



CLS 332 - Lecture 1

Instrumental Analysis

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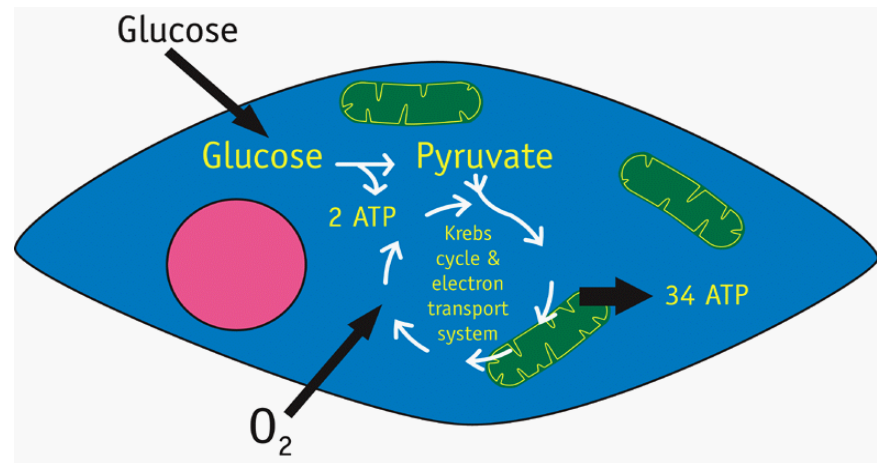
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The Clinical Process

- Pre-Analytical
 - Sample collection and storage
- Analytical
 - Analytical goals & quality assurance (QA)
- Post-Analytical
 - Reference range determination

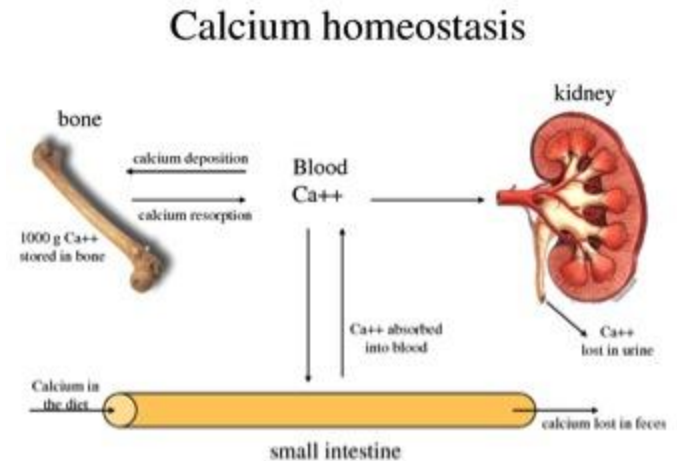
Clinical Analytes

- Intermediary Metabolites
 - Glucose, Lactate, Ammonia
- Enzymes
 - AST, CK, serum cholinesterase
- Isoenzymes
 - CK-MB, ALP, LDH



Clinical Analytes

- Proteins
 - Albumin, alpha1-antitrypsin, immunoglobulins (Ig)
- Electrolytes
 - Na, K, Cl, Li
- Trace Elements
 - Cu, Zn, Al, Se, Au, Hg



Clinical Analytes

- Steroid Hormones
 - Oestradiol, Cortisol, Aldosterone
- Peptide Hormones
 - Growth Hormone, Gastrin, IGF₁
- Drugs
 - Digoxin, Phenytoin, Cocaine
- Gases
 - O₂, CO₂, CO



Range of concentrations

0.1 mol/L → 0.000,000,000,000,001 mol/L

- mmol/L (10^{-3})
 - Sodium 140 mmol/L
 - Glucose 7 mmol/L
- umol/L (10^{-6})
 - Urate 400 umol/L
 - Bilirubin 10 umol/L
 - Amiodarone 1 umol/L
- nmol/L (10^{-9})
 - Thyroxine 120 nmol/L
 - Adrenaline 20 nmol/L
- pmol/L (10^{-12})
 - 1,25-Vitamin D 100 pmol/L
 - Free Thyroxine 15 pmol/L
 - PTH 1pmol/L
- fmol/L (10^{-15})
 - ADH AVP ?

