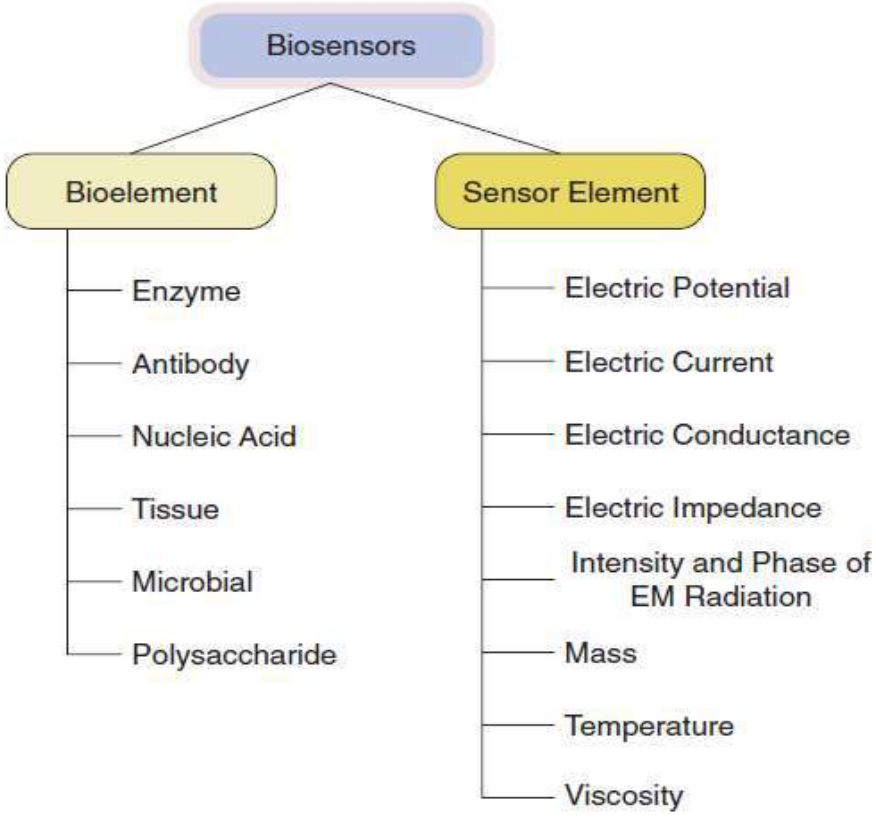
2) An enzyme electrode is a biosensor

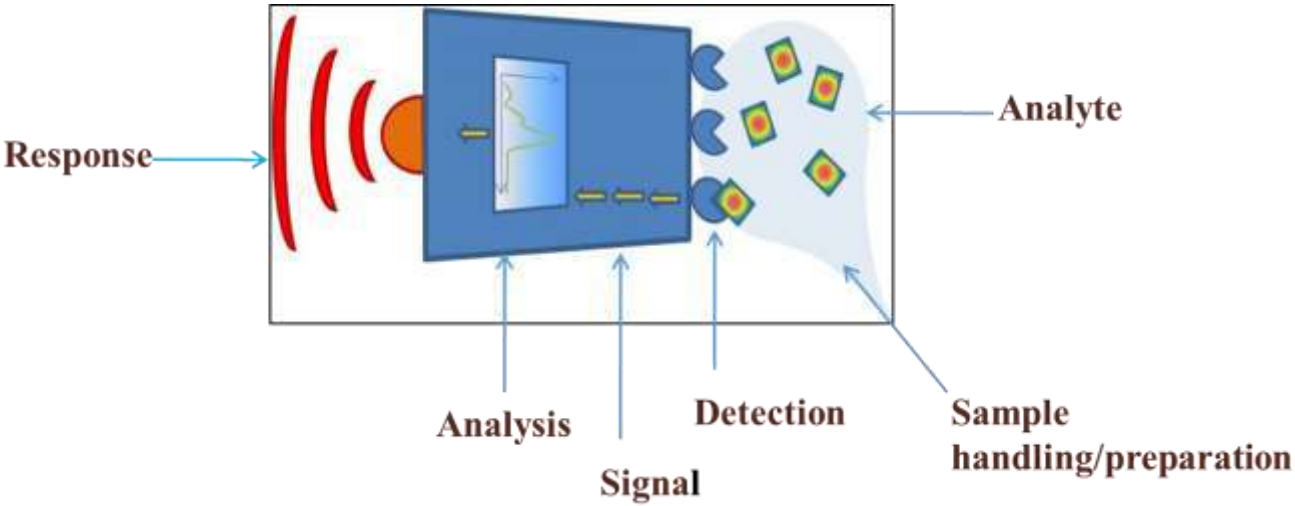
COMPONENTS


Analyte
Bioreceptor
Transducer
Measurable Signal
Enzyme
Antibody
Microorganism
Cdl
Electroactive Substance
pH Change
Heat
Light
Mass Change
Electrode
Semiconductor pH Electrode
Thermistor
Photon Counter
Piezoelectirc Device
Electric Signal
Molecularly Recognizing Materials
Signal Trasducers
Detector

