



# Trust but always Verify:

---

**Best practices in spectrophotometric measurement**

**L S Theobald Lecture  
15<sup>th</sup> May 2003**



# A journey in analytical science

---

- Sourced in classical analysis (ABC)
- Inspired by qualitative analysis
- Progressed via colorimetry
- Matured in spectrophotometry
- Continued quest for correctness and traceability in spectroscopic measurements

## Sourced in classical analysis

---

- ICI General Chemicals Division  
Widnes 1962-1967
- 'Techniques Course' Widnes Tech.  
12 months before you were 'acceptable'  
within the analytical laboratory
- Classical gravimetric and volumetric  
analysis (Over 150 procedures against  
'unknown' samples from Vogel 3<sup>rd</sup>  
Edition)
- Trained analyst

# Traceability in classical analysis

---

## Gravimetric analysis

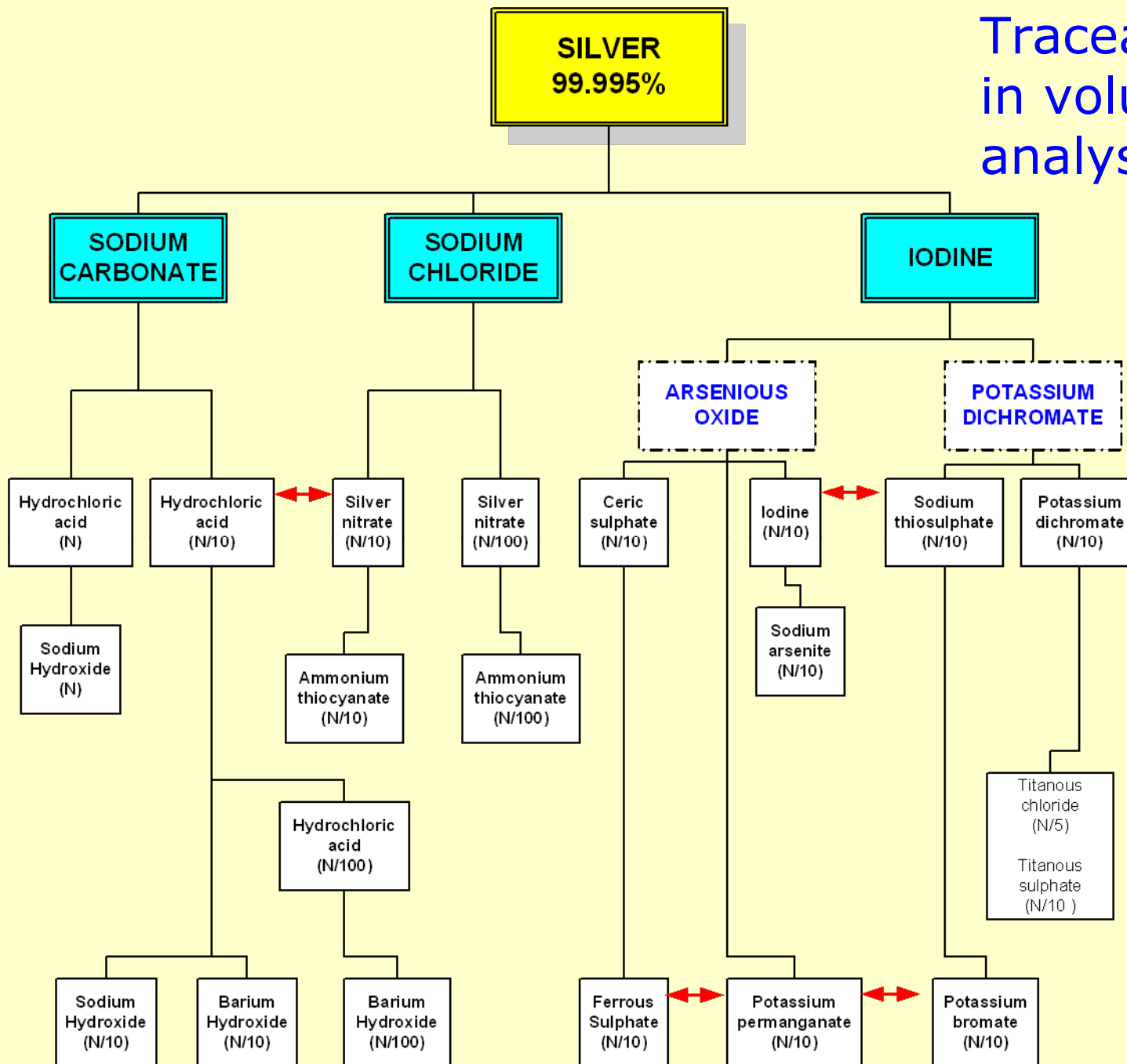
- Traceable masses
- Method of swings on 2 pan balances
- Substitution
- Buoyancy correction

## Volumetric analysis

- Calibration of pipettes
- Calibration of burettes with drainage correction
- Calibration of volumetric flasks

Chemical Analysis –The Working Tools  
Analytical Chemists' Committee of ICI  
2<sup>nd</sup> Edition 1961

# Traceability in volumetric analysis



# Qualitative analysis

---

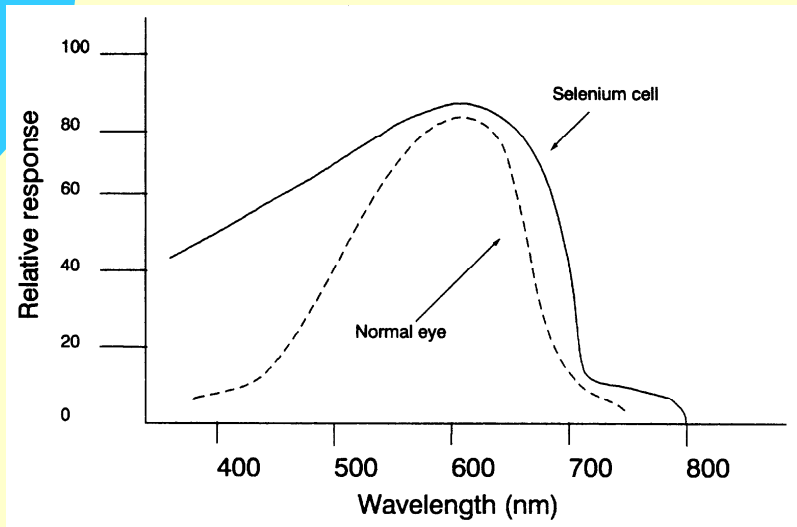
- The flame test; low cost atomic emission (Flame and bead tests data have been restored in the 84<sup>th</sup> Edition, 2003-2004, CRC Handbook of Chemistry and Physics)
- Calibration of spectrometers
  - 'Raies Ultimes'
  - Dispersion curves

# Bunsen spectroscope

---



# The eye as a detector



<i>Approximate wavelength range (nm)</i>	<i>Colour of reflected light</i>	<i>Colour of absorbed light</i>
400-456	Violet	Yellow-green
465-482	Blue	Yellow
482-487	Greenish-blue	Orange
487-493	Blue-green	Red-orange
493-498	Bluish-green	Red
498-530	Green	Red-purple
530-559	Yellowish-green	Reddish-purple
559-571	Yellow-green	Purple
571-576	Greenish-yellow	Violet
576-580	Yellow	Blue
580-587	Yellowish-orange	Blue
587-597	Orange	Greenish-blue
597-617	Reddish-orange	Blue-green
617-780	Red	Blue-green



# Absorptiometry; the eye as a detector

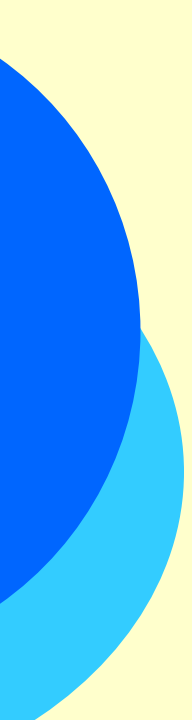
---

- Nessler tubes
  - Iron by thioglycollic acid
  - Standard series
  - Visual interpolation
- Nessleriser Glass standards
  - 'Permanent' standard
  - Traceable (?)
  - Samples only
- Lovibond tintometer

# Nessleriser standards

---





# Absorptiometry; the photocell as a detector

---

- Hilger Spekker absorptiometer
- Measurement of transmittance
- Calibration curves and the problem of absorbance
- Filter selection
  - Measurement
  - Band width
  - Stray light