Matrices (fluids, etc)

- Blood

- Whole Blood (*lead*)
- Serum (*thyroxine*)
- Plasma (*potassium*)
- Red Cells (*haemoglobin A_{1c}*)
- White Cells (*enzymes hexaminidase A&B, DNA*)
- Dissolved gases (*O₂, CO₂, CO*)



Matrices (fluids, etc)

- Urine
 - Calcium
 - Oxalate
 - Urate
 - Total Protein
 - Bilirubin
 - Aldosterone
 - Cortisol
 - Porphyrin

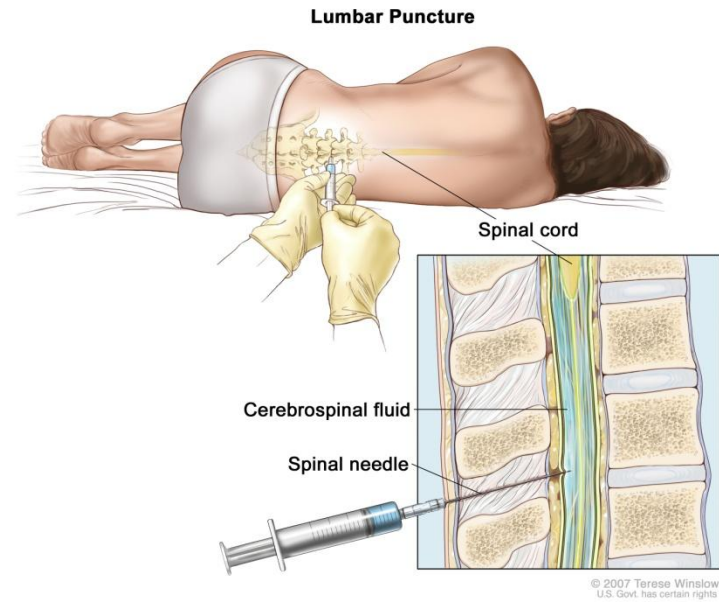


Matrices (fluids, etc)

- Cerebrospinal Fluid (CSF)

Usually from lumbar puncture

- Glucose
- Lactate
- Total Protein
- Immunoglobulin (IgG)
- Oligoclonal Bands

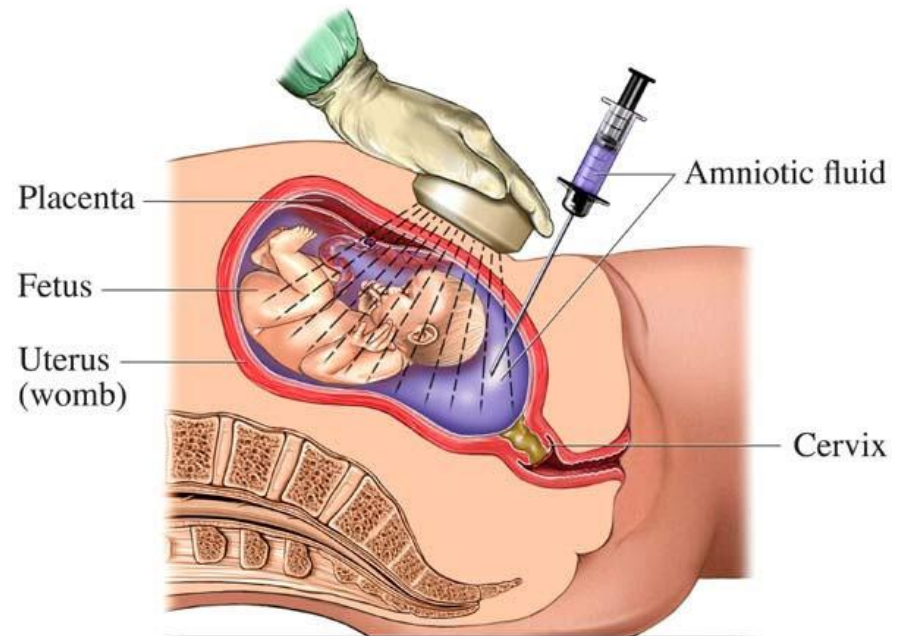


Matrices (fluids, etc)

- Amniotic Fluid
 - Alpha Fetoprotein (*AFP*)
 - Chromosome typing (*Downs' Syndrome*)
- Pleural, Pericardial, Ascitic, Nose Fluid