ELEMENTS OF BIOSENSORS



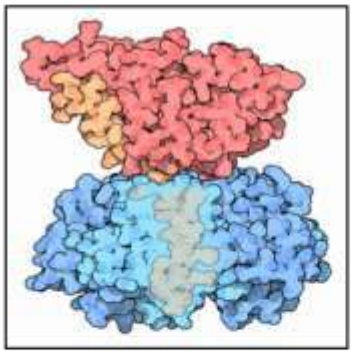




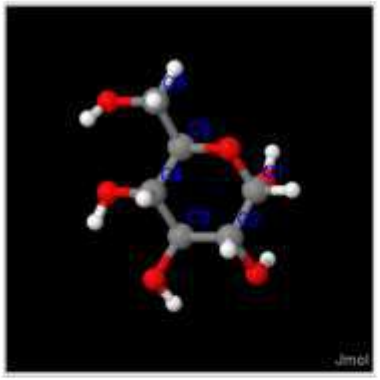
THE ANALYTE: (What do you want to detect?)

Molecule

Protein, toxin, peptide, vitamin, sugar, metal ion



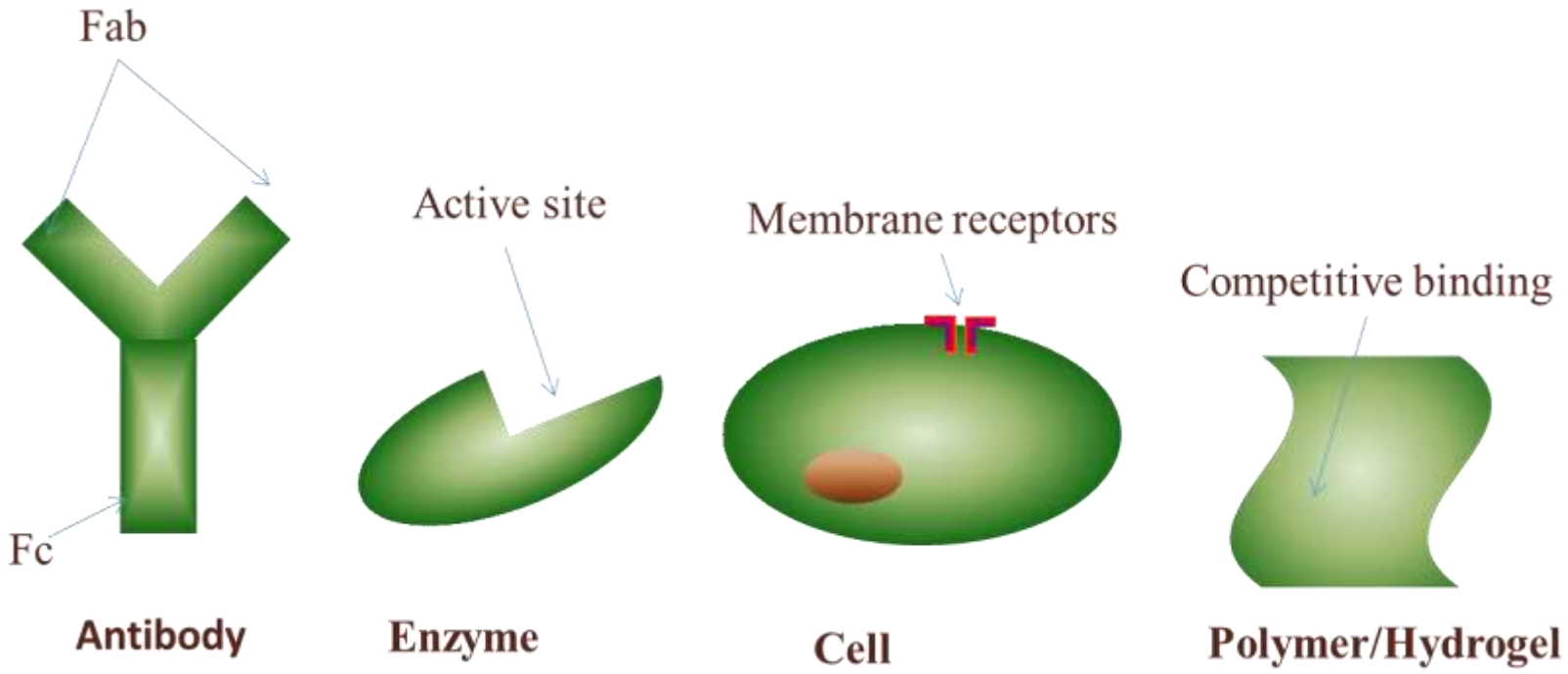
Cholera toxin



Glucose

DETECTION/RECOGNITION:

(How do you specifically recognize the analyte?)



WORKING PRINCIPLE

- Analyte diffuses from the solution to the surface of the Biosensor.
- Analyte reacts specifically & efficiently with the Biological Component of the Biosensor.
- This reaction changes the physicochmical properties of the Transducer surface.
- This leads to a change in the optical/electronic properties of the Transducer Surface.
- The change in the optical/electronic properties is measured/converted into electrical signal, which is detected.