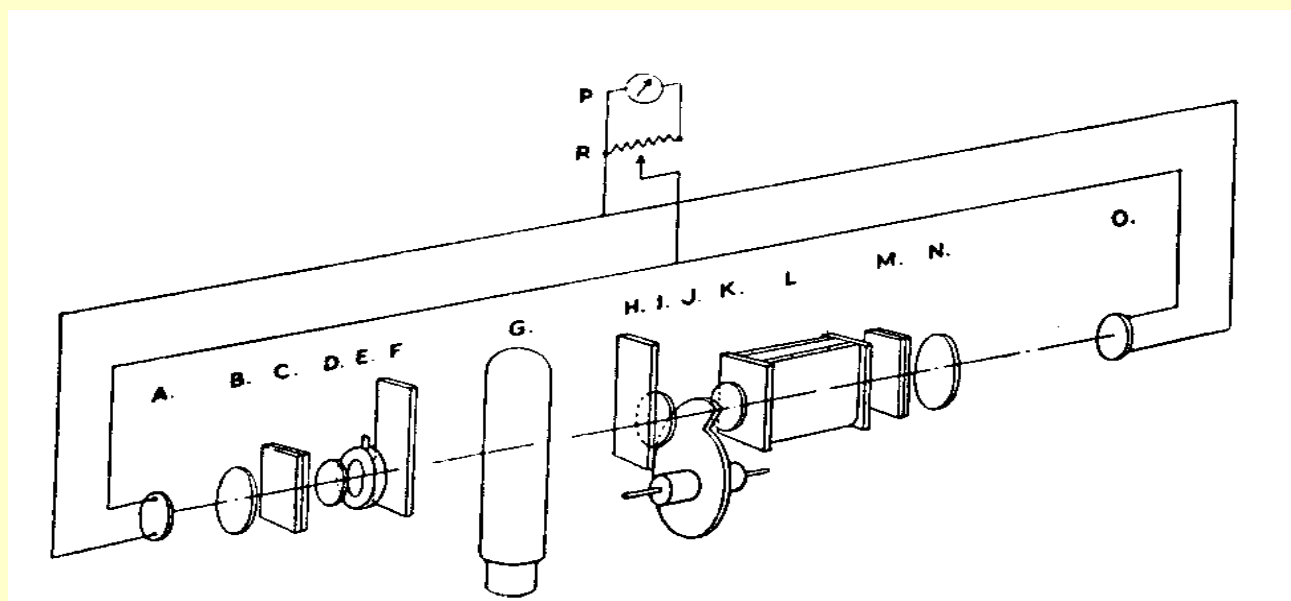
# Hilger Spekker Absorptiometer

---

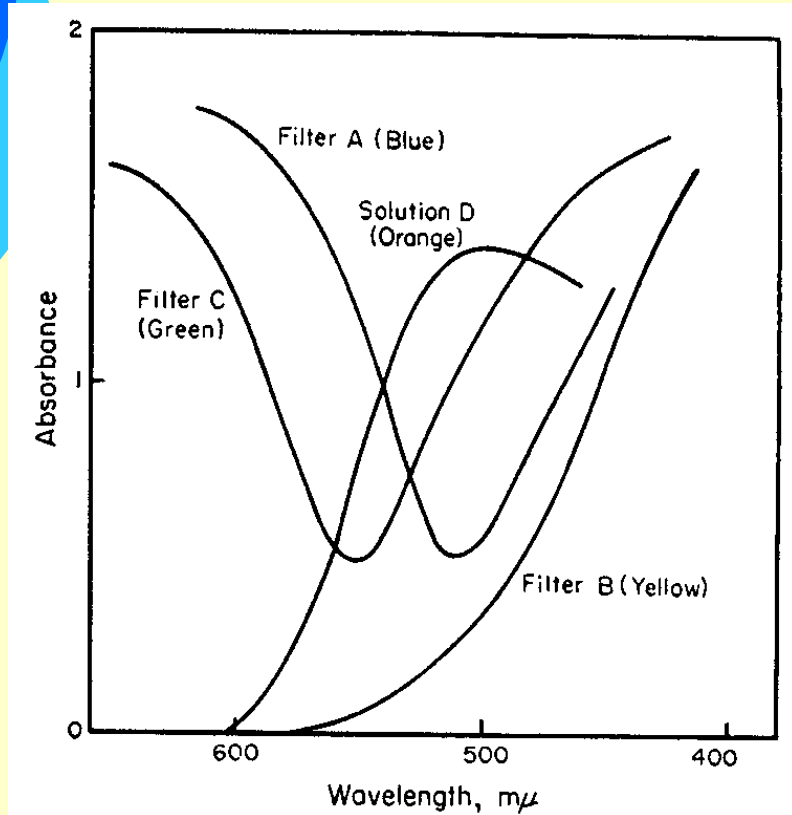


# Hilger Spekker Absorptiometer

- Light from lamp G illuminates 2 photocells A & O via optical null
- F & H are heat absorbing filters
- M is the wavelength filter
- I is the variable window
- The sample is in the cell L



# Band-pass filters for filter photometers



- The chromogenic reagent yields an orange solution (D)
- Filter A will be satisfactory
- Filters B & C transmit considerable radiation where the solution has little absorption

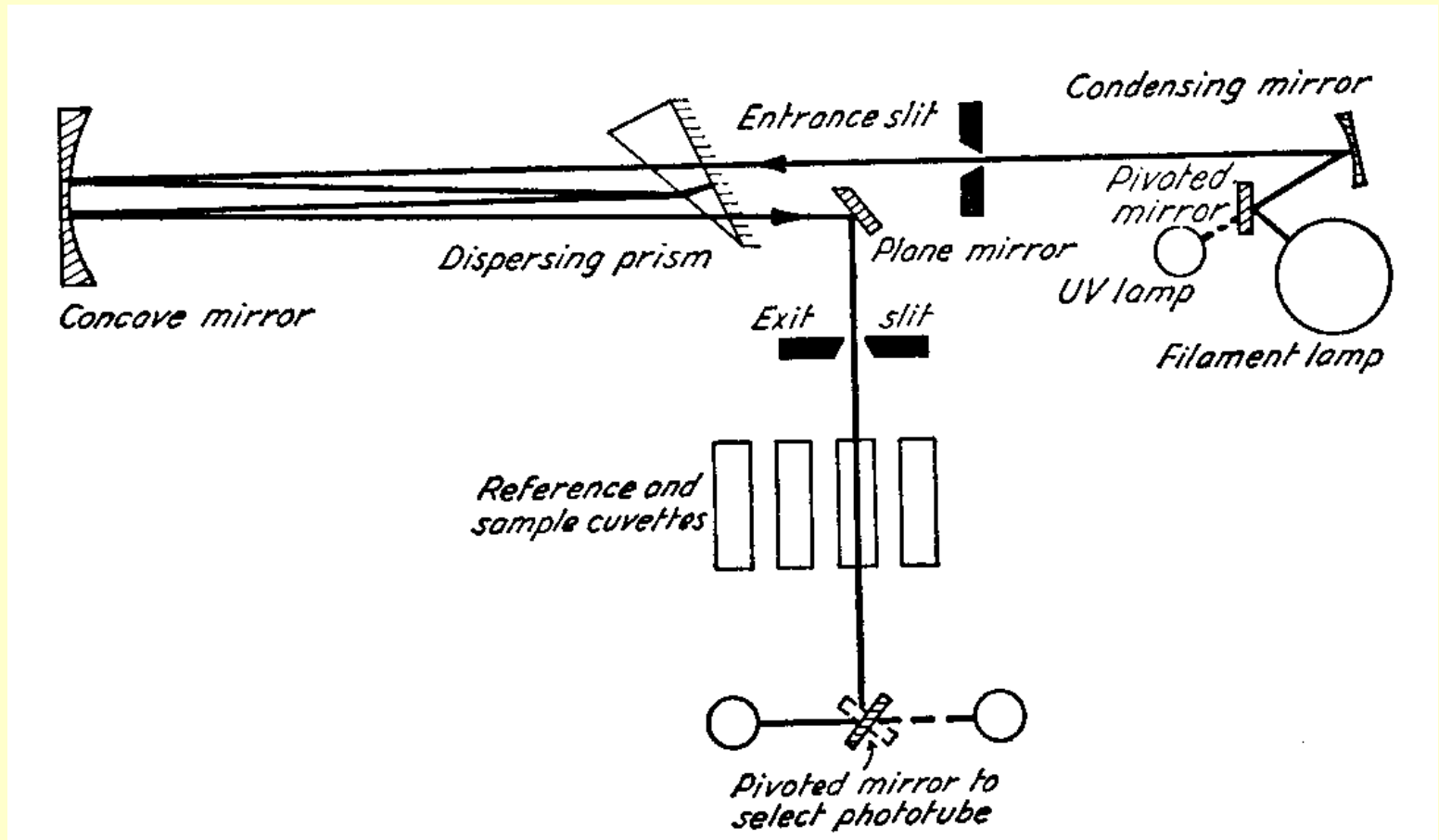


# Loughborough University of Technology

---

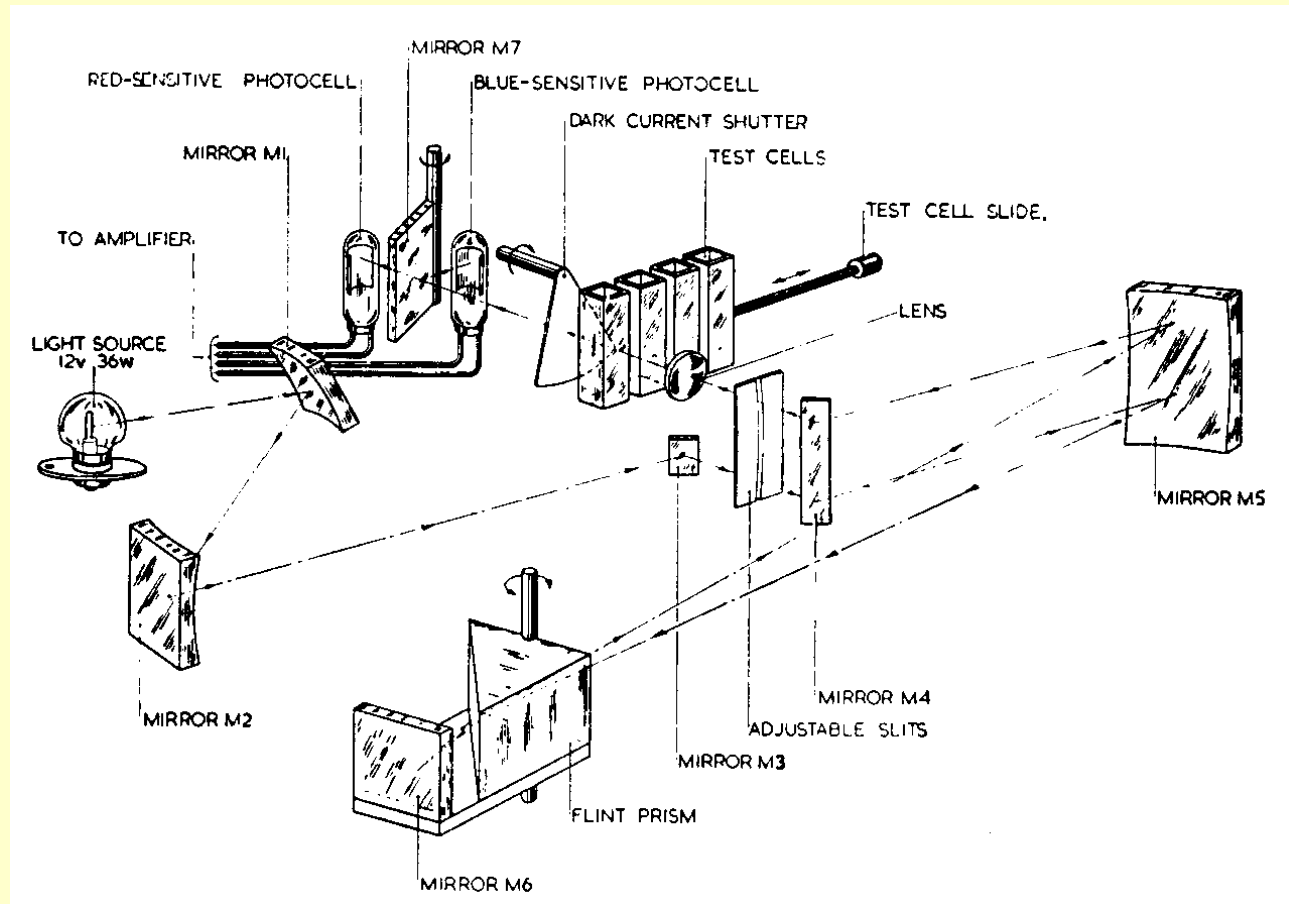
- 1967 to 1972
- The Photoelectric Spectrometry Group Bulletin
- Basic dyes as chromogenic reagents
- Elwell Ward 1971
- Society for Analytical Chemistry

