Calcium quantitation by atomic spectroscopy

Textbook 6th ed. Chapter 10

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A Validated Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) Method to Estimate Free Calcium and Phosphorus in *In Vitro* Phosphate Binding Study of Eliphos Tablets

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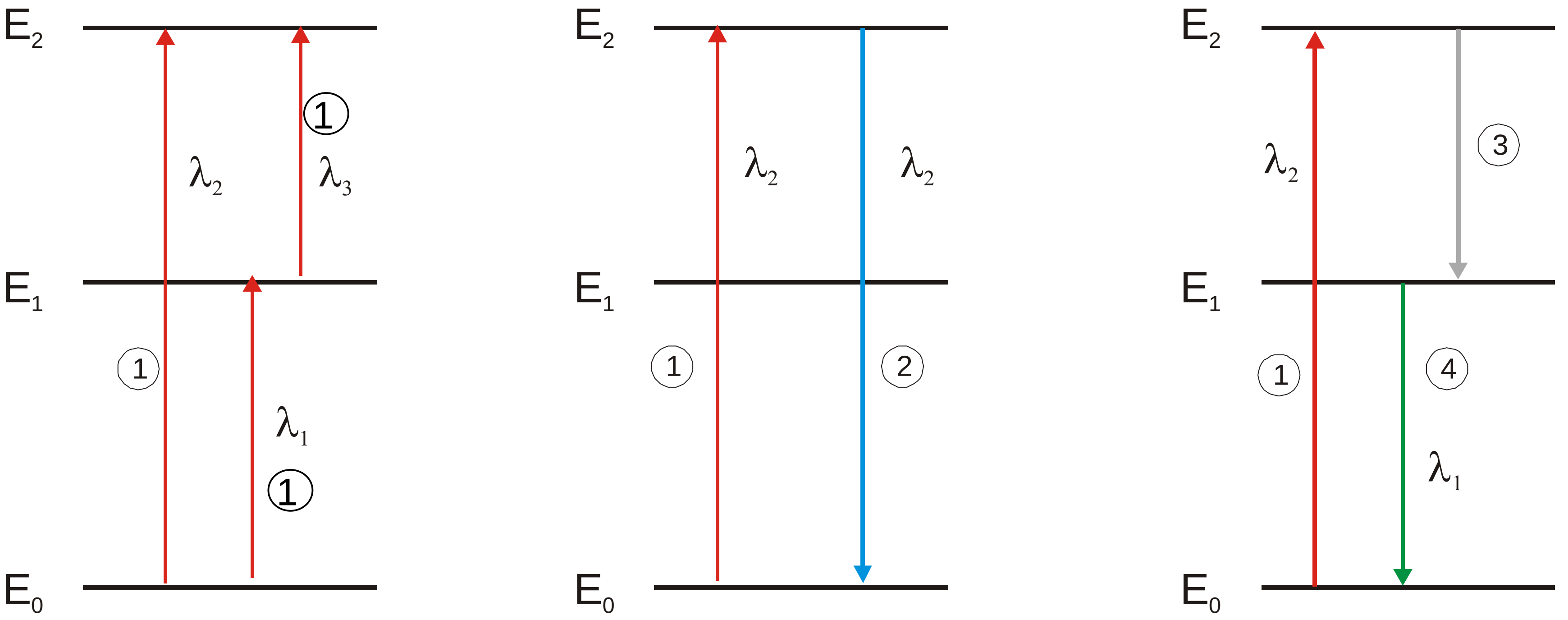
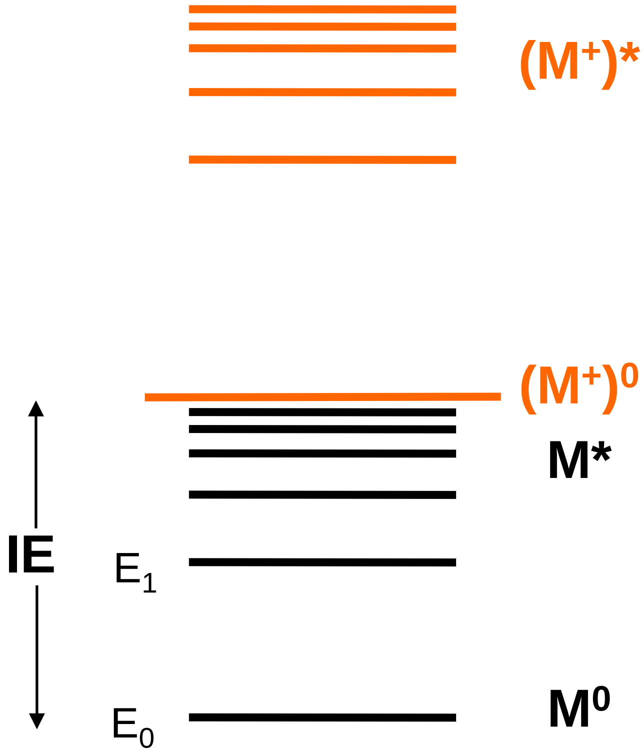
Basics of Atomic Spectroscopy

- Atomic structure and spectra
- Major atomic spectroscopic methods
- Atomic (optical) emission spectroscopy (AES or OES)

Reminder: Absorption and Emission



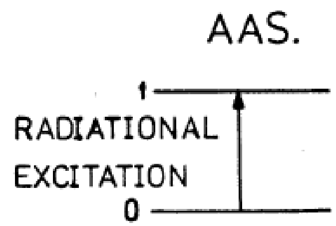
Energy States of Atoms

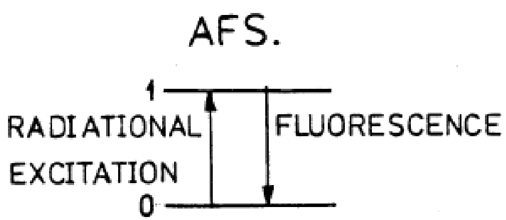


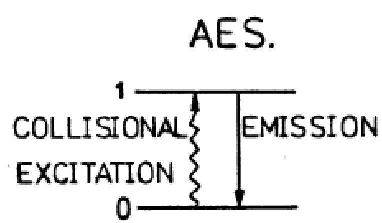
Energy state of an atom (and ion)

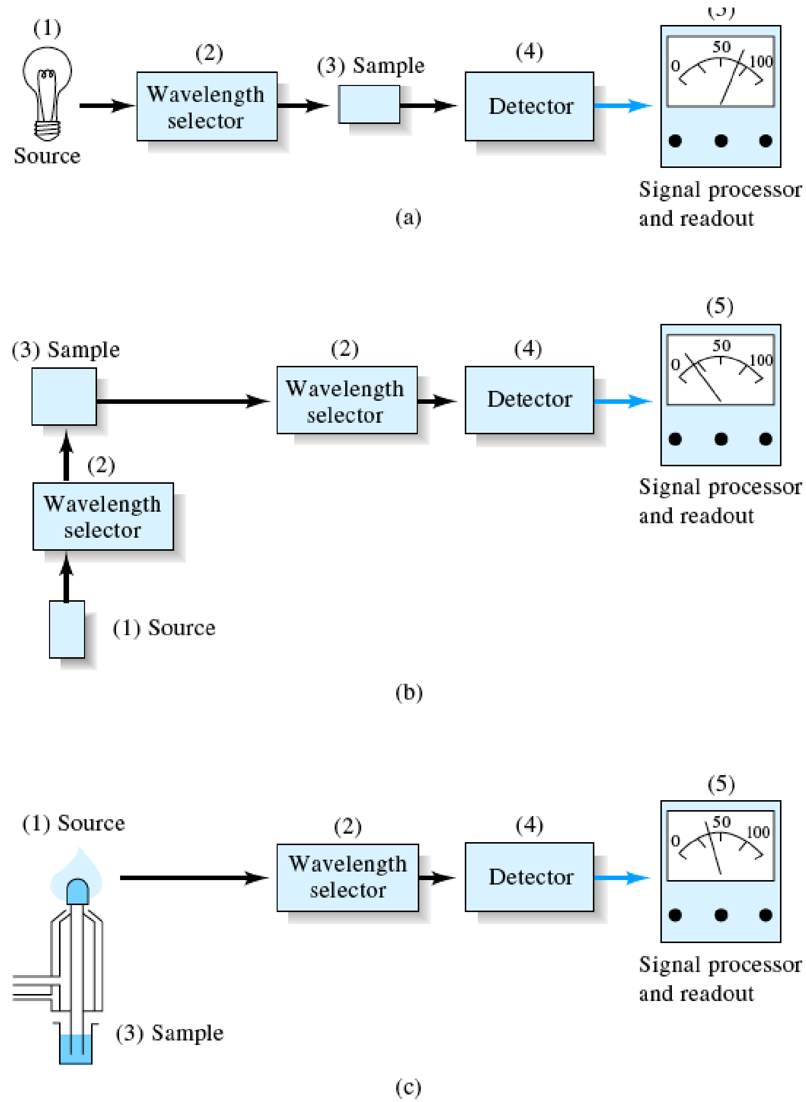
- Only electronic energy levels; no vibrational or rotational sublevels.
- Sharp absorption/ emission lines.
- Highly selective.
- Only determine elements; no molecular information.