ADVANTAGES

- Highly Specific.
- Independent of Factors like stirring, pH, etc.
- Linear response, Tiny & Biocompatible.
- Easy to Use, Durable.
- Require only Small Sample Volume.
- Rapid, Accurate, Stable & Sterilizable.

TYPES

1. Calorimetric/Thermal Detection Biosensors.
2. Optical Biosensors.
3. Resonant Biosensors.
4. Piezoelectric Biosensors.
5. Ion Sensitive Biosensors.
6. Electrochemical Biosensors.
    I. Conductimetric Sensors.
    II. Amperometric Sensors.
    III. Potentiometric Sensors.

1. CALORIMETRIC / THERMAL DETECTION BIOSENSORS.

- Uses Absorption / Production of Heat.
- Total heat produced/absorbed.
- Temp. measured by Enzyme Thermistors.

Advantages:

- No need of Frequent recalibration.
- Insensitive to the Optical & Electrochemical Properties of the sample.

Uses:

Detection of: (1) Pesticides & (2) Pathogenic Bacteria.

2. OPTICAL BIOSENSORS

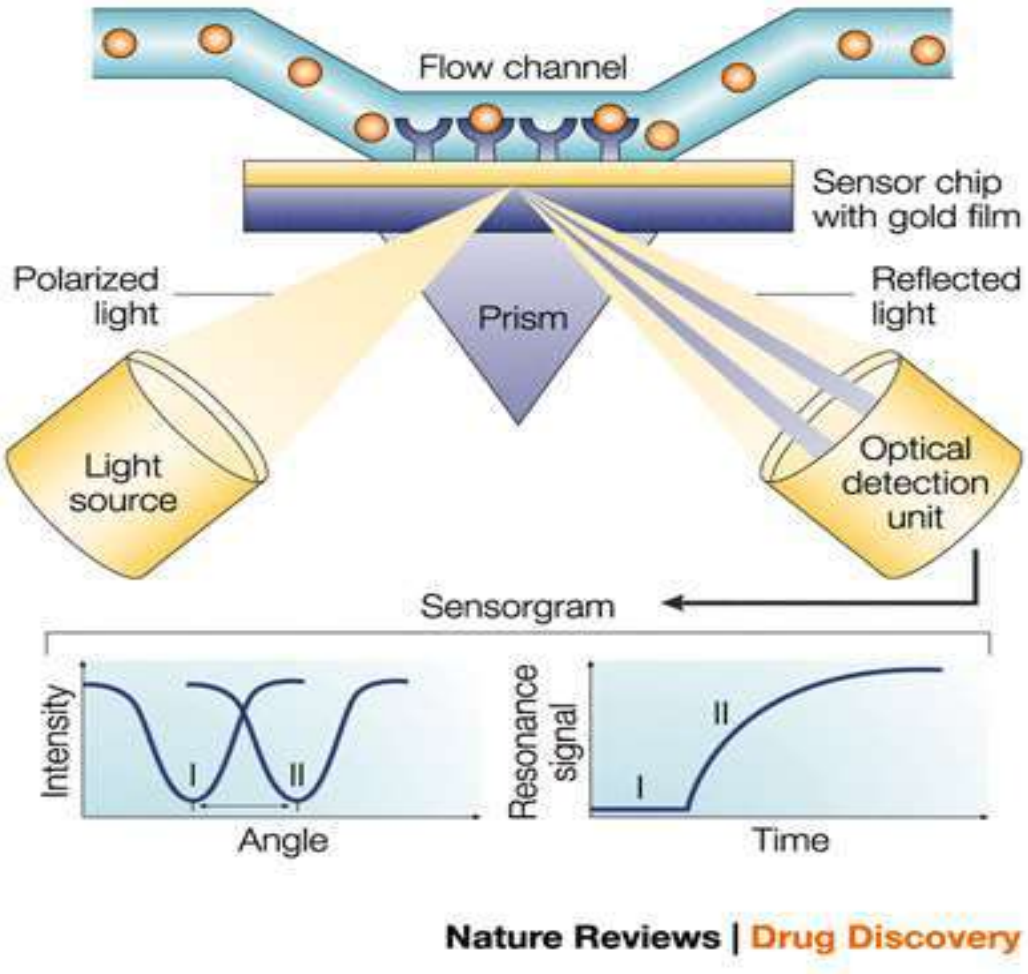
- Colorimetric for *colour* - Measures change in Light Adsorption.
- Photometric for *Light Intensity* - Detects the Photon output.

3. RESONANT BIOSENSORS

- An Acoustic Wave Transducer is coupled with Bio-element.
- Measures the change in Resonant Frequency.

4. PIEZOELECTRIC BIOSENSORS

- The prefix piezo- is Greek for 'press' or 'squeeze'.
- A piezoelectric sensor is a device that uses the piezoelectric effect, to measure changes in pressure, acceleration, temperature, strain, or force by converting them to an electrical charge.
- Acoustics is the interdisciplinary science that deals with the study of all mechanical waves in gases, liquids, and solids including topics such as vibration, sound, ultrasound and infrasound.