# Hilger & Watts Uvispeck





# Unicam SP 600



# Basics of spectrophotometry

---

- Theoretical aspects
- Nuts & bolts
- Confidence in the numbers!
- J R Edisbury (1966)  
'Practical hints on absorption spectrometry 200 -800m $\mu$ '

# Beer Lambert Law Assumptions

---

- ✓ **The radiation is perfectly monochromatic**
- ✓ **There are no uncompensated losses due to scattering or reflection**
- ✓ **The radiation beam strikes the cuvette at normal incidence**
- ✓ **There are no molecular interactions between the absorber and other molecules in solution**
- ✓ **The temperature remains constant**



## Two More Assumptions

---

- ✓ **The instrument works properly**
- ✓ **The person operating it is competent**

# Beer's Law and Instrument Linearity

---

The voltage generated by the detector is a function of the power of the incident radiation falling on it;  $V = I^\beta$

Beer's law

$$I = I_0 e^{-\epsilon cb} \quad \text{or} \quad A = \log\left(\frac{I_0}{I}\right) = \epsilon cb$$

Combining these two equations we get

$$V = I^\beta = (I_0 e^{-\epsilon cb})^\beta = I_0^\beta e^{-\epsilon\beta cb}$$

and hence the measured absorbance  $A$  is

$$\log\left(\frac{V_0}{V}\right) = \epsilon\beta cb$$

and therefore a proportional change in  $c$  or  $b$  only confirms that  $\beta$  is constant and does not confirm unity