AAS, AES (OES), and AFS

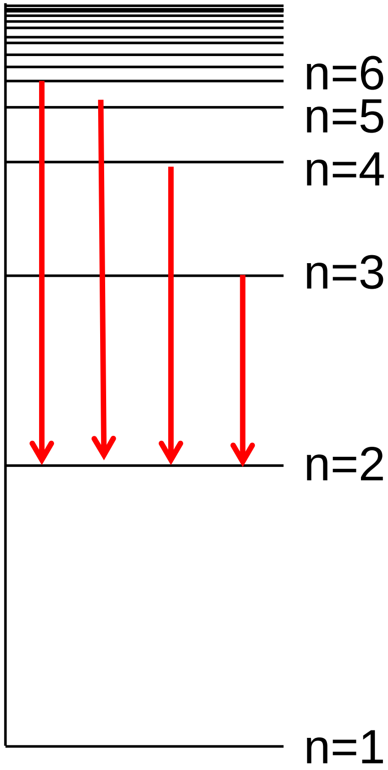




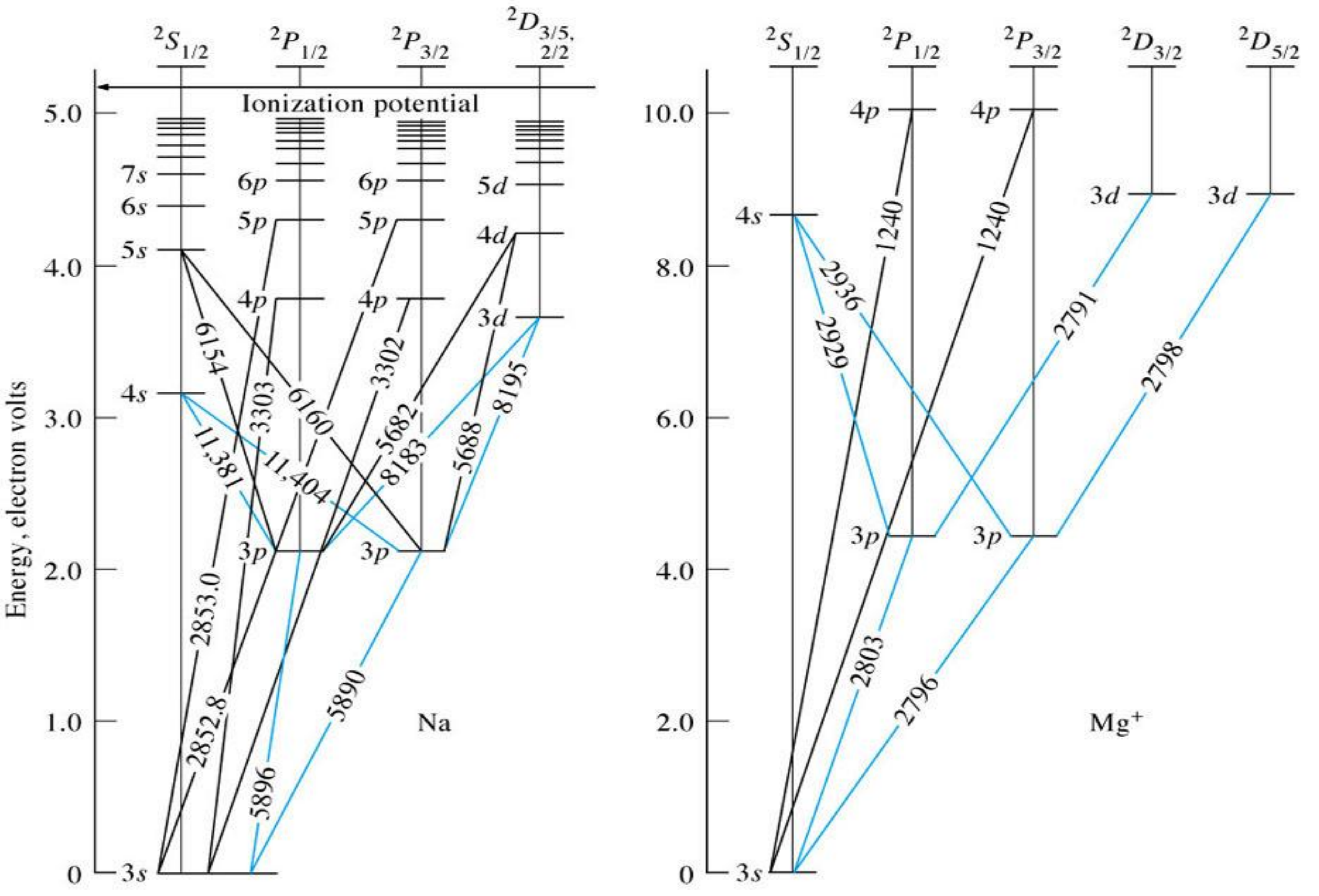




Line emission spectrum of H atom

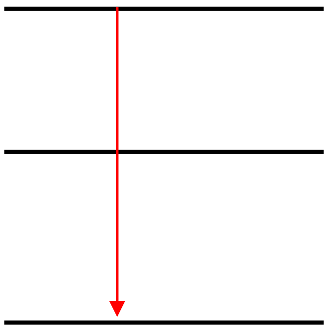


Atoms other than H: Grotrian diagrams



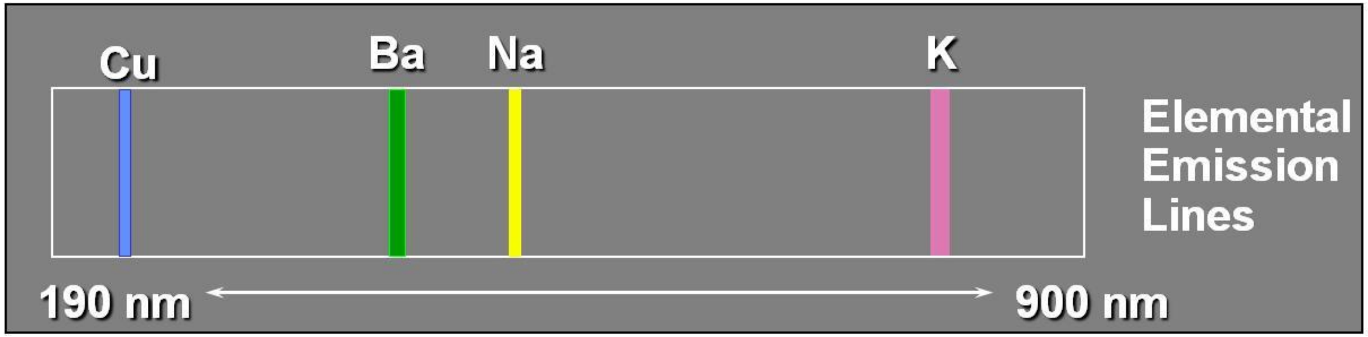
Atomic Emission Spectroscopy (AES or OES)

Once brought to an excited state, an atom (or ion) is not stable and decays back to lower energy levels by emitting electromagnetic radiation.



Atomic emission

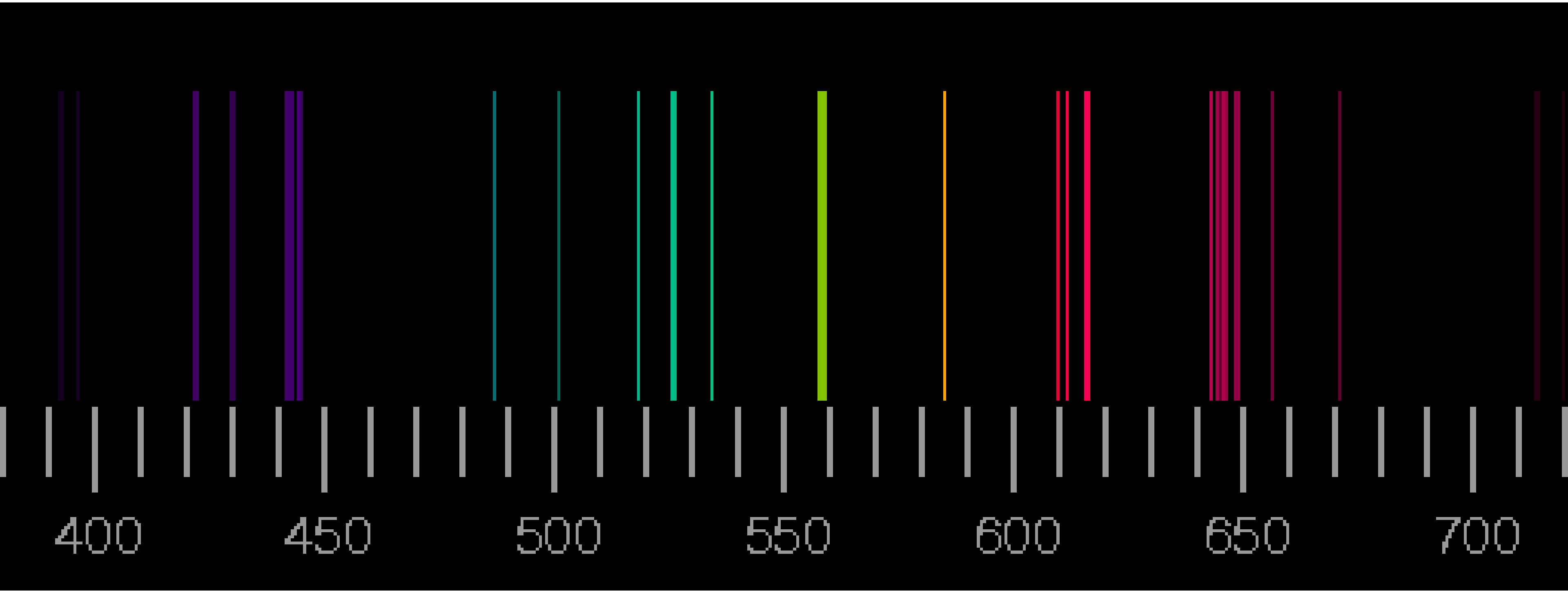
- Each excited element emits radiation at unique and characteristic wavelengths



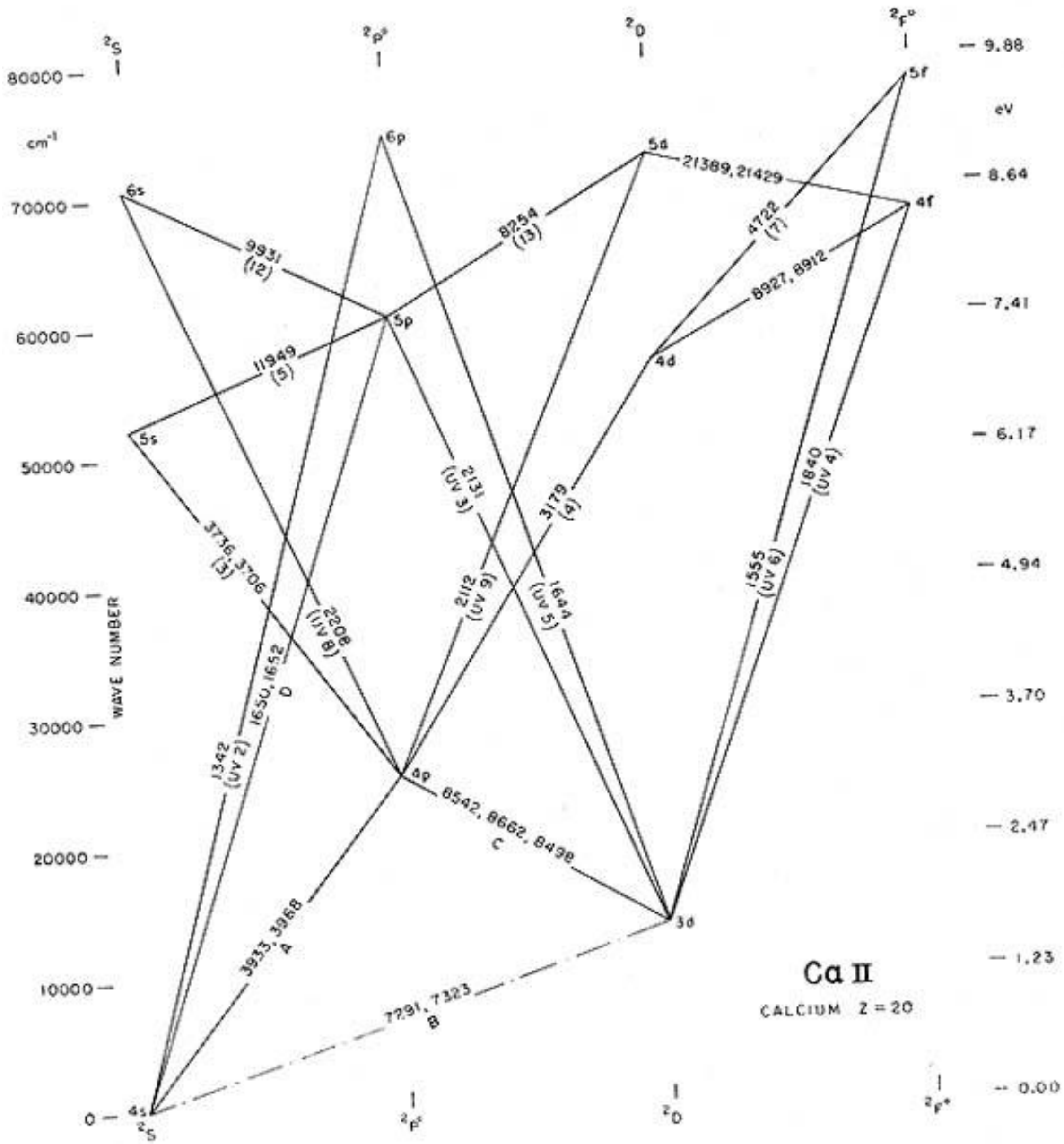
- For each element, different emission wavelengths have different intensities