5. ION SENSITIVE BIOSENSORS

- Are semiconductor FETs with ion-sensitive surface.
- Surface Electrical Potential changes when the ions & semiconductors interact.
- Measures the Change in Potential.

Uses: pH Detection.

6. ELECTROCHEMICAL BIOSENSORS.

Principle

Many chemical produce or consume ions or ês causing some change in the electrical properties of the solution that can be sensed out & used as a measuring parameter.

Following sensor uses:

I. Conductimetric Sensors.
II. Amperometric Sensors.
III. Potentiometric Sensors.

Uses: Detection of :

I. Hybridized DNA
II. DNA- binding Drugs
III. Glucose Concentration.

- In electronics and electrical engineering, a **ramp** generator is a function generator that increases its output **voltage** up to a specific value, called a **ramp**. Among multitude of other uses, it is used in electrical generators or electric motors to avoid jolts when changing a load.

I- CONDUCTIMETRIC SENSORS

1. Measures Electrical Conductance/Resistance of the solution.
2. Conductance Measurements have relatively Low Sensitivity.

II- AMPEROMETRIC BIOSENSORS

1. High Sensitivity Biosensor.
2. Detects electroactive species present in the biological test samples.
3. Measured Parameter – Current.

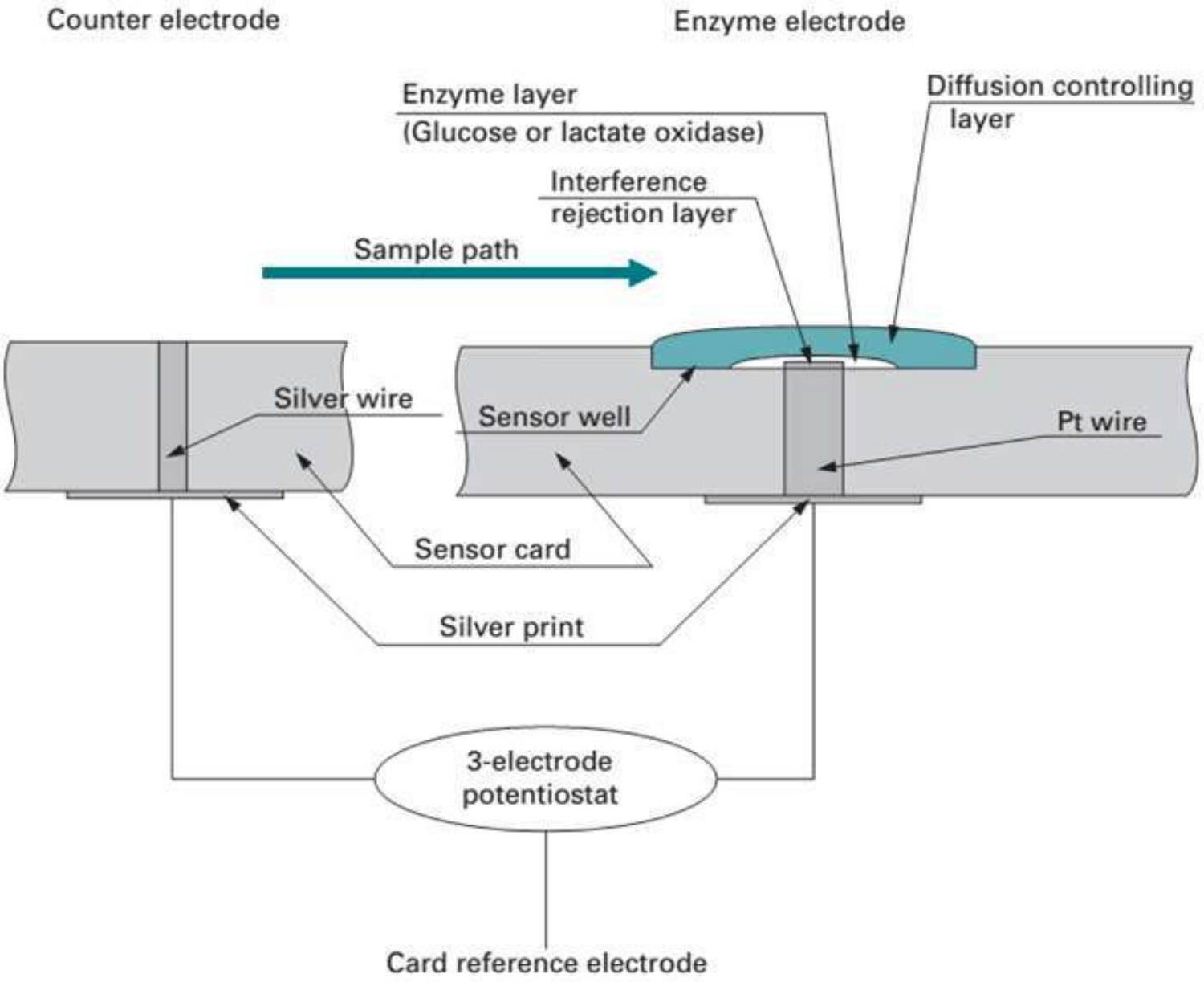
III- POTENTIOMETRIC SENSORS

1. **Working Principle –** When ramp voltage is applied to an electrode in solution, a current flow occurs because of electrochemical reactions.
2. **Measured Parameter –** Oxidation / reduction Potential of an Electrochemical reaction.