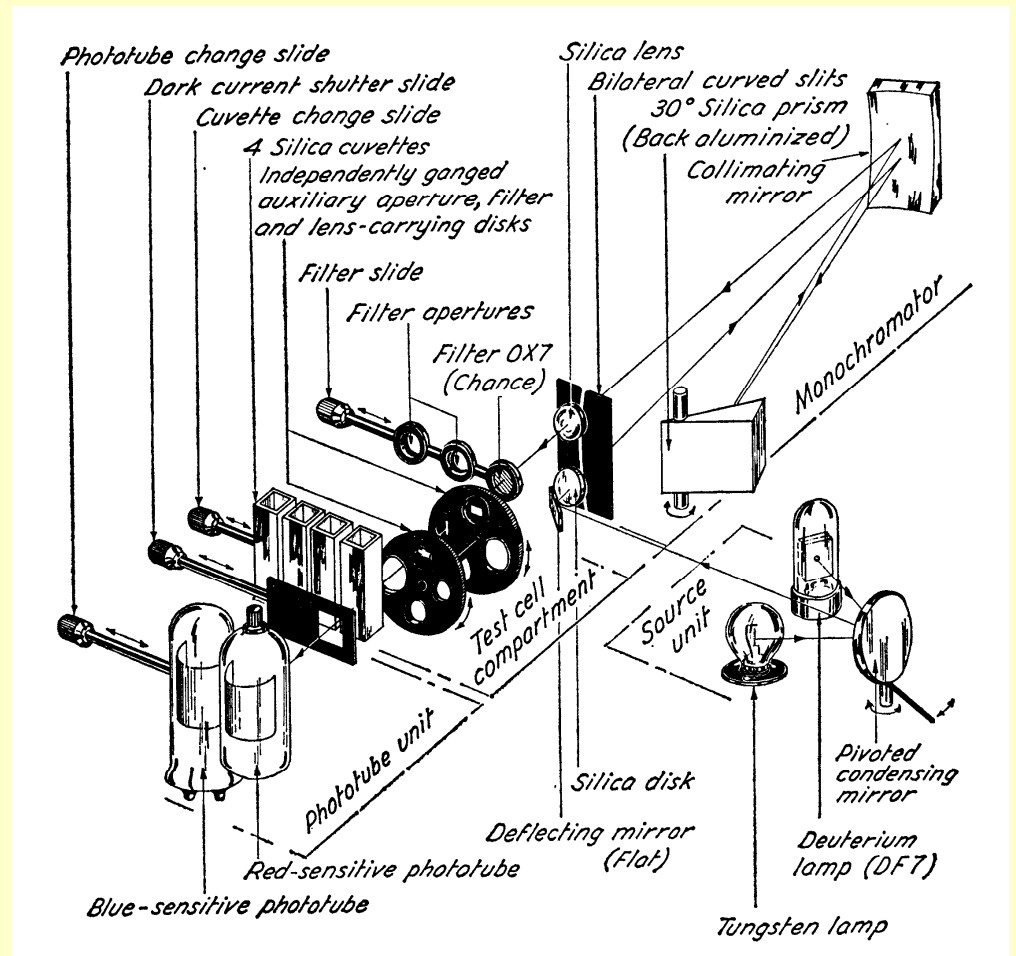


# Nuts and bolts

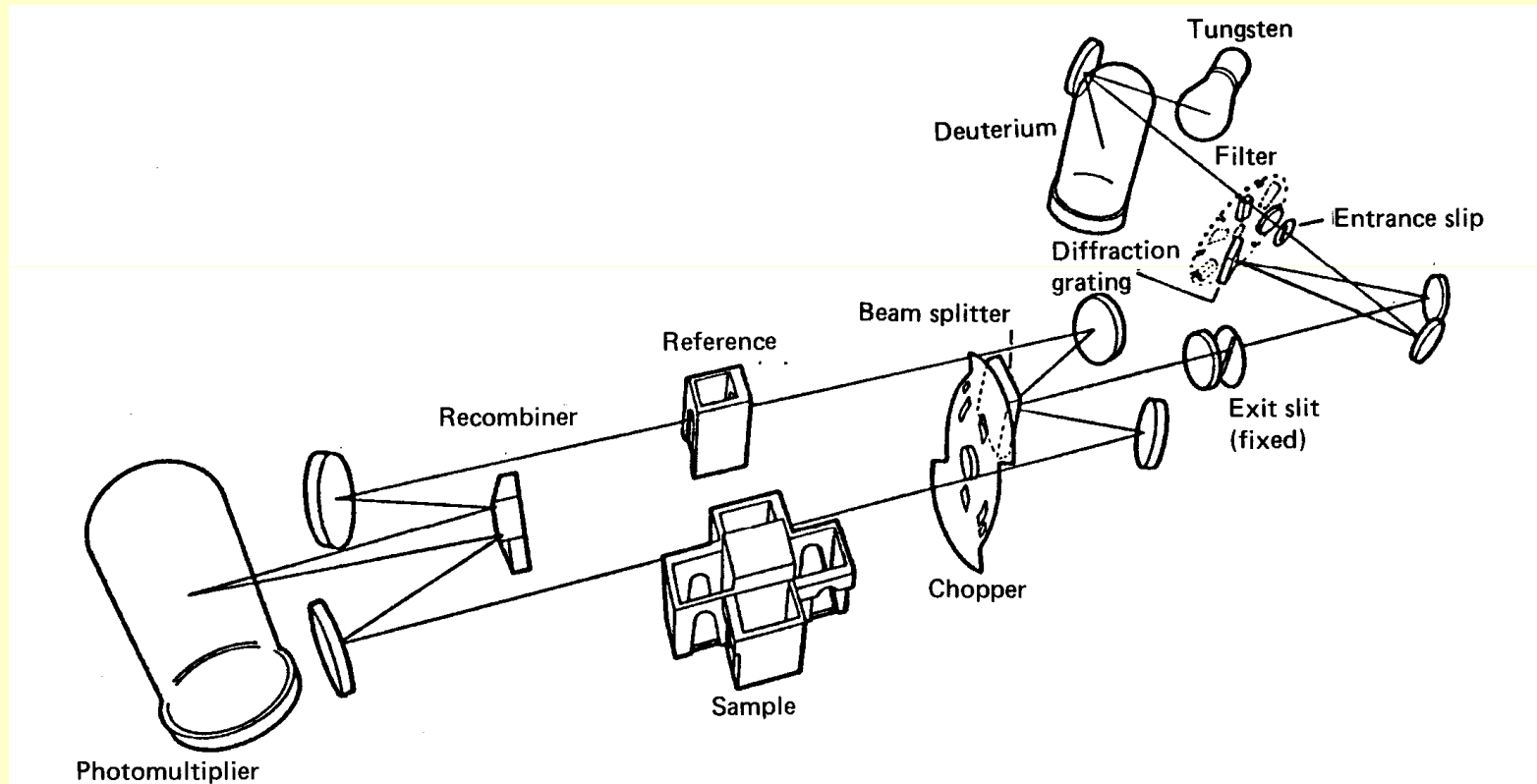
---

- Know your instrument
- Importance of optical design

# Pye Unicam SP500

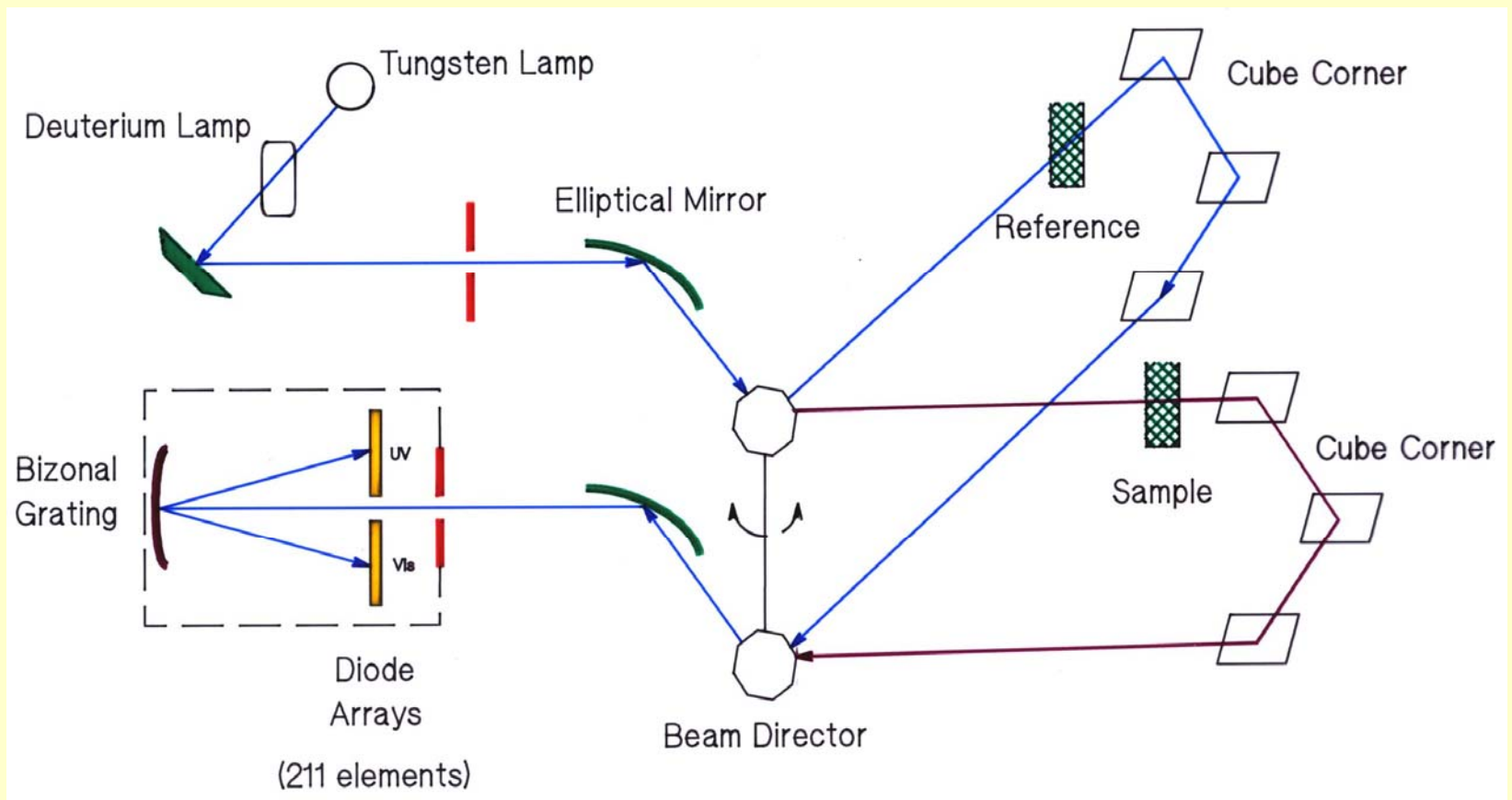


# Double beam recording design





# HP 8450 diode array spectrometer



# The rise of spectrophotometry

---

- Photoelectric Spectrometry Group
  - 1948
  - Beckman model DU & Unicam SP 500
  - UVSG
  - 50<sup>th</sup> anniversary in 1998

# UVSG monographs

---

1. Standards in Absorption Spectrometry  
Ed. C. Burgess & A. Knowles, 1981
2. Standards in Fluorescence Spectrometry  
Ed. J. N. Miller, 1981
3. Practical Absorption Spectrometry  
Ed. A. Knowles & C. Burgess 1984
4. UV Spectroscopy; Techniques, instrumentation,  
data handling  
Ed. B.J. Clark, T. Frost & M.A. Russell, 1993
5. Standards and Best Practice in Absorption  
Spectrometry  
Ed. C. Burgess & T. Frost, 1999

## UVSG/CORM conferences

---

1. Advances in Standards and Methodology in Spectrophotometry  
Ed. C. Burgess & K.D. Mielenz, 1987
2. Spectrophotometry, Luminescence and Colour;  
Science and Compliance  
Ed. C. Burgess & D.G. Jones, 1995
3. Optical Spectrometry: Applications and Instrumentation into the 21<sup>st</sup> Century  
1998

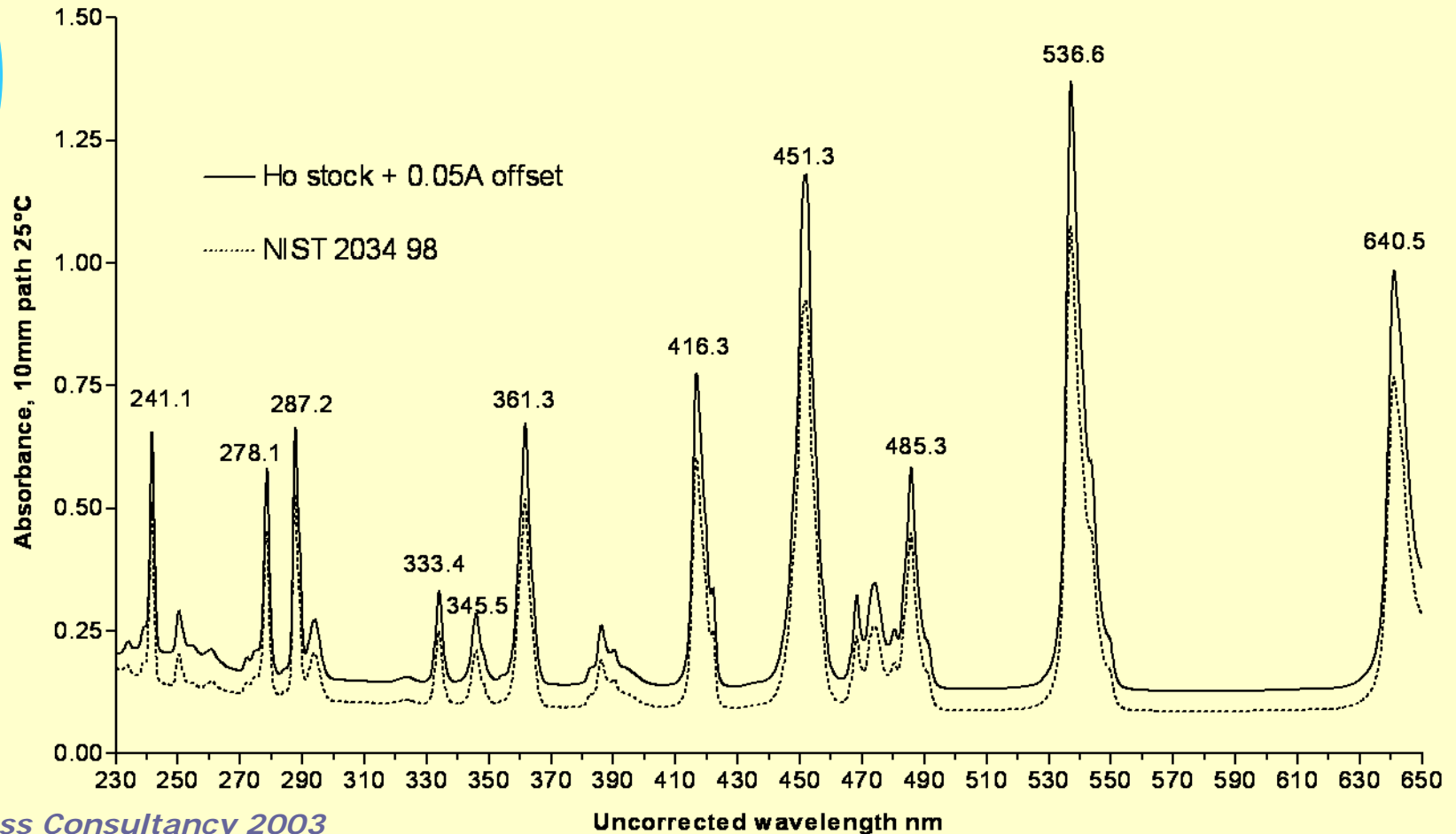
# Spectrophotometry in a regulated environment

---

## Glaxo Laboratories Ltd 1974

- Monitoring the performance of UV-visible spectrophotometers;  
UV Spectrometry Group Bulletin 5 77, 1997
- Control charts
- Standards for accuracy and precision
  - Ho(III) & Sm(III)
  - Thompson's solution
  - Sap Green solutions