- Quantitative analysis: emission intensity varies with concentration

Ca visible emission spectrum

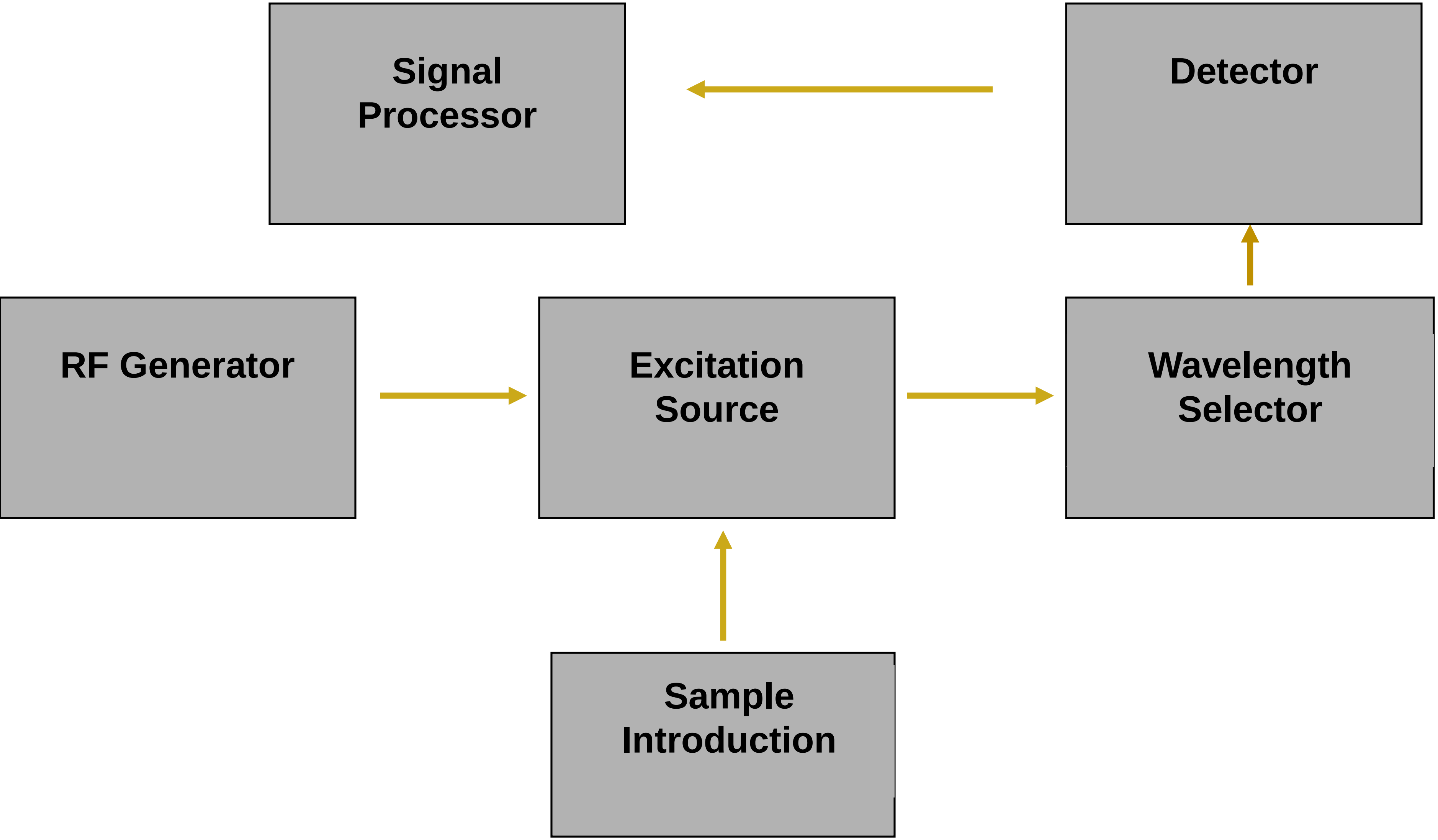


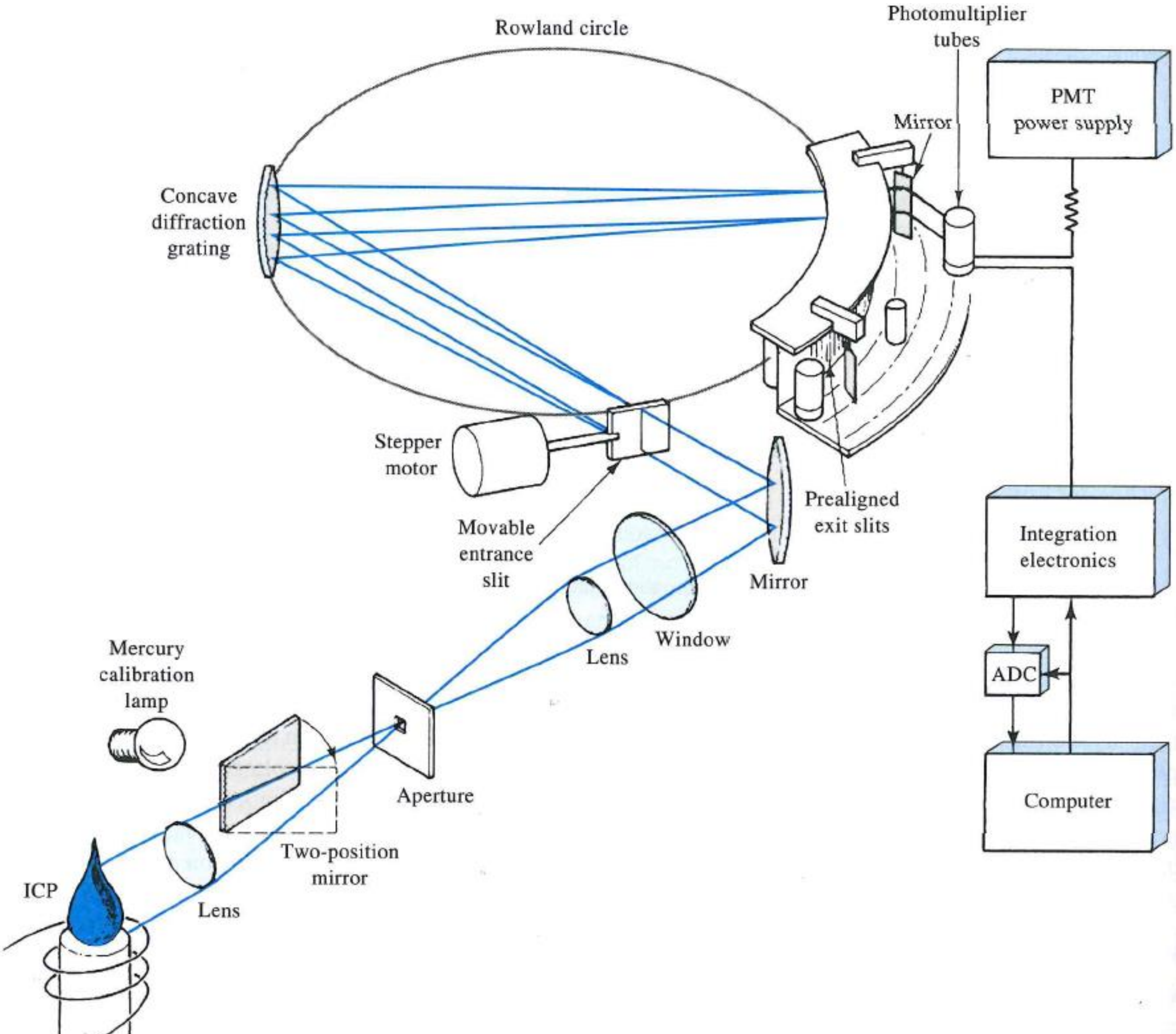
Grotrian diagram for Ca(II)



https://www.nhn.ou.edu/~jeffery/imcat/grotrian_20_01_Ca_II.php

Instrumentation for ICP-OES





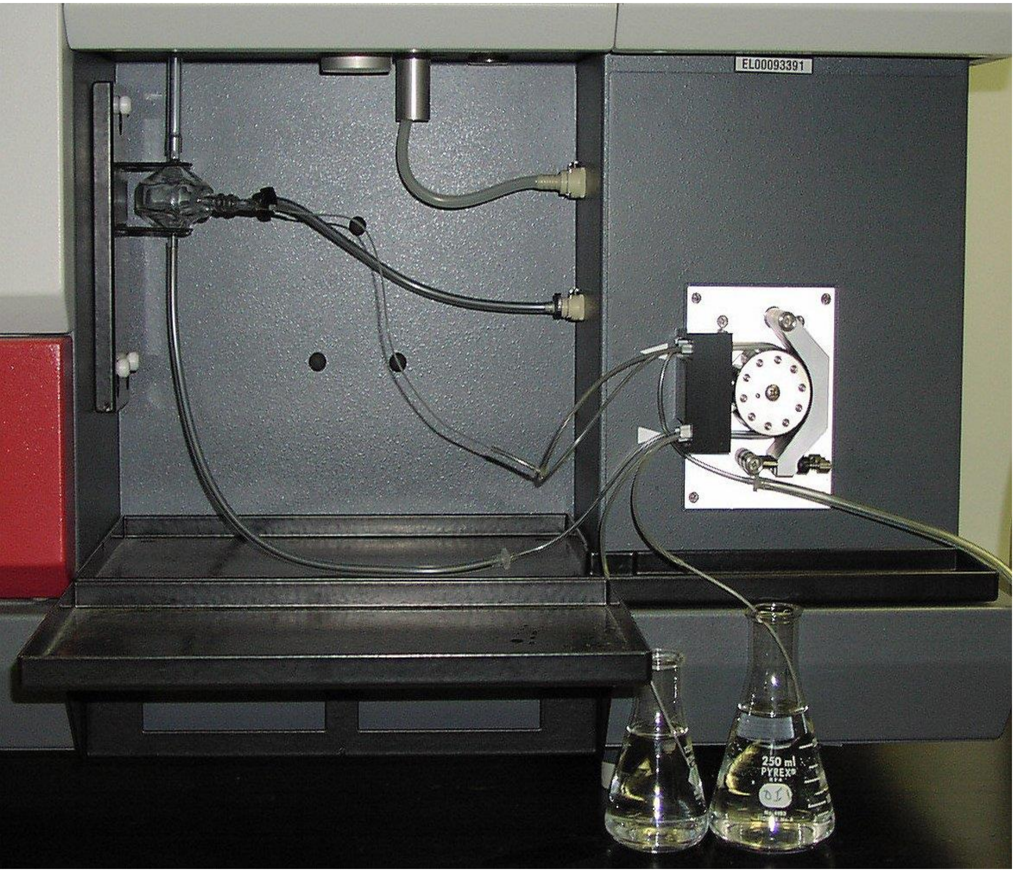
Rowland circle
Photomultiplier tubes
PMT power supply
Mirror
Concave diffraction grating
Stepper motor
Movable entrance slit
Prealigned exit slits
Mirror
Integration electronics
Window
Lens
Mercury calibration lamp
ADC
Aperture
Computer
Two-position mirror
ICP
Lens