ENZYME ELECTRODE

- The enzyme electrode is a miniature chemical transducer which functions by combining an electrochemical procedure with immobilized enzyme activity.

- This particular model uses glucose oxidase immobilized on a gel to measure the concentration of glucose in biological solution and in the tissue in vitro.



Micro sensor analyte detectors. (a) The GEM 3000 Sensor Card; (b) the Amperometric Glucose/Lactate Sensors. (Reproduced by permission of IL Critical Care, Lexington, USA.)

IMMUNOELECTRODES

ANTIBODY-BASED BIOSENSORS

A biosensoris a device that is composed of a biological element and a physicochemical transduction part which converts signal reception by the biological entity into an electrical impulse.

A number of biosensor devices are available that use enzymes as the biological part of the device. The enzyme is used to catalyse a chemical reaction which generates an electrical charge at an electrode.

Antibodies have the potential to be excellent biological molecules to use for this technology as they can be developed to detect virtually any molecule. The main problem with developing this technology with antibodies has been the lack of adequate physicochemical transduction systems.

Three methods have been developed that will provide a signal from antibody binding and these are likely to produce a new generation of biosensors in the future.

1- SURFACE PLASMON RESONANCE
2- FIBRE OPTIC SENSORS
3- PIEZOELECTRIC CRYSTAL

1- SURFACE PLASMON RESONANCE

- Antibodies may be bound onto thin layers of gold which in turn are coated onto refractive glass slides.

- If the slides are illuminated at a precise angle with fixed-wavelength Laser light then electron waves are produced on the surface of the gold. This is known as surface plasmon resonance and only occurs if the incident angle and wavelength of light are precisely right.

- If the antibody binds antigen then the surface plasmon resonance pattern is changed and a measurable change in emitted energy is observed.

2- FIBRE OPTIC SENSORS

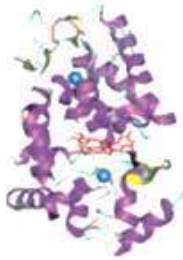
- Fibre optic sensors have also been developed which rely on the natural ability of biological materials to fluoresce with light at defined frequency.
- The reaction vessel is coated with antibody and the fibre optic sensor used to illuminate and read light scatter from the vessel.
- The sample is then applied and the sample vessel washed.
- The fibre optic sensor is again used to illuminate and read backscatter from the vessel.
- Changes in the fluorescence will give a change in the observed returned light.

2- PIEZOELECTRIC CRYSTAL

- A third approach relies on changes in crystals as a result of surface molecules bound to them.
- Piezoelectric crystals generate a characteristic signature resonance when stimulated with an alternating current.
- The crystals are elastic and changes to their surface will produce a change in the signature resonance.
- The binding of antigen to antibody located on the surface of the crystal can be sufficient to alter the signature and therefore induce a signal indicating that antigen has been detected by antibody.