# Ho(III) as a wavelength standard



# Qualification and calibration of spectrophotometers

---

## Basis for measurement

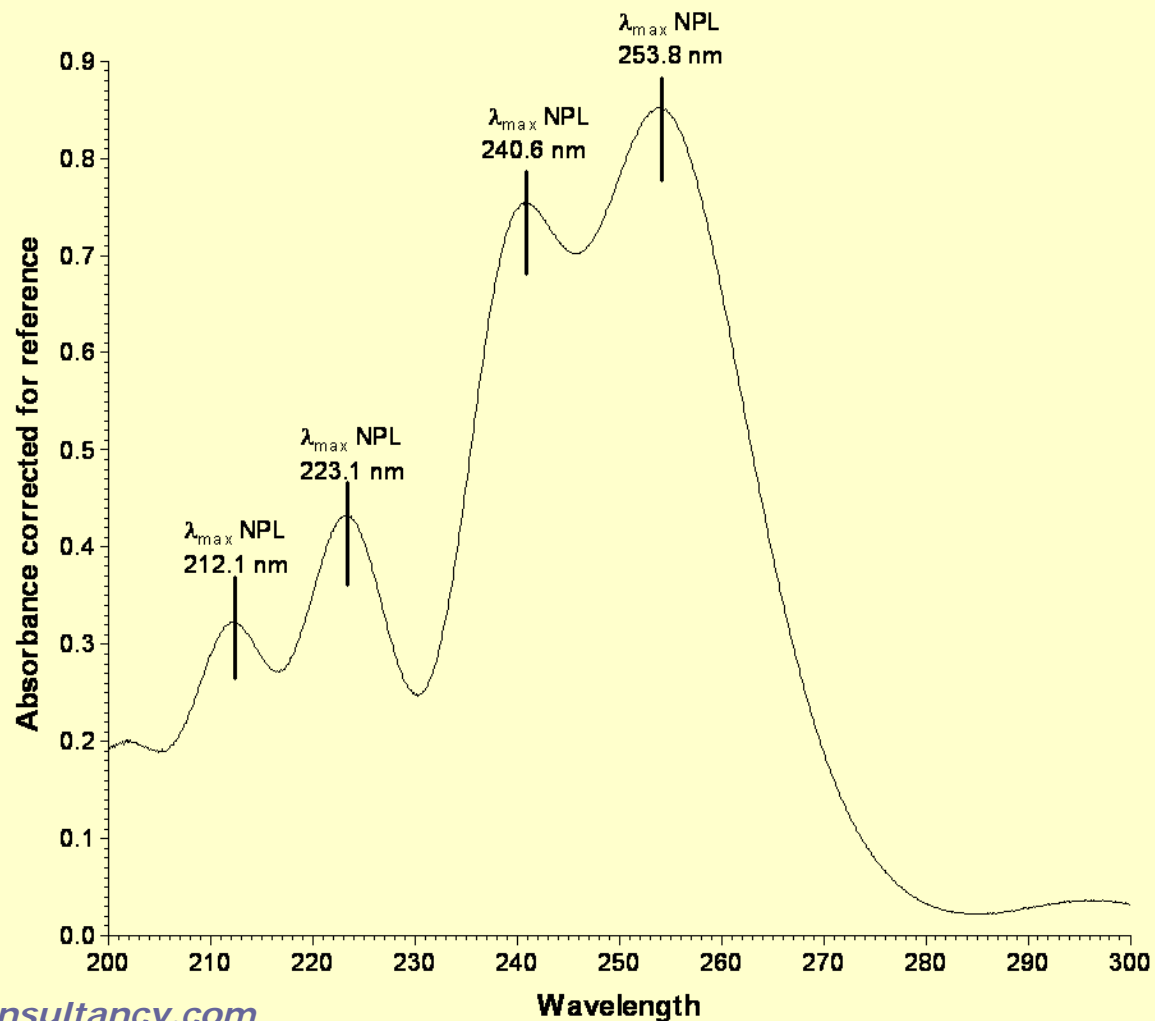
- **Wavelength scale**
  - Operational range
  - Accuracy & precision
- **Photometric scale**
  - Operational range
  - Accuracy & precision (Linearity)
  - Noise and drift
- **Resolution**
- **Stray light**

# Traceable standards





# Starna RM-RE





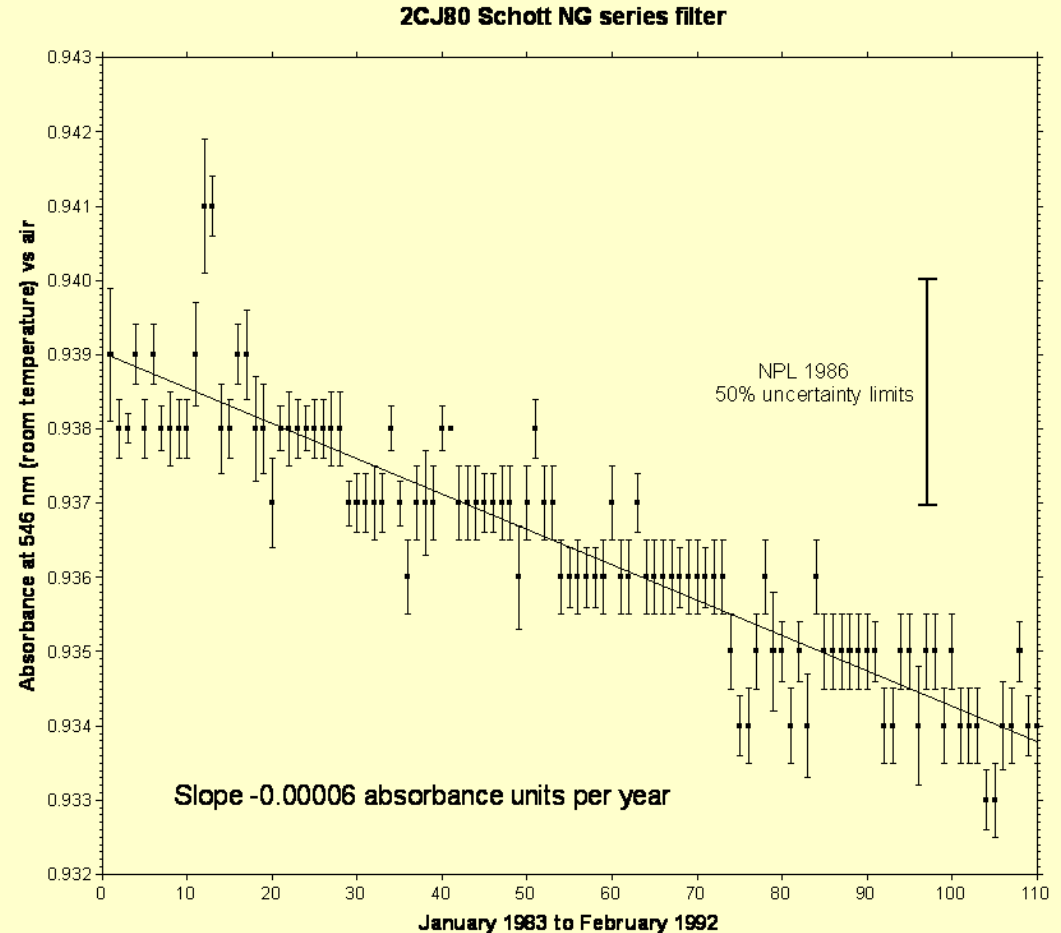
## Long term photometric stability

---

- How reliable is the instrument over its working life?
- How stable is the standard?
- How stable is the standardising laboratory?

# HP 8450 life time photometric accuracy

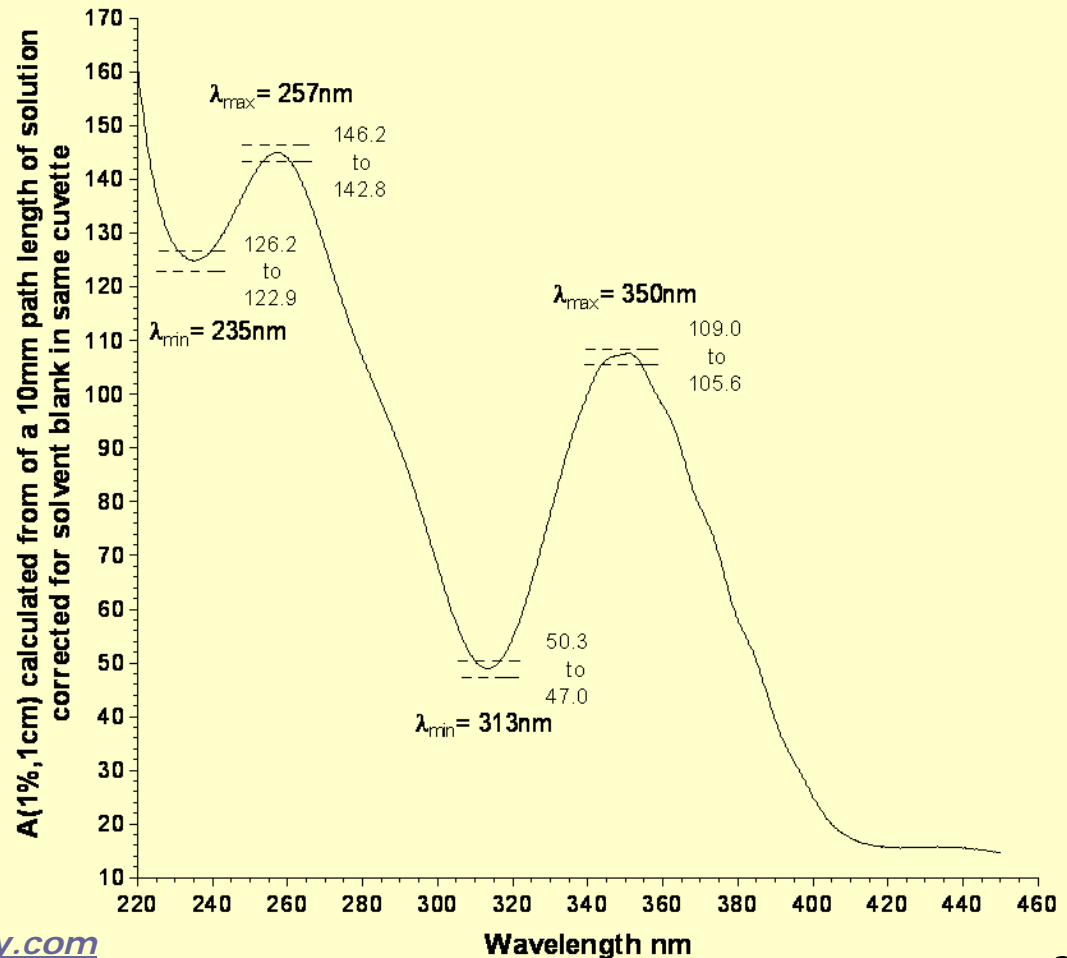
- NPL calibrated neutral density filter
- 546nm checked daily
- Nine years (110 months)
- 2161 data points
- Monthly averages
- 95% CLs