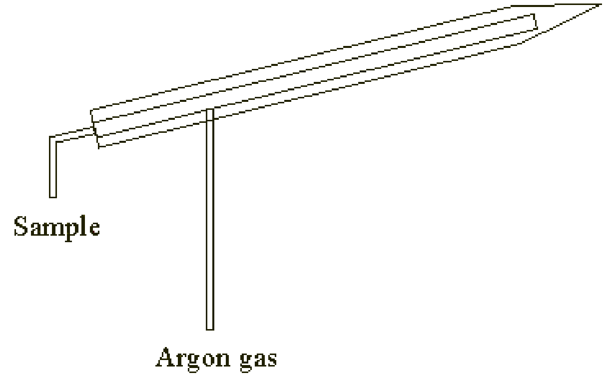
(for last slide)

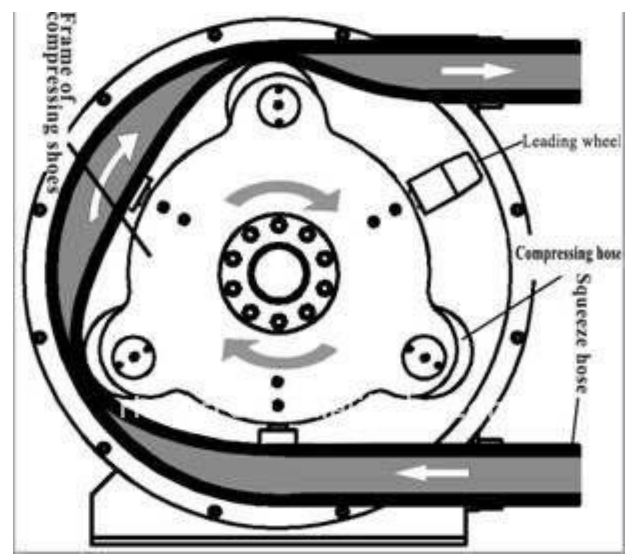
FIGURE 10-8 Direct-reading ICP emission spectrometer. The polychromator is of the Paschen-Runge design. It features a concave grating and produces a spectrum around a Rowland circle. Separate exit slits isolate each spectral line, and a separate photomultiplier tube converts the optical information from each channel into an electrical signal. Notice the radial viewing geometry. PMT = photomultiplier tube. (From J. D. Ingle Jr. and S. R. Crouch, *Spectrochemical Analysis*, p. 241, Upper Saddle River, NJ: Prentice-Hall, 1988, with permission.)

Varian Vista 725 ICP-OES

Sample Introduction System





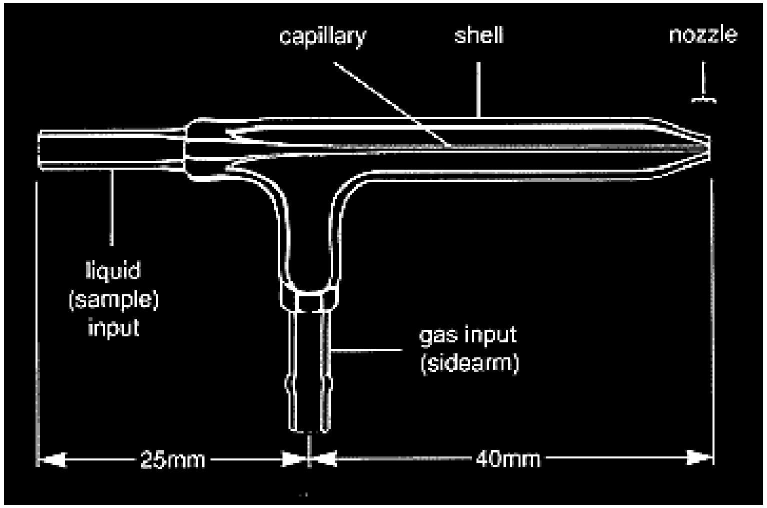


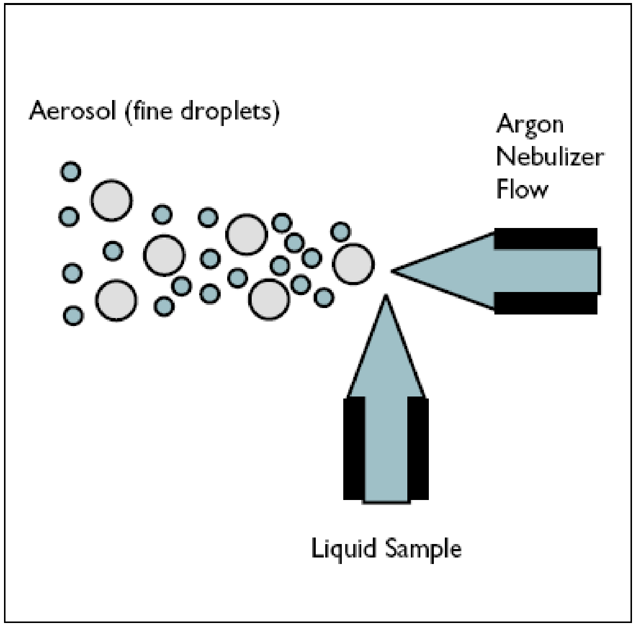
Nebulizer

Mix the liquid sample with the nebulizer gas to produce a fine sample aerosol

Many types available:

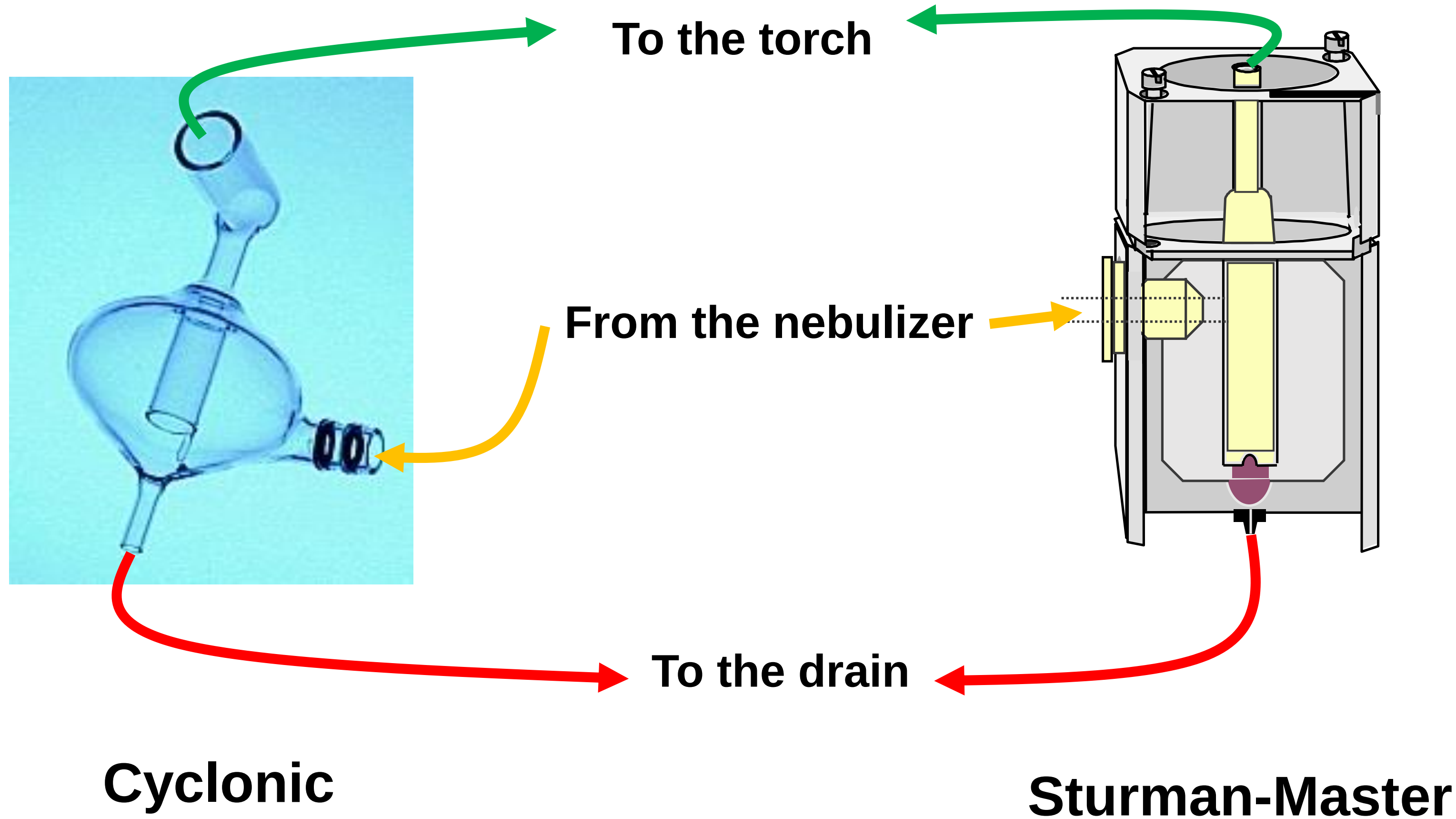
- Glass concentric
- V-groove





Spray Chamber

Separate sample aerosol droplets by size and transport only the smallest droplets into the torch (~3%). The remaining goes to the waste container



Excitation Sources

Traditionally based on

- Flame

- Arc and spark, and

- Plasma

Plasma offers:

(i) enhanced atomization/excitation

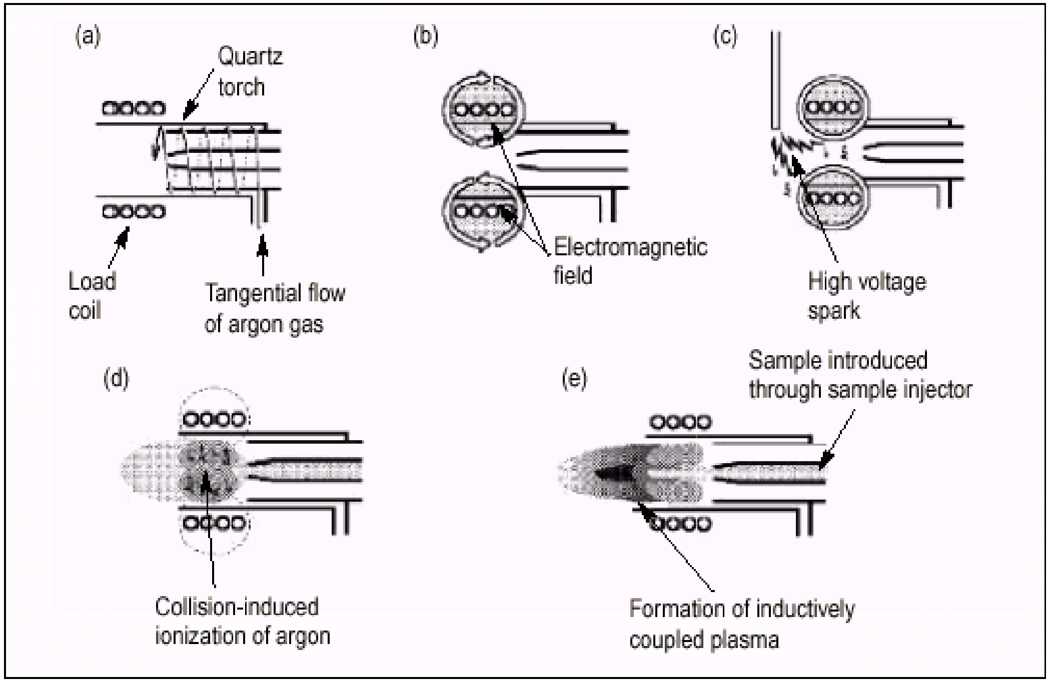
(ii) wider range of elements

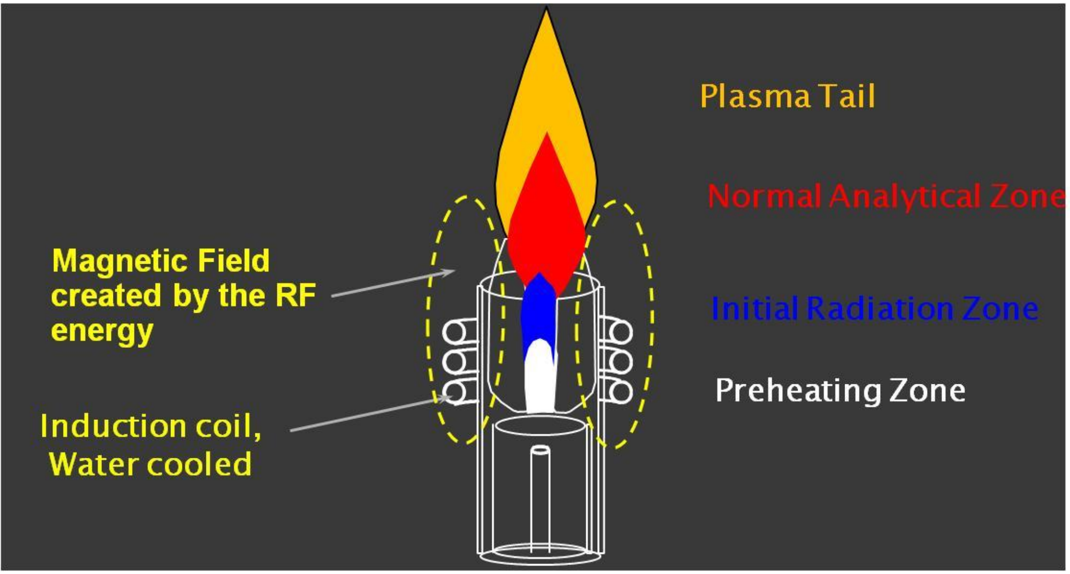
(iii) emission from multiple species simultaneously

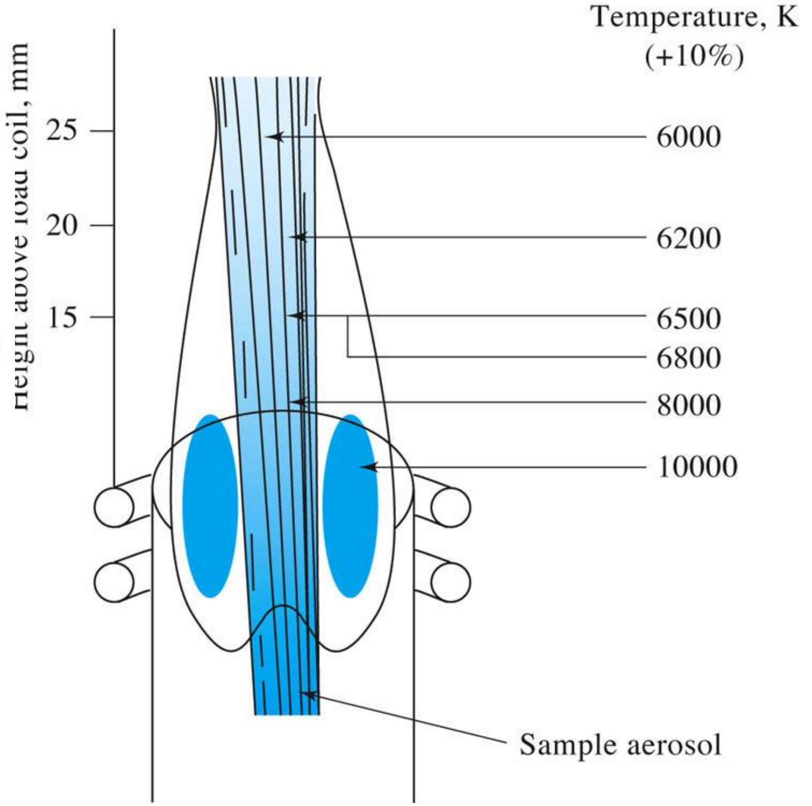
Plasma Excitation Source

A *plasma* is a high-energy, ionized gas composed of electrons (negative) and positively charged ions.

How is ICP produced?




Plasma Tail
Normal Analytical Zone
Magnetic Field created by the RF energy
Initial Radiation Zone
Preheating Zone
Induction coil, Water cooled


Temperature, K
(+10%)
Height above load coil, mm
25
20
15
6000
6200
6500
6800
8000
10000
Sample aerosol

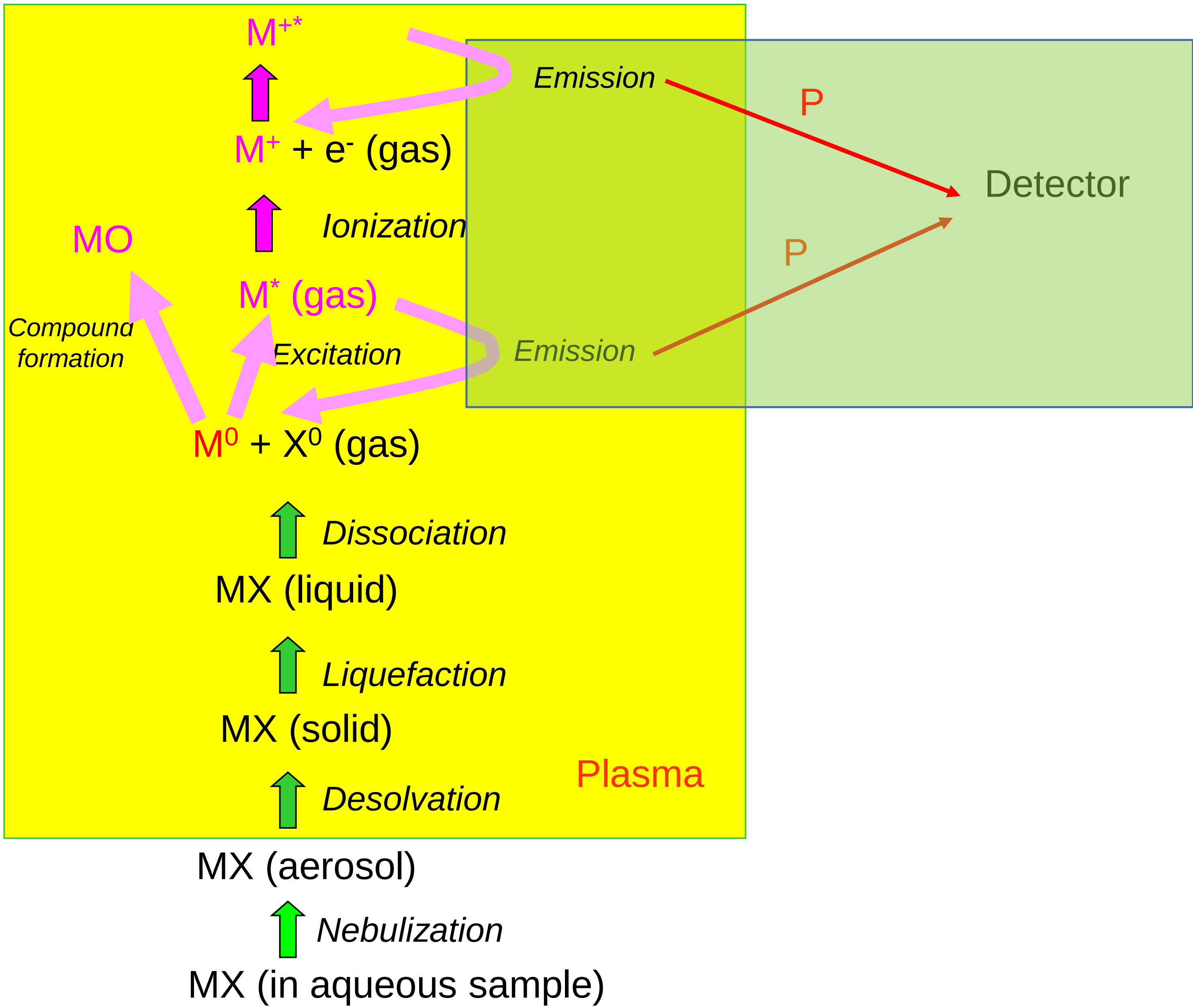
A Plasma is NOT a Flame!!!

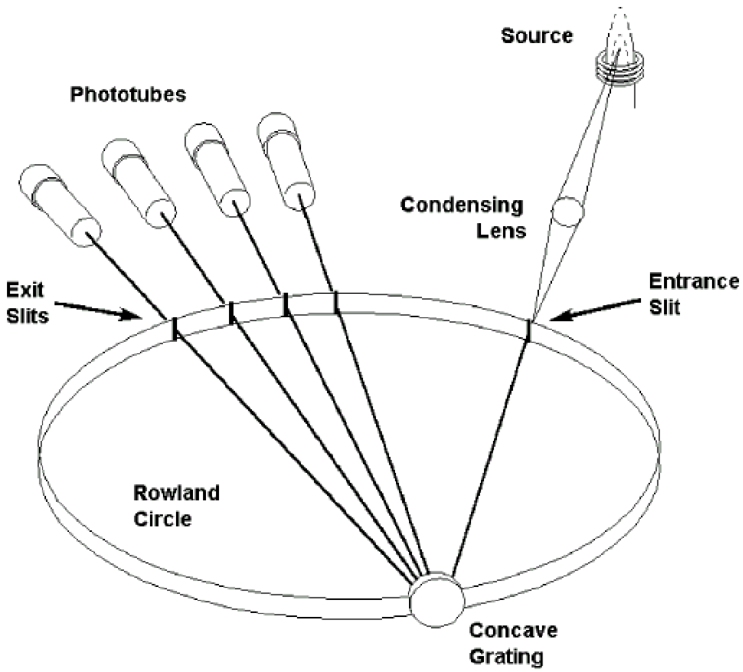
- A plasma is a very hot “cloud” of gas, gaseous ions & electrons

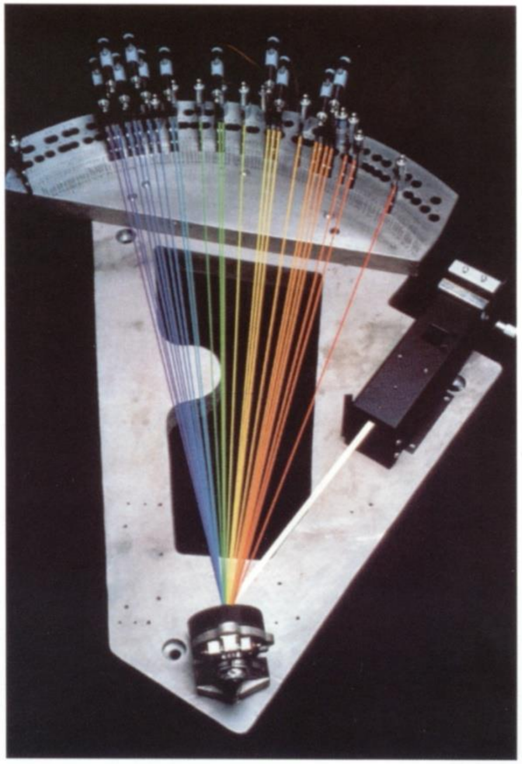
A Plasma is NOT a Flame!!!