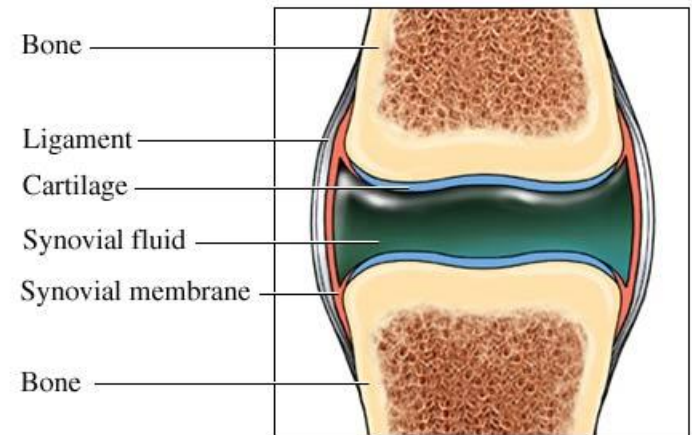
Transudate (effusion) / Exudate

- Albumin
- Total Protein



Matrices (fluids, etc)

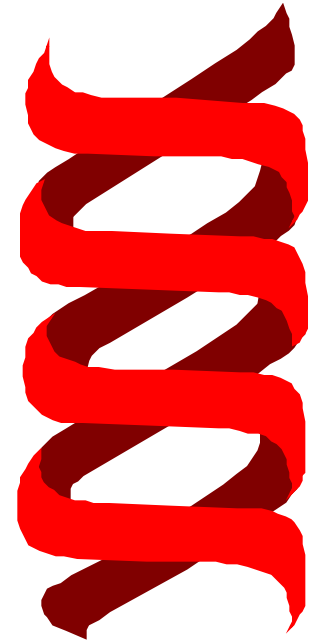
- Synovial (Joint) Fluid
 - Lactate
- Saliva
 - Testosterone, 17-OH Progesterone



Cross section of a healthy joint

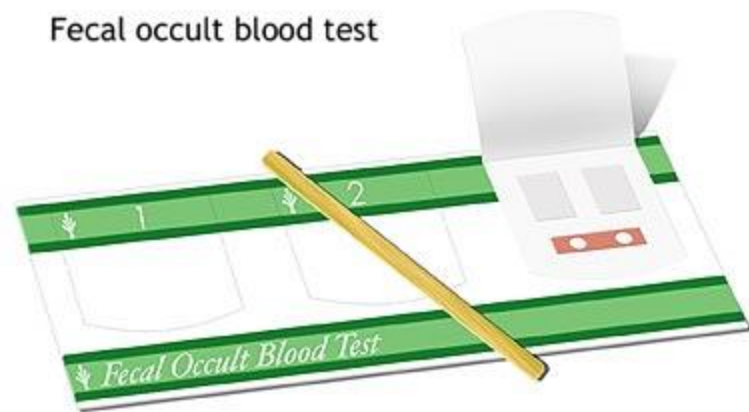
Matrices (fluids, etc)

- Chorionic Villous
 - Chromosomes
- Skin
 - Fibroblasts (enzymes, DNA)
- Stones (kidney, biliary)
 - Cysteine, Magnesium, Oxalate



Matrices (fluids, etc)

- Hair
 - Hair strands (trace elements, drugs)
 - Hair follicles (DNA, enzymes)
- Milk, Semen
- Faeces
 - Blood, fat, trypsin



Units

- Moles (SI)
 - mmol/L, mmol/24hrs, mmol/dL
- Mass
 - g/L, mg/L, mg/dL, ppm
- Pressure
 - kilopascals (kpa)
- Mixed
 - Timed
 - Urine collections /24hrs
 - Creatinine clearance mL/min
 - mmol/g
 - Tissue concentrations
 - mmol/mmol, mg/mmol
 - Albumin in urine, mg/mmol creatinine



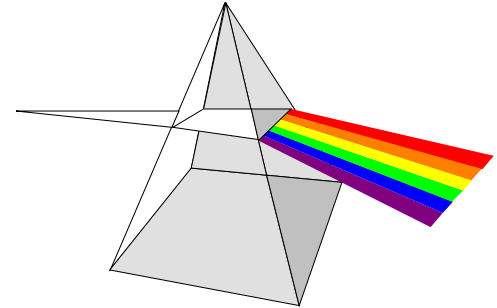
Units

- Mixed
 - Timed
 - Urine collections /24hrs
 - Creatinine clearance mL/min
 - mmol/g
 - Tissue concentrations
 - mmol/mmol, mg/mmol
 - Albumin in urine, mg/mmol creatinine