# Potassium dichromate

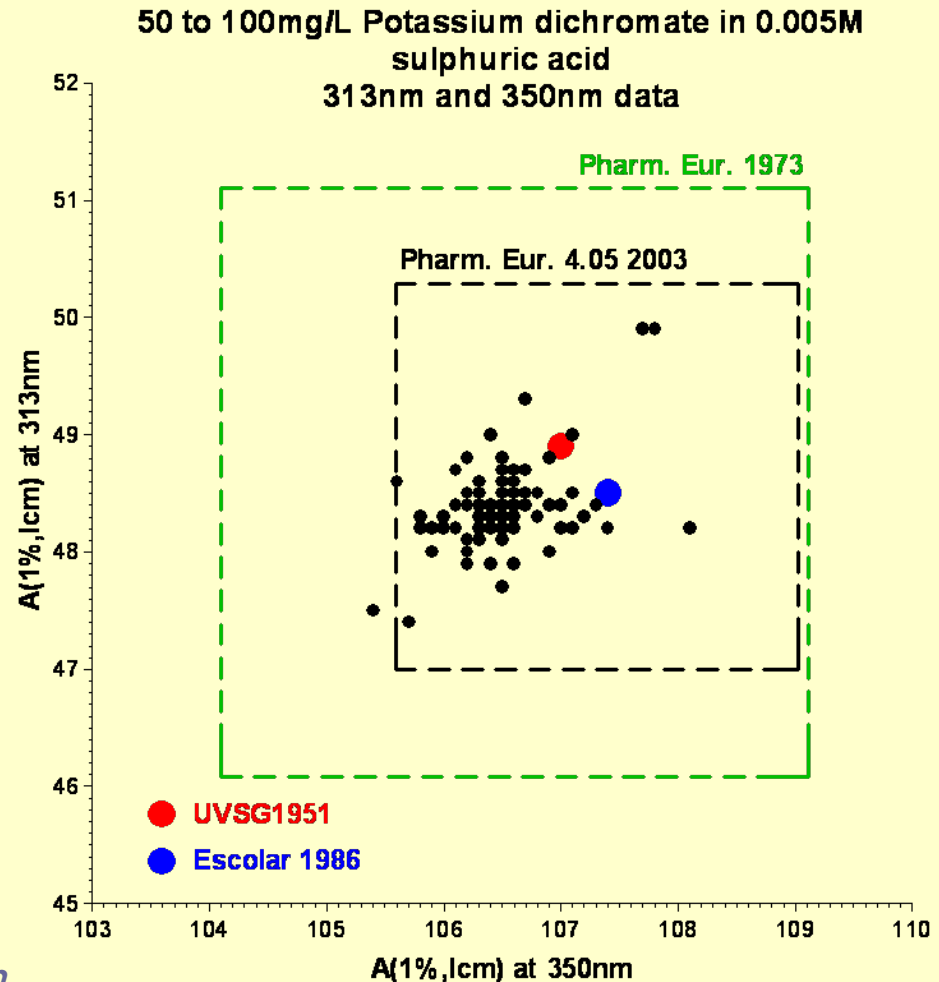
European Pharmacopoeia 4.5 2003

- 2 maxima/minima pairs
- One close to the visible
- One in 'bomb alley' of UV region
- Easy to prepare (60mg in 0.005M sulphuric acid)



# HP 8450 photometric accuracy

- acid dichromate
- Four years (47 months)
- Duplicates
- Satisfactory performance
- Reading slightly low at 350nm
- No correlation max/min

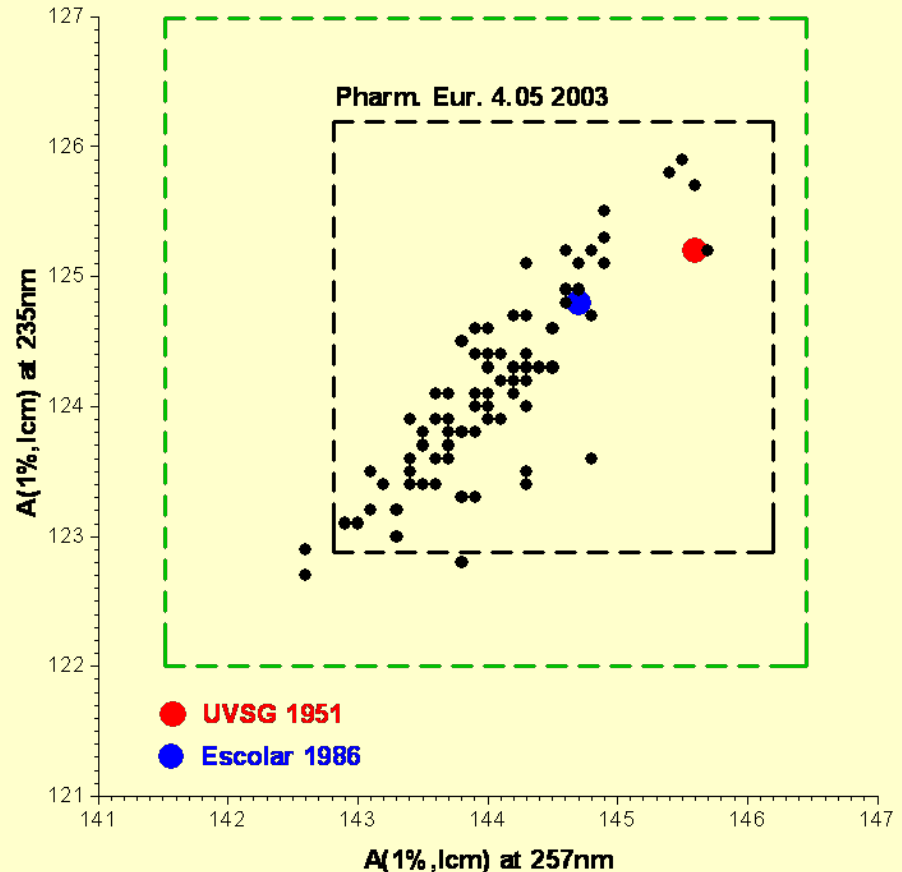


# HP 8450 photometric accuracy

- acid dichromate
- Four years (47 months)
- Duplicates
- Less satisfactory performance in UV
- Correlation max/min

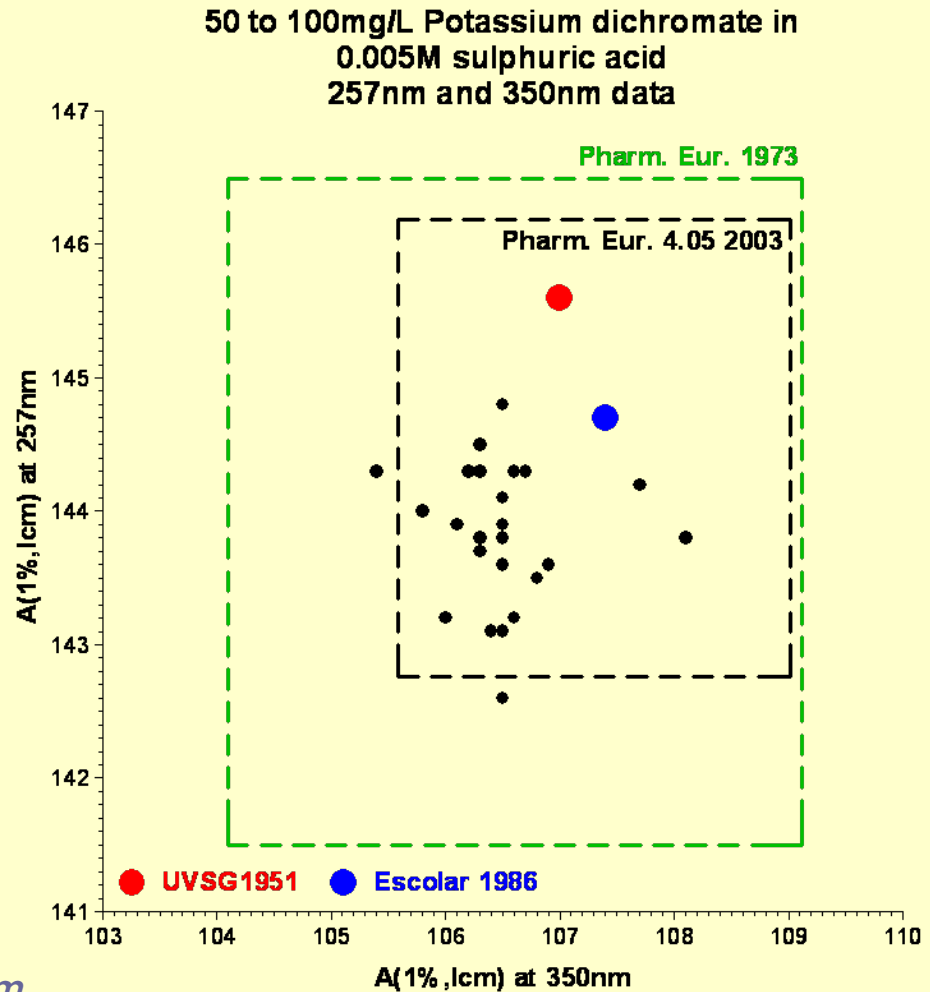
50 to 100mg/L Potassium dichromate in 0.005M sulphuric acid  
235nm and 257nm data