- A plasma is a very hot "cloud" of gas, gaseous ions & electrons
- Net electrical charge, the plasma is neutral
- Collisional heating: 6 000 – 10 000 K
- Self-sustaining plasma
- There are no combustible gases in a plasma; it does not support combustion

Excitation Process

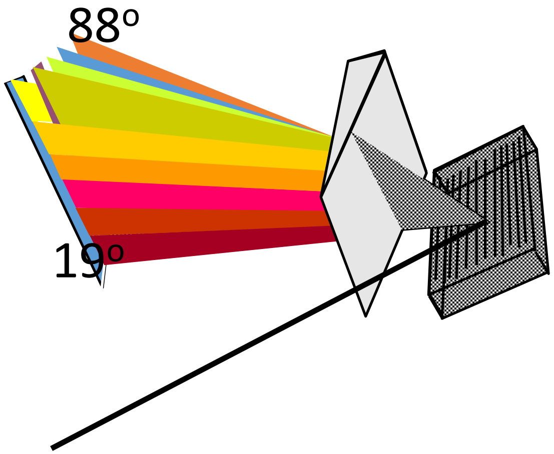


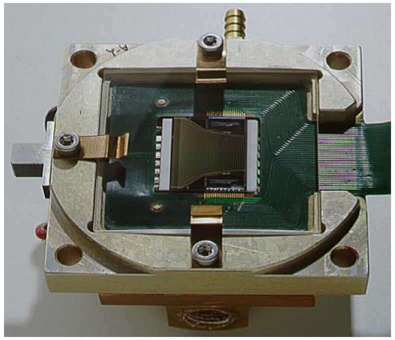




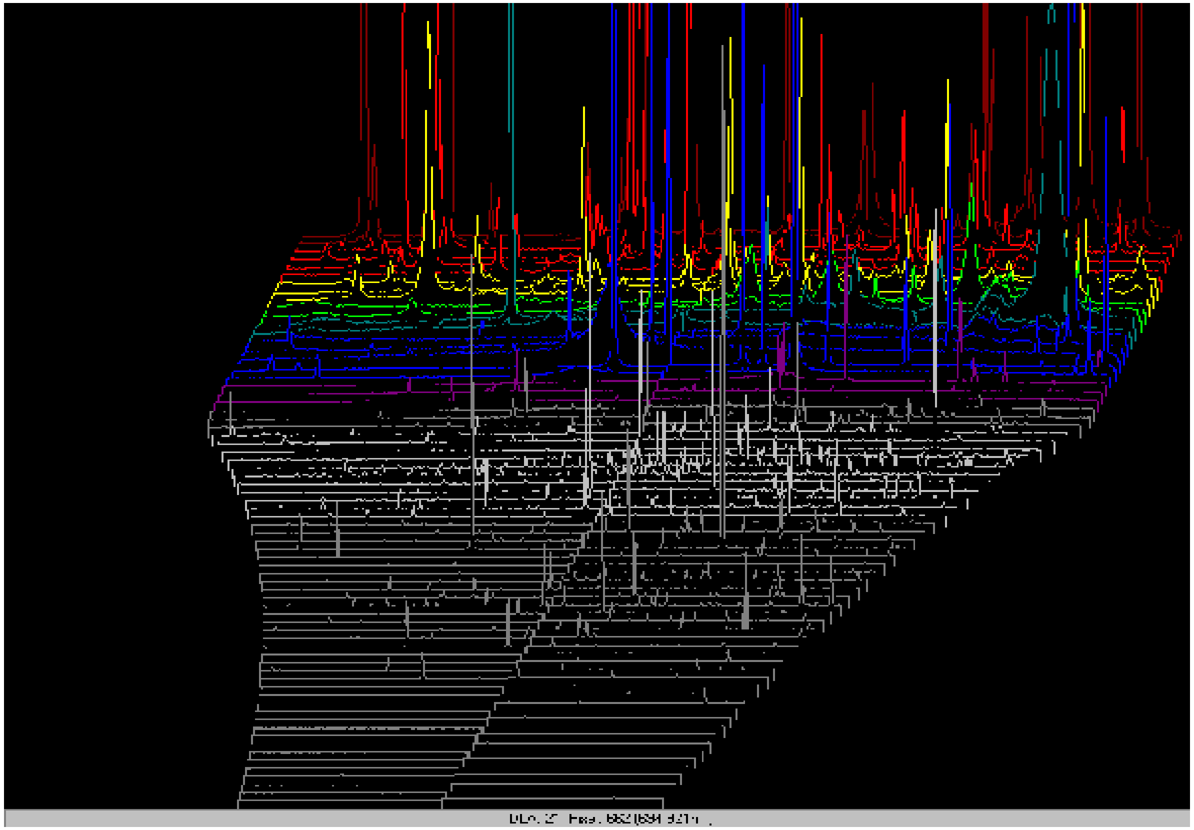
Rowland Circle Polychromator

Charge-coupled device (CCD) solid state detector in ICP-OES





Varian



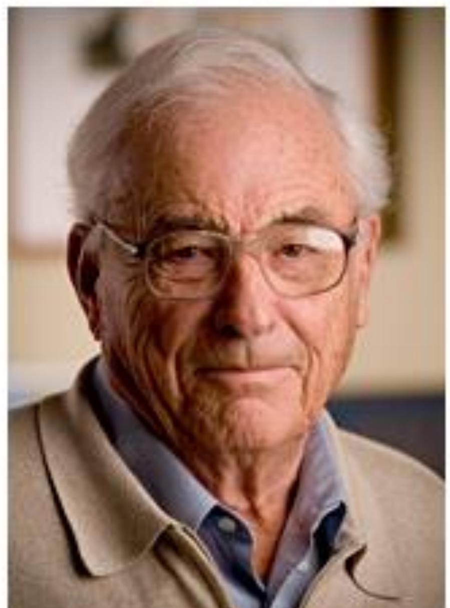
The Nobel Prize in Physics 2009

"for groundbreaking achievements concerning the transmission of light in fibers for optical communication"

"for the invention of an imaging semiconductor circuit – the CCD sensor"

Photo: Richard Epworth

Charles K. Kao



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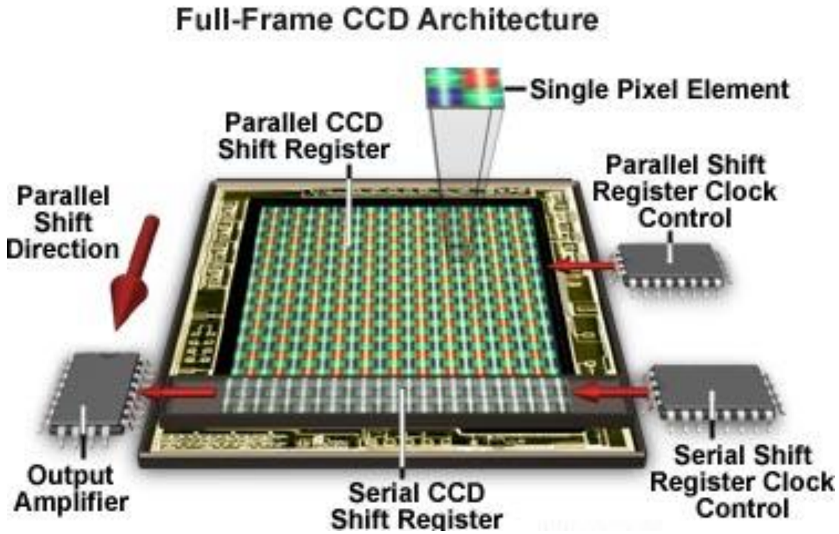
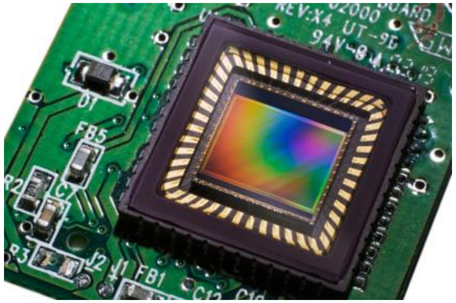
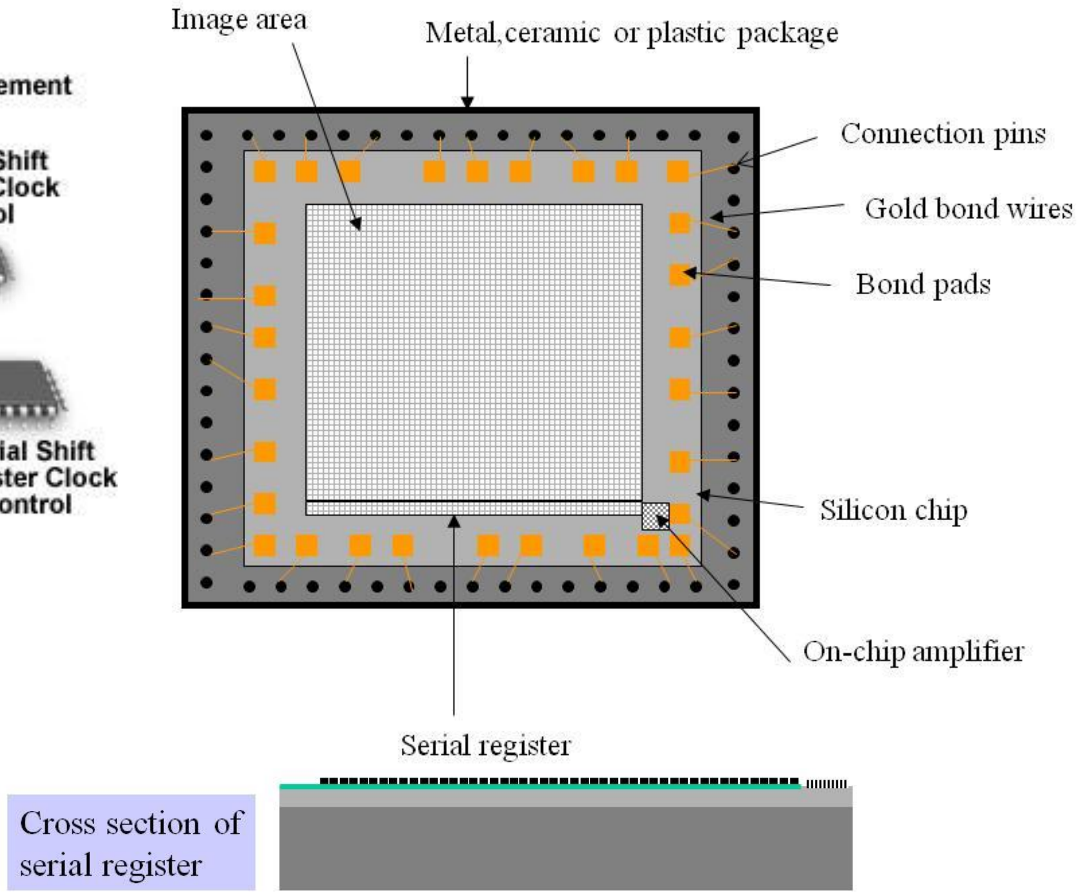
George E. Smith

The CCD is positioned at the focal plane of the beam. An image then builds up that consists of a pattern of electric charge. At the end of the exposure this pattern is then transferred, one pixel at a time, by way of the serial register to the on-chip amplifier. Electrical connections are made to the outside world via a series of bond pads and thin gold wires positioned around the chip periphery.