Techniques

- **Spectrophotometry - colorimetry**

- Analyte colour (**bilirubin**)
- Coloured product of a reaction
 - Analyte itself reacts (**creatinine**)
 - Enzyme product (**LDH, measure NADH @ 340nm**)
 - Secondary product of enzyme product (**ALP - PNP**)
 - Antibody antigen reaction - colloid (**IgG**)
 - Antibody immobilised, enzyme linked Ab. (**T₄**)



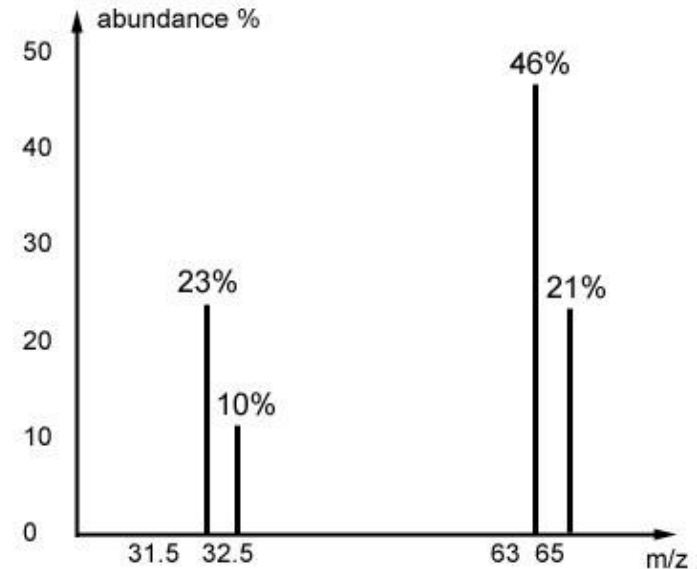
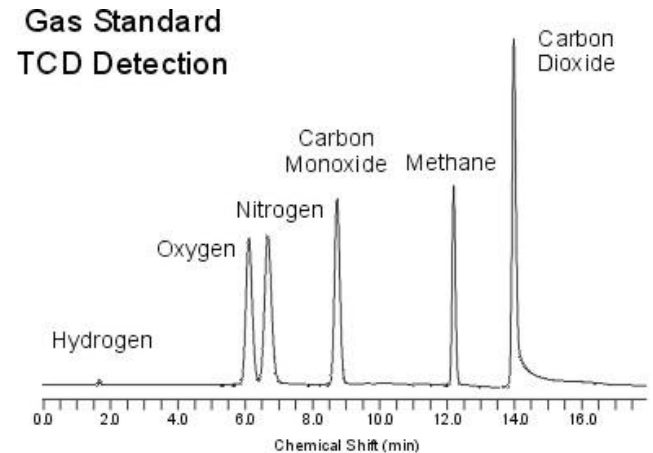
Techniques

- **Chromatography**

- Gas Chromatography (GC)
- High Performance Liquid Chrom. (HPLC)
- Thin Layer Chromatography (TLC)

- **Mass spectrometry**

- GC Mass Spectrometry (GC-MS)
- HPLC Mass Spectrometry (HPLC-MS)
- Tandem Mass Spectrometry (MS-MS)
 - Time-of-flight
 - Quadrupole
 - Quadrupole ion trap
 - Linear quadrupole ion trap
 - Fourier transform ion cyclotron resonance
 - Orbitrap

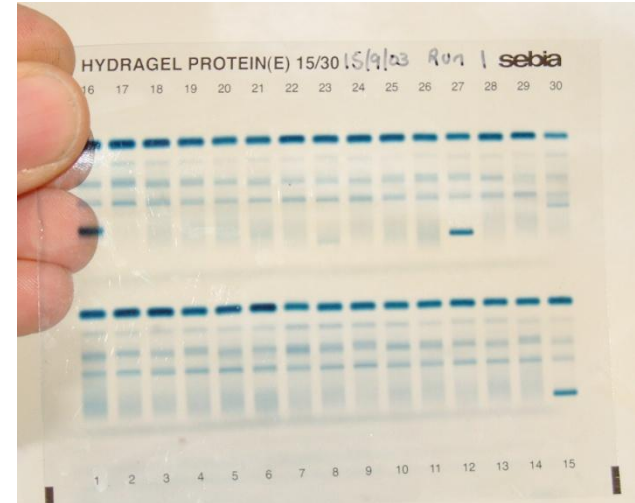


Techniques

- **Electrophoresis**

- Proteins (size and charge)
- Enzymes (CK, LDH & ALP isoenzymes)