Pharm. Eur. 1973



# HP 8450 photometric accuracy

- acid dichromate
- Four years (47 months)
- Duplicates
- Maxima to maxima
- No correlation





# Technology transfer standards

---

- Stable
- Non hazardous
- UV & Visible range
- Easy to use

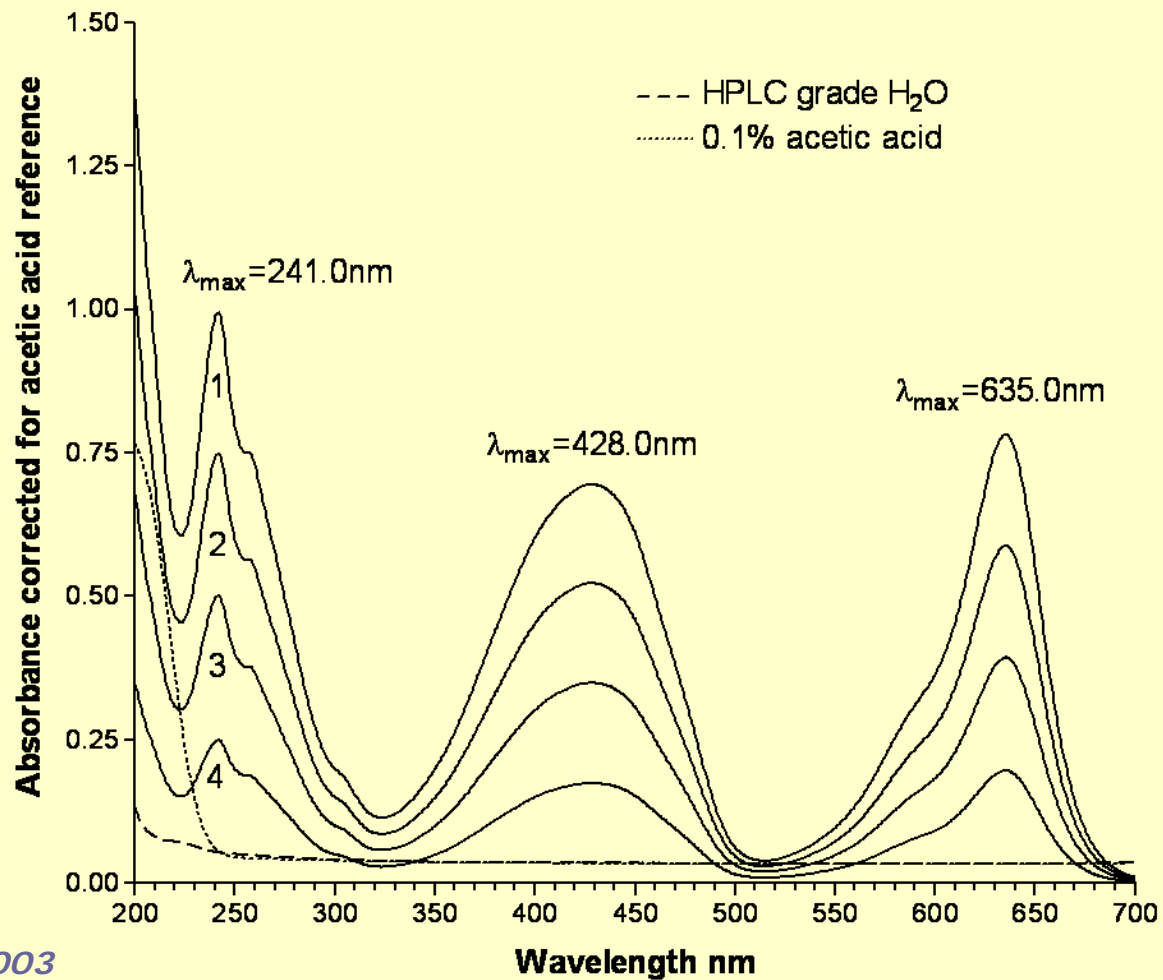


# Sap Green 1979

---



# Sap Green Linearity



# Comparison of scanning and DAS spectrophotometers 1990

---

- 7 DAS (HP8450 & HP8452)
- 6 Scanning instruments (PE Lambda 5, 6 and 15)
- All labs had the 'same' solution of Sap Green





# Spectrophotometric methods

---

The instrument is OK but what about the method?

## Optimum conditions

- Best precision
- Best accuracy

# Precision and noise

---

The relative standard deviation of concentration

$$RSD_c = \frac{s_c}{c} = \frac{0.4343s_T}{T \log_{10} T}$$

however  $s_T$  is a function of  $T$

Three main sources;

1. Electronics, which is a constant (Johnson Noise)

$$s_T = k_1$$

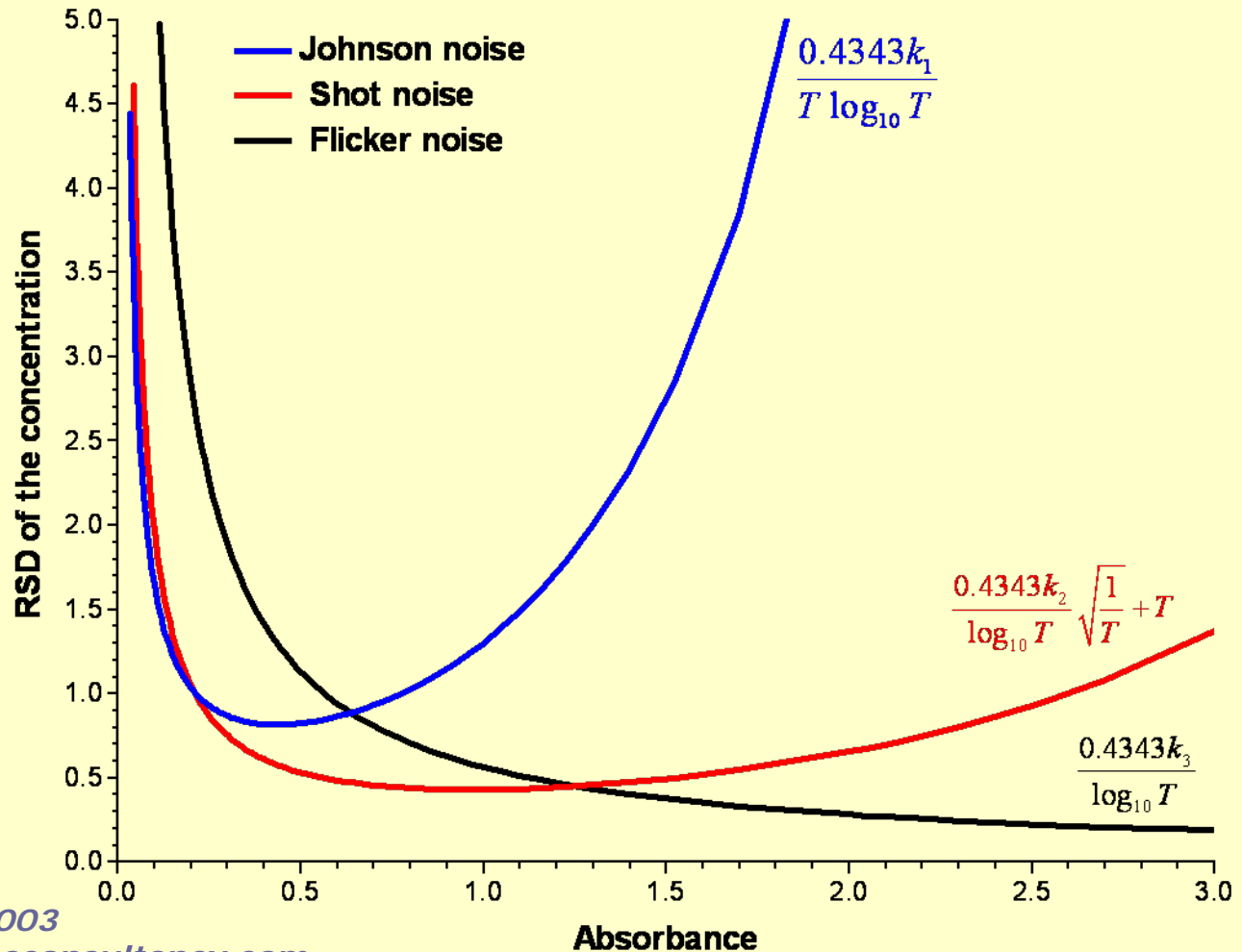
2. Detector, photon statistics (Shot noise)