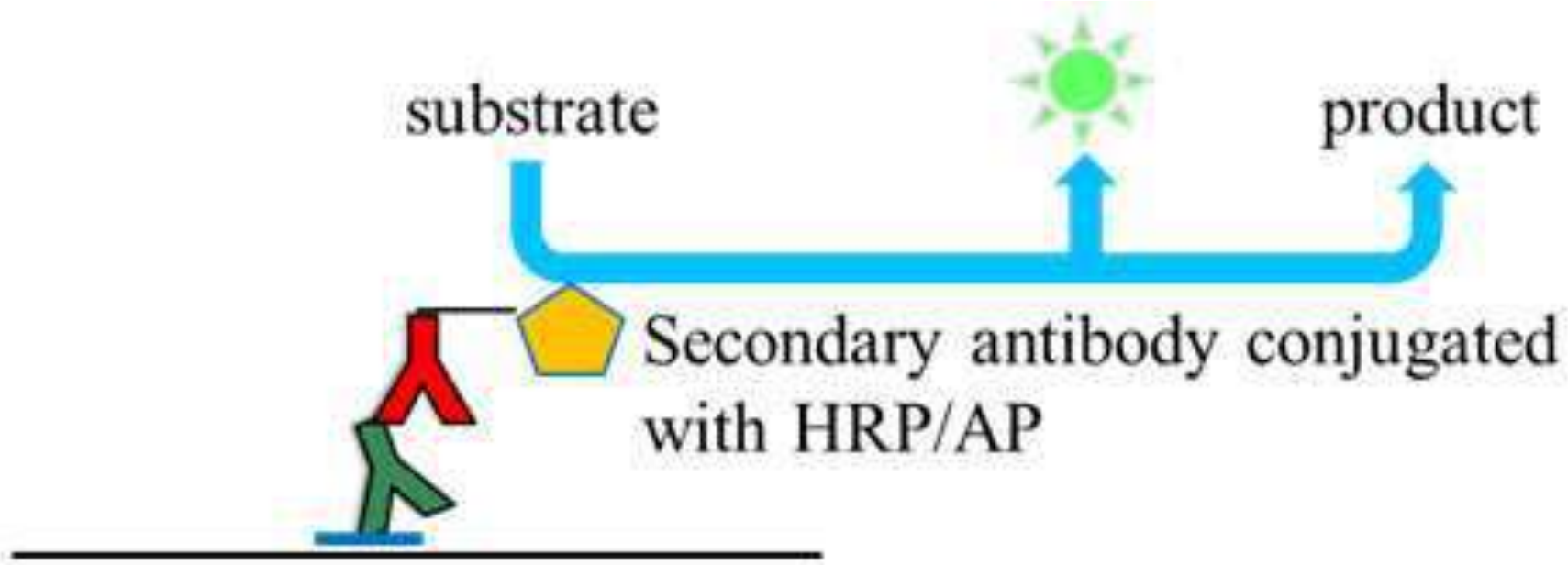
IMMUNOELECTRODES

The enzyme **horseradish peroxidase** (**HRP**)



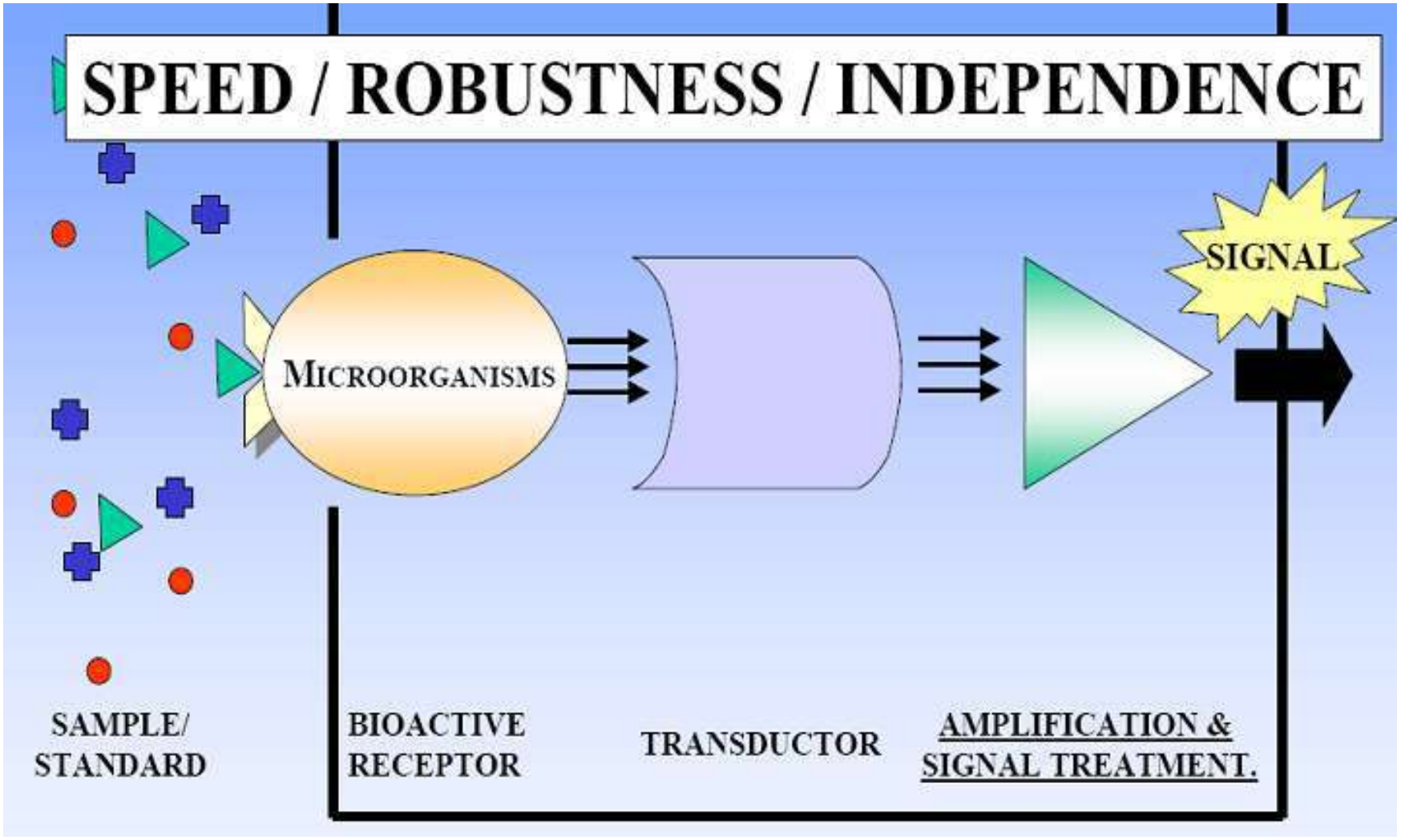
Horseradish peroxidase
(EC 1.11.1.7)