- Protein electrophoresis ('protein strip')
- Immunoelectrophoresis (characterize paraproteins)
- Isoelectric focusing (CSF screen for MS)
- 2D electrophoresis
- Capillary zone electrophoresis (Beckman)



Techniques

- **Immunoassay**

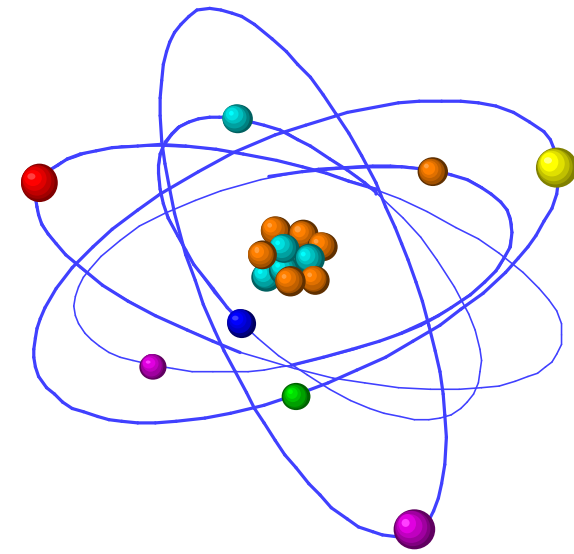
- Label

- Radio-labeled
 - Enzyme-labeled
 - Fluorimetric
 - Chemiluminescent



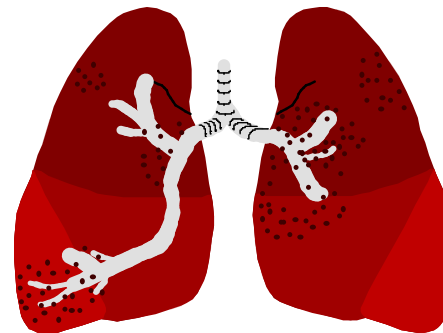
Techniques

- Atomic absorption
 - (copper, zinc)
 - Flameless atomic absorption (Hg)
- Atomic emission
 - (Sodium, Potassium, Lithium)
 - Flame photometry
 - Inductively coupled plasma emission
 - ICP-MS (multi analyte)



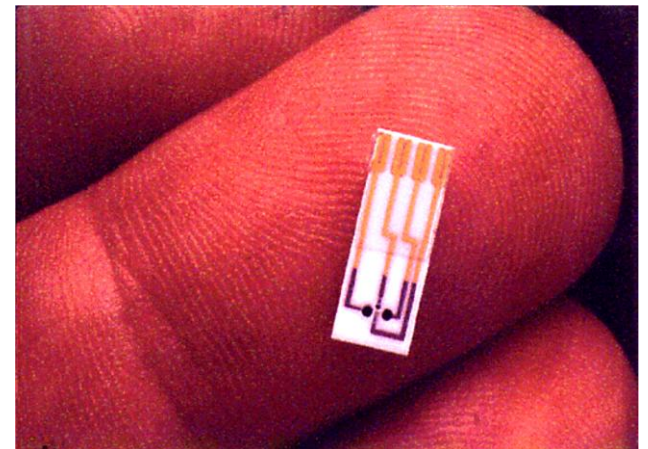
Techniques

- Ion Specific Electrodes
 - Blood Gases
 - pH, O₂, CO₂
 - Ions
 - Sodium, Potassium, Lithium, Chloride
 - Metabolites
 - Glucose, Lactate, Urea



Instruments

- Versatile general laboratory
 - Manual spectrophotometers
- Single analyte
 - Glucose analysers, Osmometers, Hb_{A1C}
- Analyte profile
 - Blood gas analysers