$$s_T = k_2 \sqrt{T^2 + T}$$

3. Cuvette positioning errors and source flicker

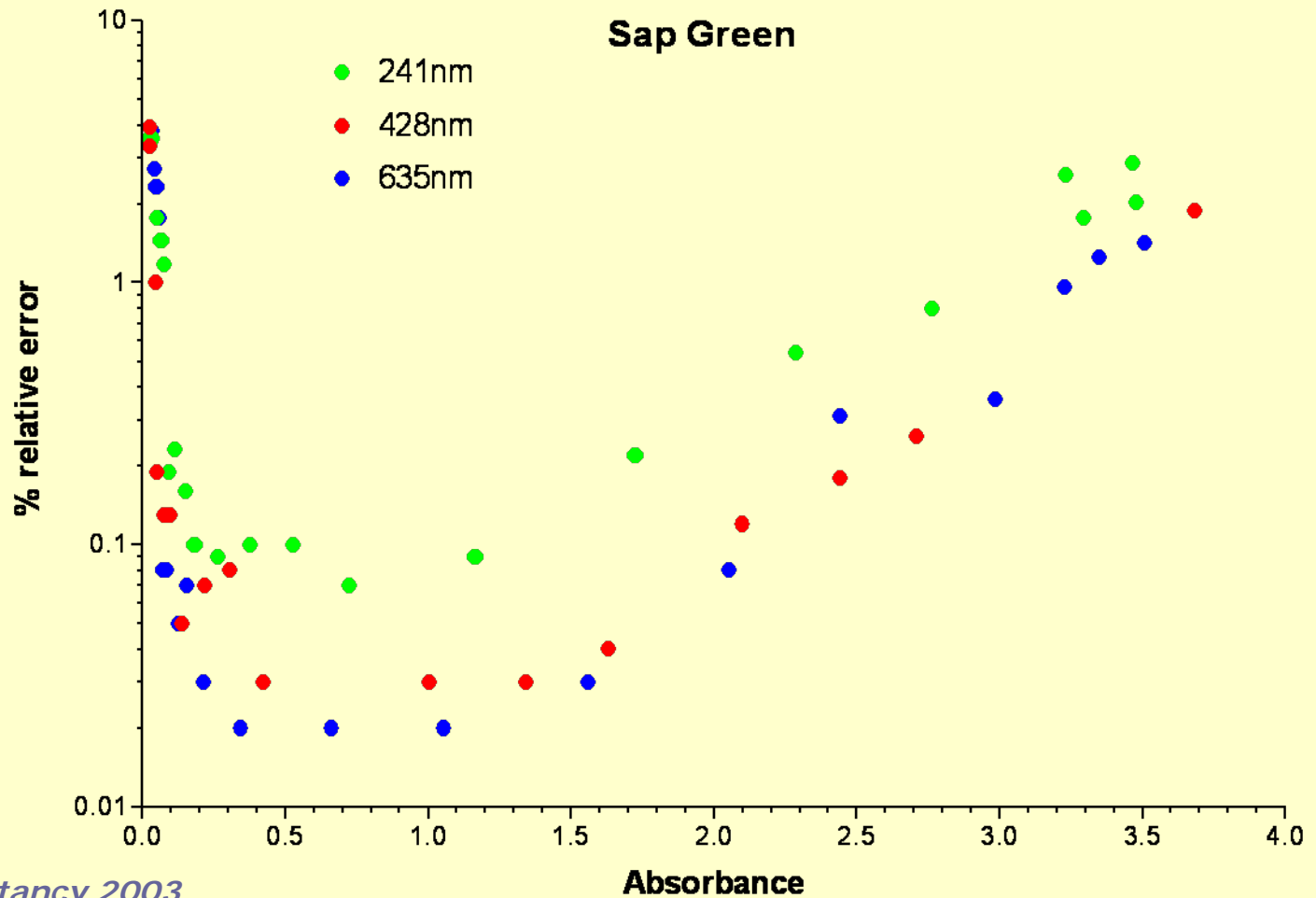
$$s_T = k_3 T$$

# Precision and noise





# Relative errors; HP 8450 diode array spectrometer



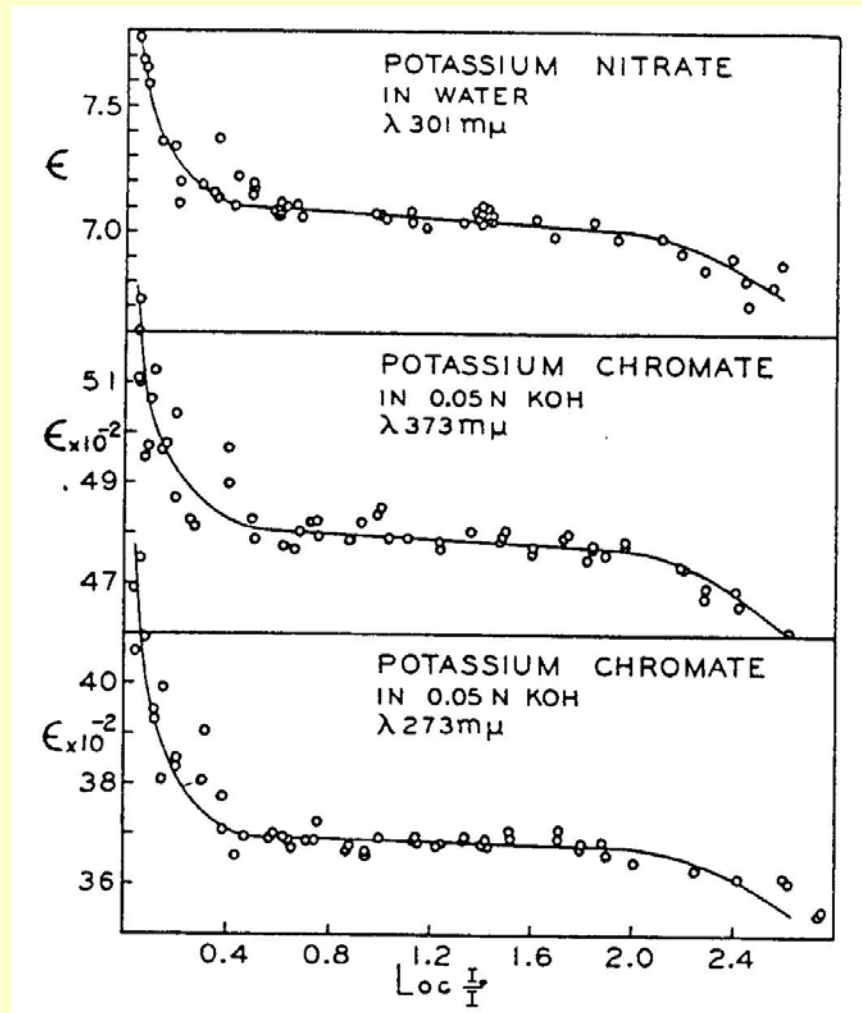
# Accurate values of absorbance

---

- Setting standard values
- Minimising bias
- Less out of specification results
- Forgotten lessons
  - Ringbom-Ayres plots
  - VFG method

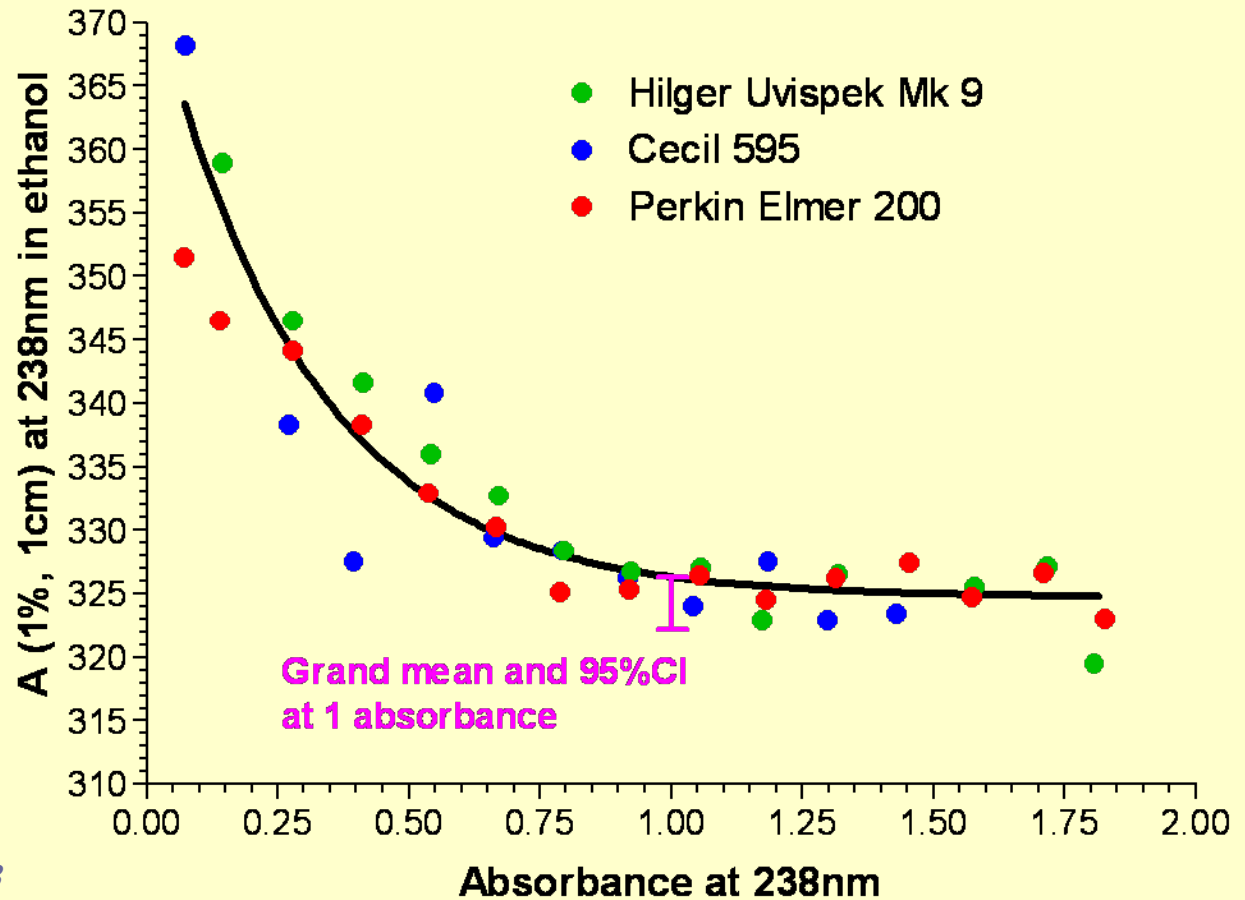
# Vandenbelt, Forsyth and Garrett Ind. Eng. Chem. (Analytical Edition) 1945

- variation of molar extinction coefficient with absorbance
- Impact on standard values
- Beckman DU working absorbance range 0.5 to 1.9 for broad bands



# VFG plot

## Betamethasone 17 valerate 1976





## In conclusion

---

- Spectrophotometry is alive if not well
- Standards are becoming more important
- Trust your instruments and your methods BUT always verify
- The journey continues...

# NIR wavelength for the future?

TS5 & SRM 2035 (offset)  
8<sup>th</sup> January 2003; Jasco V570 1nm SBW; 100nm min<sup>-1</sup>