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Scientific Research

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Received June 24, 2011; *revised July* 25, 2011; *accepted August* 2, 2011

Abstract

An ICP-OES method was developed to estimate Ca and P as part of an *in vitro* phosphate binding study of Eliphos Tablets.

The method can detecting Ca and P in the presence of other trace elements.

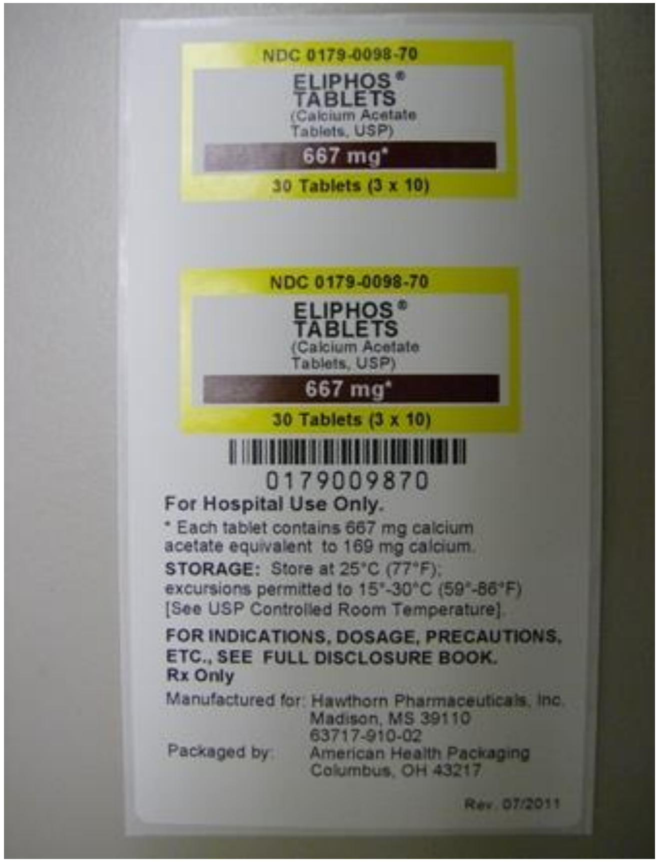
The wavelength was monitored for Ca and P at 317.933 nm and 213.677 nm, respectively.

The *in vitro* binding studies were performed for Eliphos Tablets at eight different phosphate concentrations by incubating at 37.0°C. Analysis was performed using a validated method to estimate free Ca and P.

The objective of the study is to provide an alternate *in vitro* method to estimate the binding capacity of calcium acetate tablets and avoid the expensive *in-vivo* bio clinical studies.

Introduction

Calcium acetate is the active ingredient in Eliphos Tablets™ and is indicated for haemodialysis and peritoneal dialysis. In kidney disease, blood levels of phosphate may raise, leading to bone problems. Calcium acetate binds phosphate in the diet to lower the blood phosphate levels. This medication is used for kidney disease to control blood phosphate.



P binding is a chemical reaction between dietary phosphate and cation of the binder compound (Ca), resulting in the formation of insoluble and unabsorbable phosphate compounds.

In ICP-OES, samples experience temperatures estimated to be in the vicinity of 10 000 K.

This results in atomization and excitation of even most elements with high efficiency

Detection limits for Ca and P with ICP-OES can be orders of magnitude better than the corresponding values of other techniques.

The limit of quantitation values of most of the elements in ICP-OES are ppm, even ppb.

Experimental

The main objective of the study is to develop a suitable ICP-OES method to quantitate Ca and P in the presence of placebo (filler) and other matrix components.

The placebo contains PEG 8000, sodium lauryl sulfate and crospovidone (may contain KOH).

The other matrix is Na_3PO_4 (sodium is a possible interference).

Ca standard was prepared at working concentration 0.04733 mg/mL (0.2989 mM) in deionized water.

It was monitored at different possible emission lines of 317.933 nm, 315.887 nm, 393.366 nm, 396.847 nm, 422.673 nm and 227.546 nm.

The phosphorus standard was monitored at different possible emission lines of 213.617 nm, 214.914 nm, 178.221 nm and 177.434 nm.

NIST spectral library

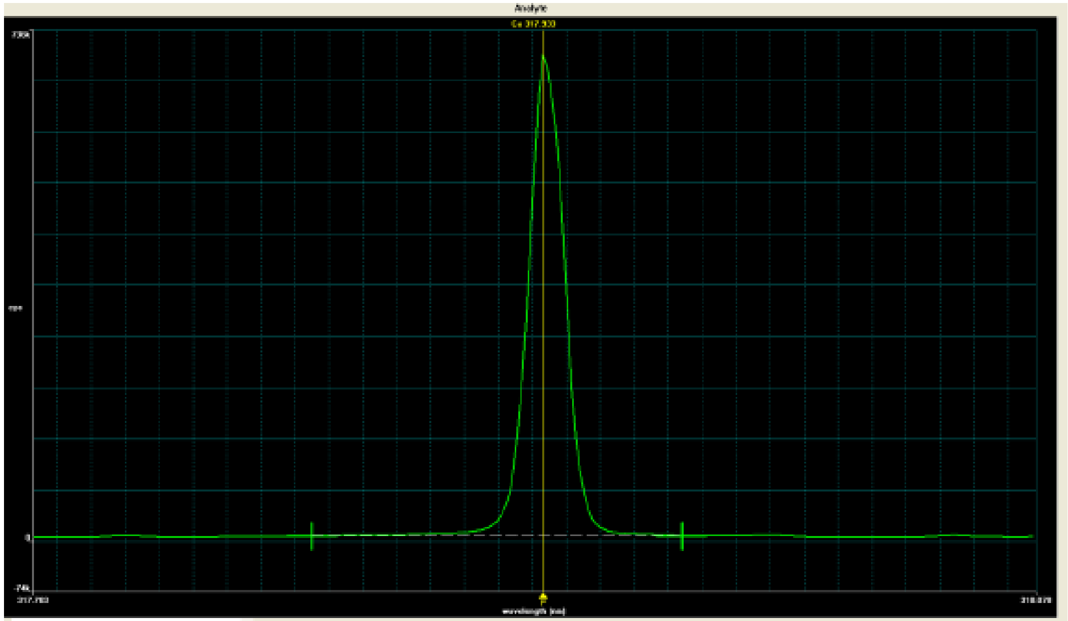


Figure 3. Typical ICP spectra of Calcium standard at working concentration of 0.0473 mg/ml (0.2989 mM) at emission line of 317.933 nm.

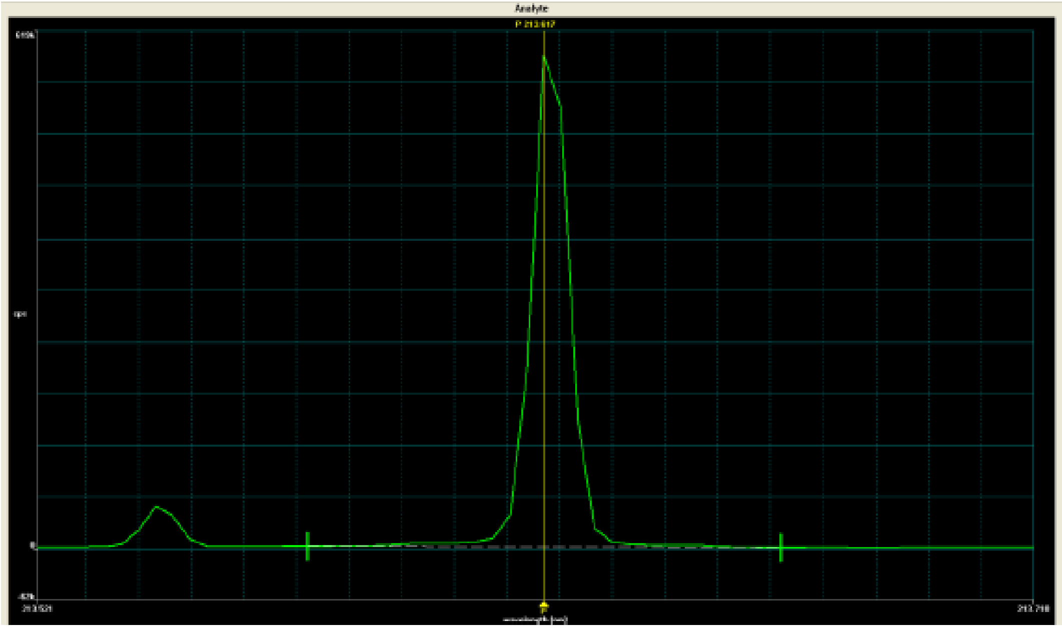
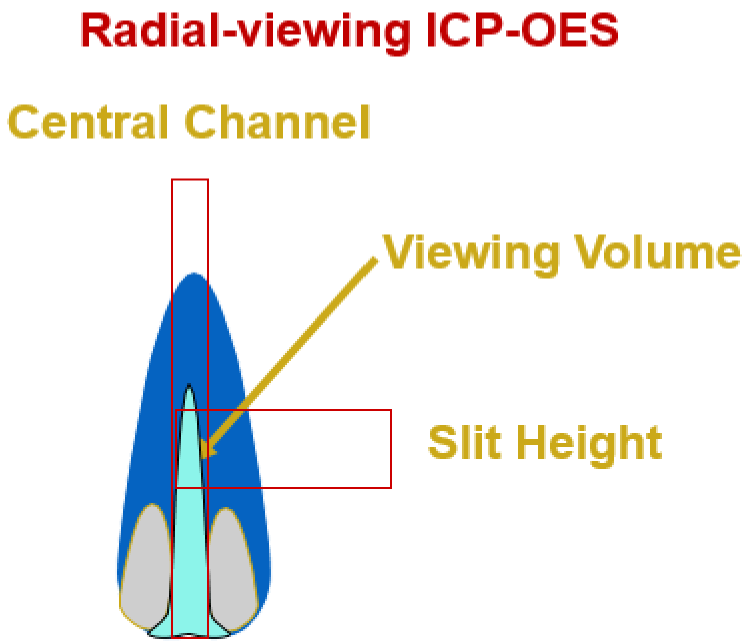


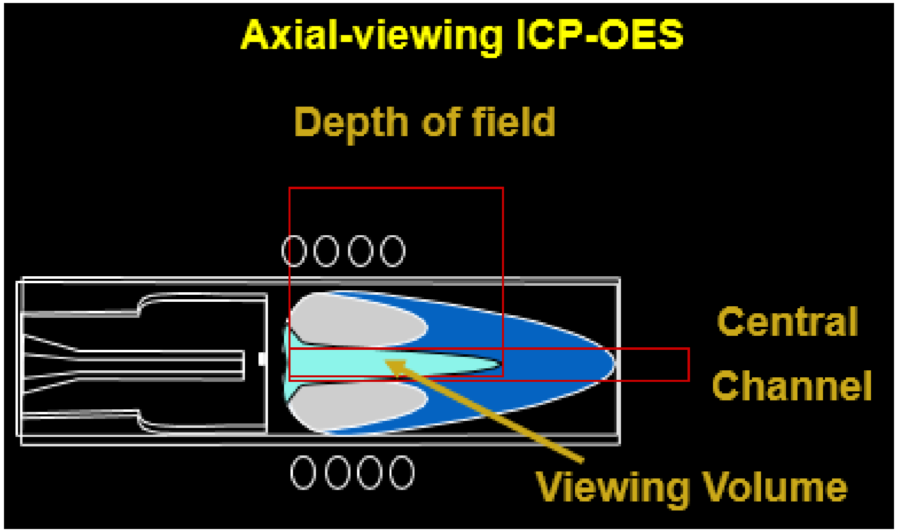
Figure 4. Typical ICP spectra of Phosphorus at concentration of 5.633 mM at emission line of 213.677 nm.