$$Substrate_{reduced} + H_2O_2 \longrightarrow Substrate_{oxidized} + 2H_2O$$



MICROBIAL BIOSENSORS

- A microbial biosensor consists of a transducer in conjunction with immobilised viable or non-viable microbial cells.
- Non-viable cells obtained after permeabilisation or whole cells containing periplasmic enzymes have mostly been used as an economical substitute for enzymes.
- Viable cells make use of the respiratory and metabolic functions of the cell, the analyte to be monitored being either a substrate or an inhibitor of these processes.
- Bioluminescence-based microbial biosensors have also been developed using genetically engineered microorganisms constructed by fusing the lux gene with an inducible gene promoter for toxicity and bioavailability testing


SPEED / ROBUSTNESS / INDEPENDENCE
MICROORGANISMS
SIGNAL
SAMPLE/
STANDARD
BIOACTIVE
RECEPTOR
TRANSDUCTOR
AMPLIFICATION &
SIGNAL TREATMENT.

Advantages

1. It able to metabolise a wide range of chemical compounds

2. It have a great capacity to adapt to adverse conditions and to develop the ability to degrade new molecules with time.

3. They are also amenable for genetic modifications through mutation or through recombinant DNA technology and serve as an economical source of intracellular enzymes.

Salmonella typhimurium as Biosensor

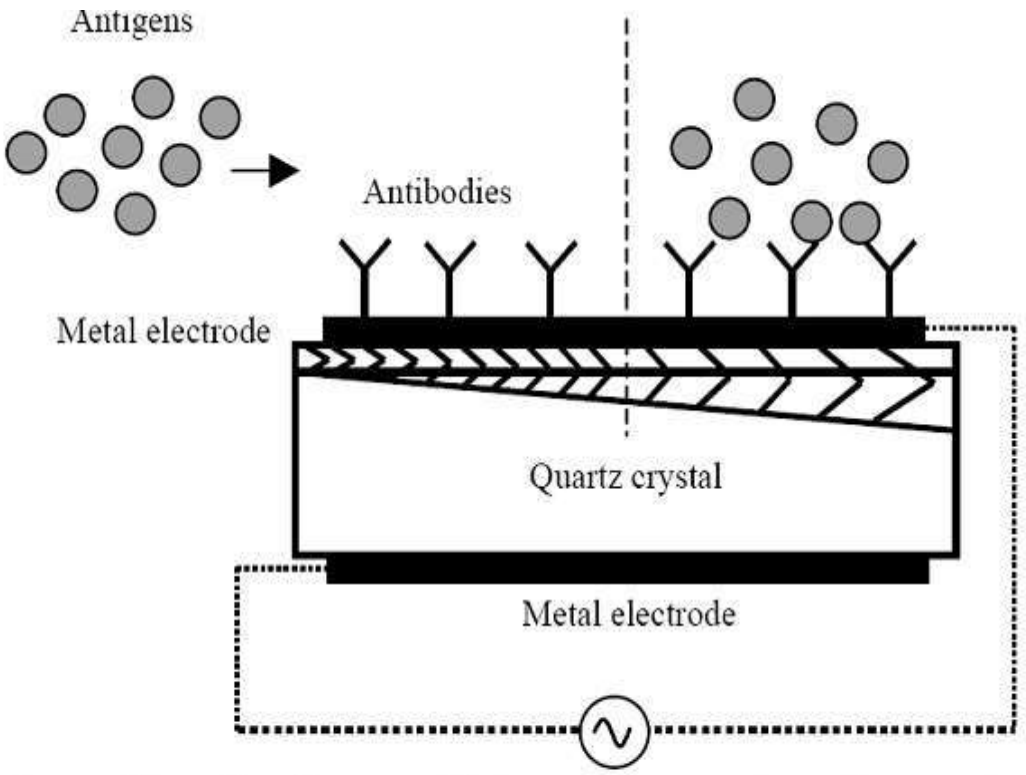
Ye et al. (1997) described a piezoelectric biosensor for detection of Salmonella typhimurium.

The device consists of a quartz crystal wafer sandwiched between two metal electrodes. These electrodes provide a means of connecting the device to an external oscillator circuit that drives the quartz crystal at its resonant frequency.

A change in mass on the surface of the electrode thus changes the resonant frequency of the quartz crystal microbalance (QCM) device.

The antibody against Salmonella was immobilised onto the gold electrode-coated quartz crystal surface through a polyethylenimine–glutaraldehyde technique.

The Salmonella cells reacted specifically and bound to the crystal surface resulting in an increase in mass that was directly related to the concentration in the solution



Piezoelectric crystal biosensor; mass deposition at the surface alters the frequency of the resonant of the crystal

PROPERTIES OF A GOOD BIOSENSOR

- **Specificity**

With biosensors, it is possible to measure specific analytes with great accuracy.