Instruments

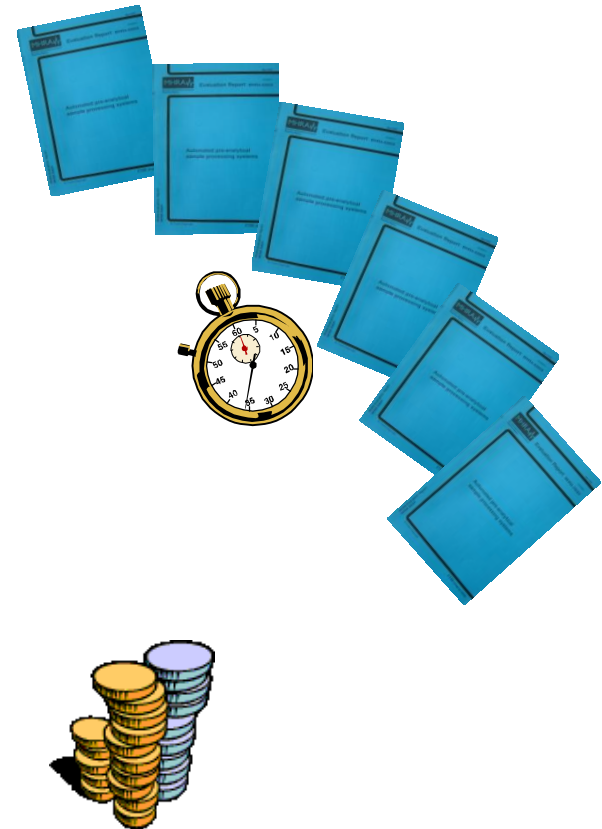
- Multi-analyte analysers
 - Spectrophotometric
 - Turbidimetric
 - Nephelometric
 - Fluorometric
 - ISE
 - Immunoassay

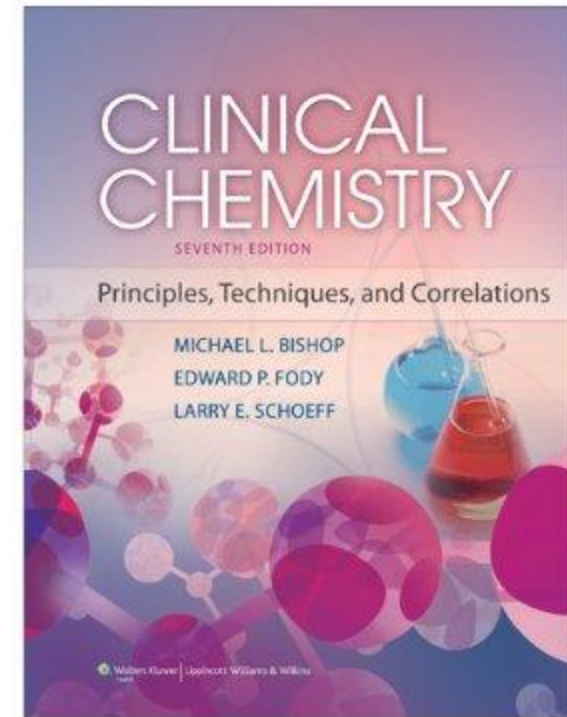
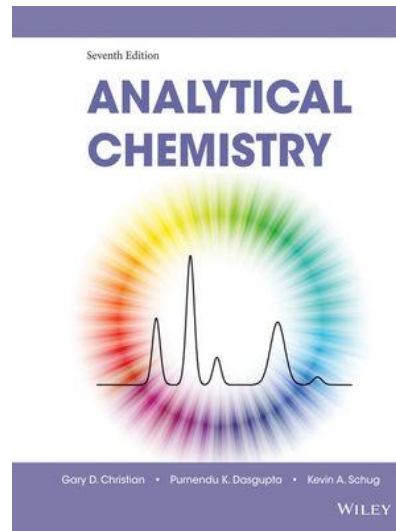
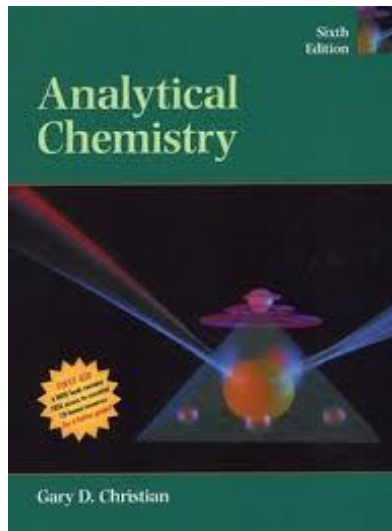


Instruments

Specification of major analysers

- Choice of analytes
- Size of instrument
- Samples / hr, Tests / hr
- Time to result
- Connects to robotic tracking system
- Ease of use
- Cost





Material for this introductory lecture were derived from presentations by:

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