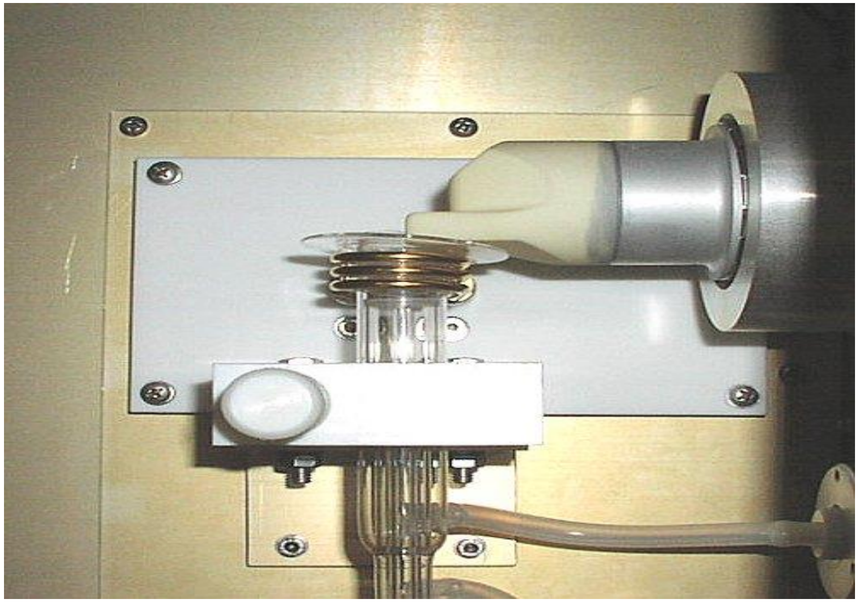
The P concentration is low and to get better sensitivity, axial view mode was selected.

In axial, the plasma view is down the central channel of the plasma and collects all the analyte emission over the entire length of the plasma.

This region is much larger than viewed radially, resulting higher intensity for phosphorus.







radial

axial

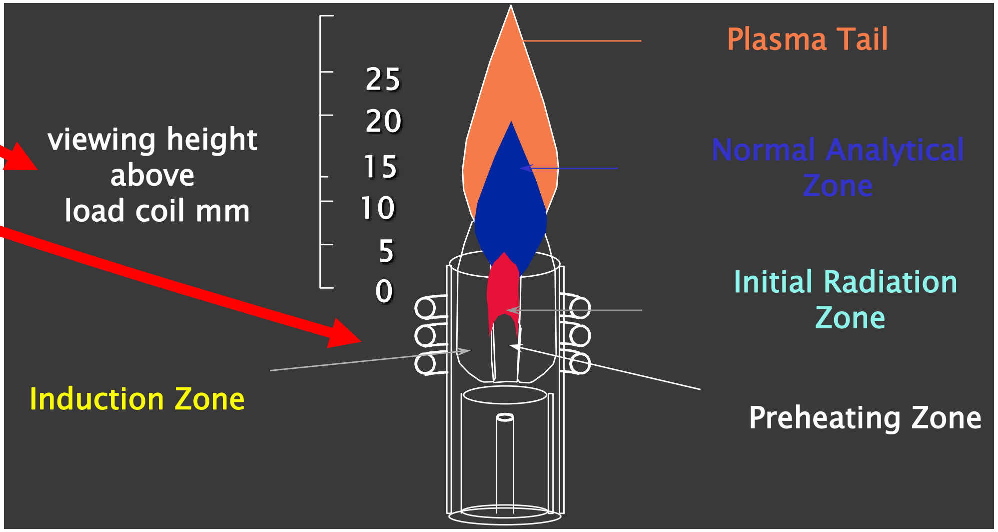
Hard vs. Soft Lines

- "Hard" Lines (<300 nm) have high energy transitions / ionization potential (>8 eV)
  - **Higher power**
  - **Higher plasma flow**
  - **Lower nebulizer pressure**

- "Soft" Lines (> 300 nm) have low energy transitions / ionization potential (< 8 eV)
  - **Low power**
  - **High nebulizer pressure**

Major Factors Affecting Line Intensities

- Viewing height
- RF Power
- Nebulizer gas flow



Linearity for P and Ca was evaluated.

Each solution was aspirated five times. The mean responses recorded for each elements were plotted against concentration.

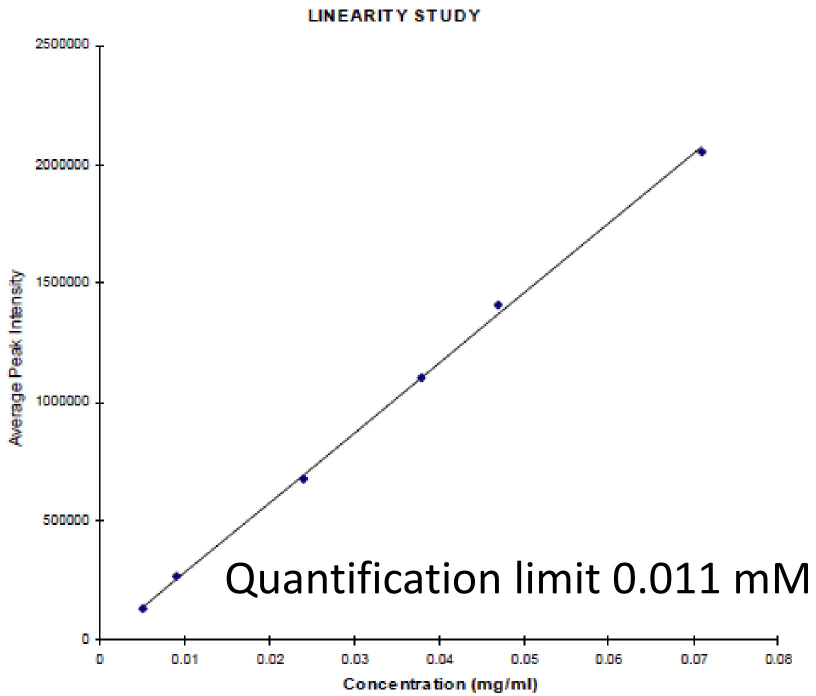


Figure 1. Linearity graph of Calcium at emission line of 317.933 nm, plotted concentration on X-axis versus average peak intensity on Y-axis.

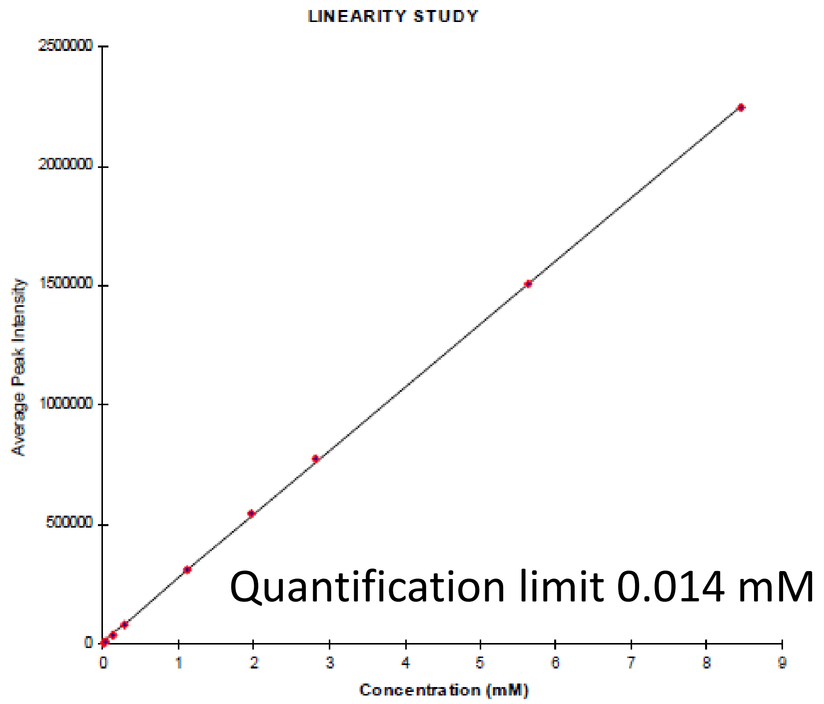


Figure 2. Linearity graph of Phosphorus at emission line of 213.677 nm, plotted concentration on X-axis versus average peak intensity on Y-axis.

Precision study

Table 1. Precision results of calcium at emission line of 317.933 nm and phosphorus at emission line of 213.677 nm.

Aspirations	Peak intensity of calcium	Peak intensity of phosphorus
1	125322.4	1502730.0
2	126382.7	1506216.4
3	128442.8	1508620.5
4	130034.5	1506005.3
5	131995.7	1498419.3
Average	128435.6	1504398.3
%RSD	2.10%	0.26%

$$\%\text{binding of phosphate} = \frac{\text{Sodium phosphate added (mM)-free phosphorus found (mM)}}{\text{Sodium phosphate added (mM)}} \times 100$$

Table 3. Average percentage binding data of Twelve tablets at each buffer stage, experimented with RF power of 1500 watts, plasma flow of 15 L/min, Auxillary flow of 0.2 mL/min, Nebuliser flow of 0.8 L/min, plasma view at radial mode for calcium, axial view for phosphorus and wavelength of 317.933 for calcium and 213.617 for phosphorus.

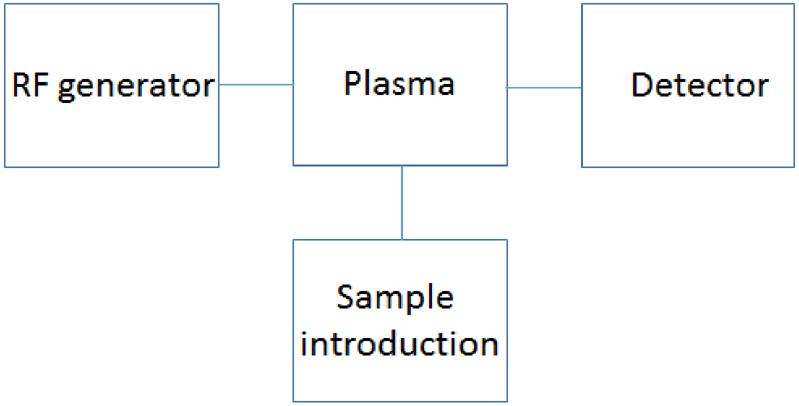
Buffer Concentration	Free calcium (mM)	Free phosphate (mM)	% Binding
0.000 mM buffer	27.241	0.000	0.0
0.028mM buffer	27.241	0.002	93.6
0.141mM buffer	27.473	0.009	93.3
0.282 mM buffer	25.012	0.008	97.0
1.127 mM buffer	22.092	0.018	98.4
1.972 mM buffer	20.494	0.014	99.3
2.817mM buffer	19.696	0.007	99.7
5.633 mM buffer	16.342	0.112	99.8

Summary: Pros and Cons of ICP-OES

Pros	Cons
Wide applicability (more than 60 elements)	Relatively insensitive; μg-mg/L level detection limits
Extremely fast; Single scan for more than 60 elements	Measuring total elemental concentration, no speciation
Few non-spectral interferences; well-known spectral interferences	
Wide linear calibration range	

Question 1:

Describe the components an ICP-OES instrument as shown in this picture.



Question 2:

Describe 3 differences between axial and radial viewing in ICP-OES.