- **Speed**

analyte tracers or catalytic products can be directly and instantaneously measured

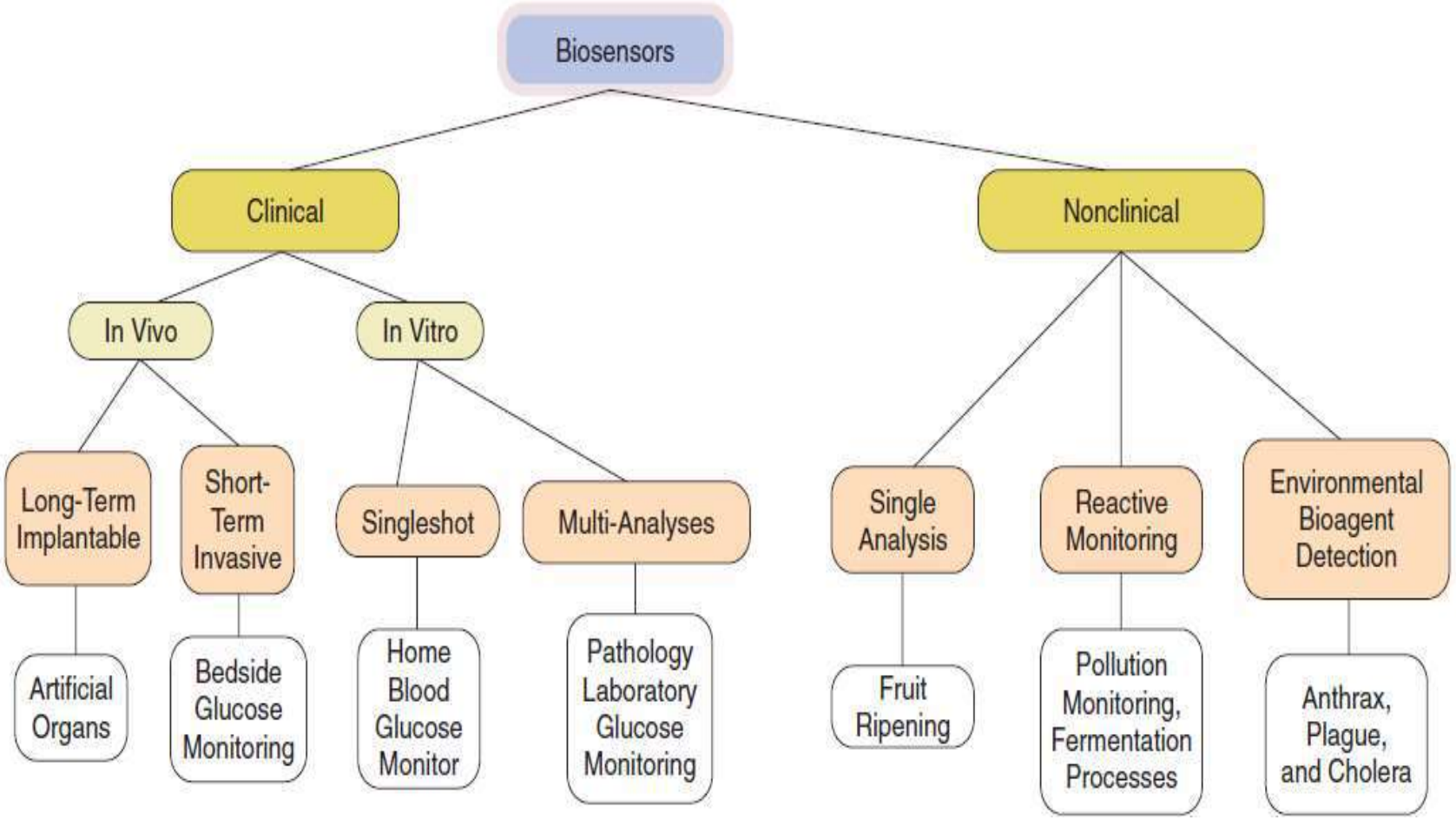
- **Simplicity**

receptor and transducer are integrated into one single sensor & the measurement of
target analytes without using reagents is possible

- **Continuous monitoring capability**

Biosensors regenerate and reuse the immobilized biological recognition element

APPLICATIONS OF BIOSENSOR



References

1. Satyanarayana, U. “Biochemistry” 5th Edition, Elsevier India 2017, ISBN: 9788131249406
2. Voet D, Voet J. .G “Biochemistry”, 4th Edition, John Wiley & Sons, Inc.,2010. ISBN: 978-0-470-57095-1
3. David L. Nelson; Michael M. Cox “Lehninger Principles of Biochemistry” Seventh Edition, Macmillan 2017, ISBN:9781464187957
4. Wilson, K., Walker, J. (eds.); Cambridge University Press, Cambridge, 2000, 784 pp., ISBN 0-521-65873-X (paperback)

All the original contributors of the concept and findings published are gratefully acknowledged while preparing the e-content for the students of Biochemistry and allied sciences