Trust but always Verify:

Best practices in spectrophotometric measurement

L S Theobald Lecture
15th May 2003

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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

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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 3rd Edition)
- Trained analyst

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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
2nd Edition 1961

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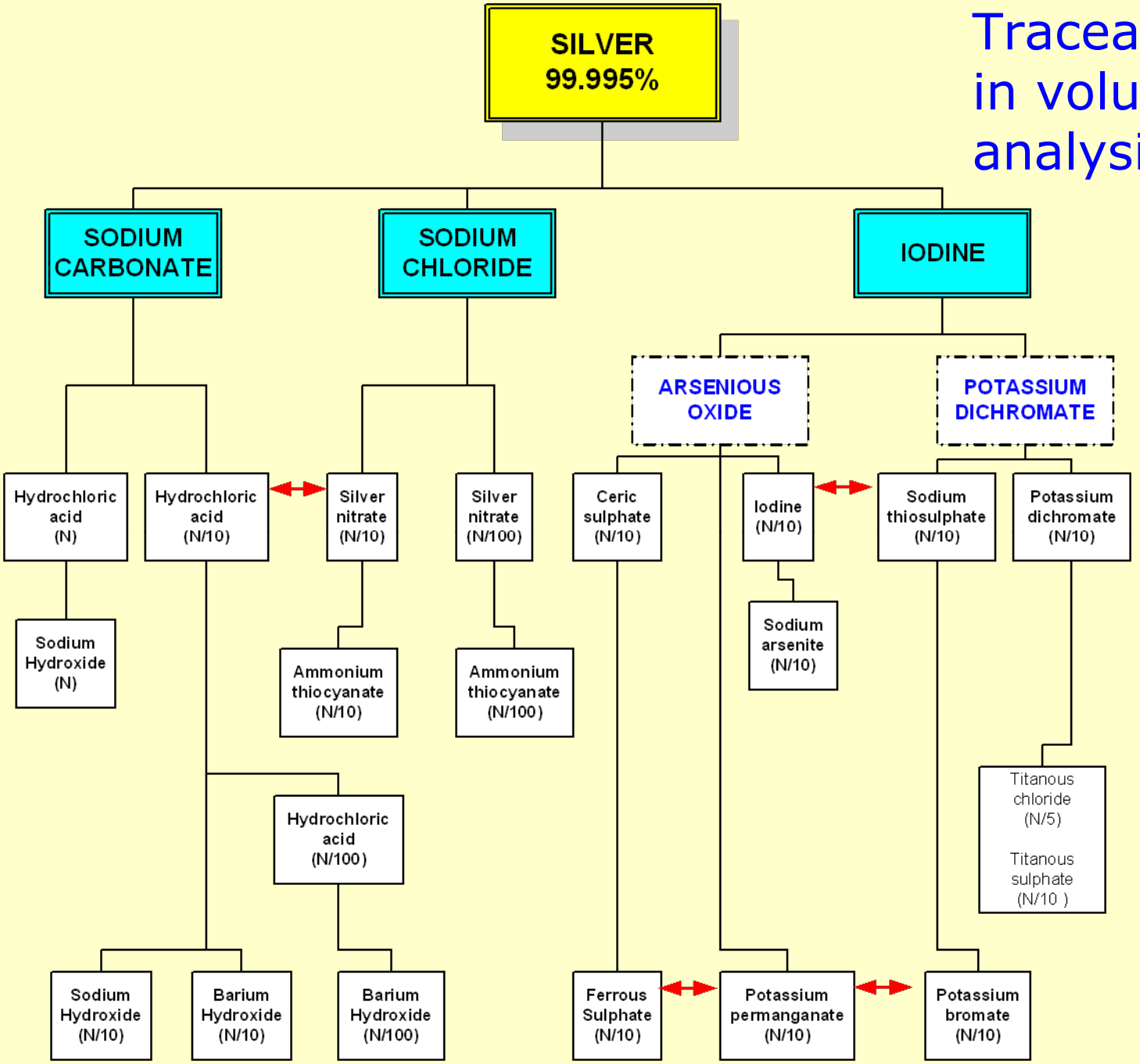
Traceability in volumetric analysis



Qualitative analysis

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

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Bunsen spectroscope

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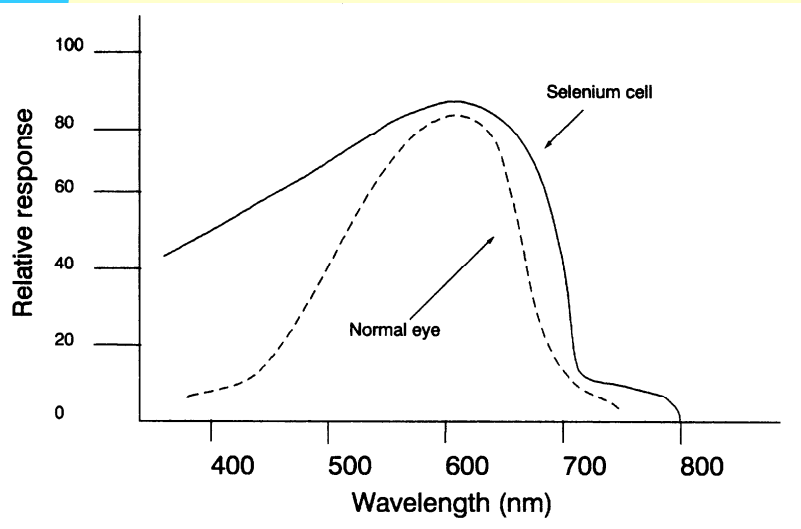
The eye as a detector



Approximate wavelength range (nm)	*Colour of reflected light*	*Colour of absorbed light*
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

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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

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Nessleriser standards

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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

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Hilger Spekker Absorptiometer

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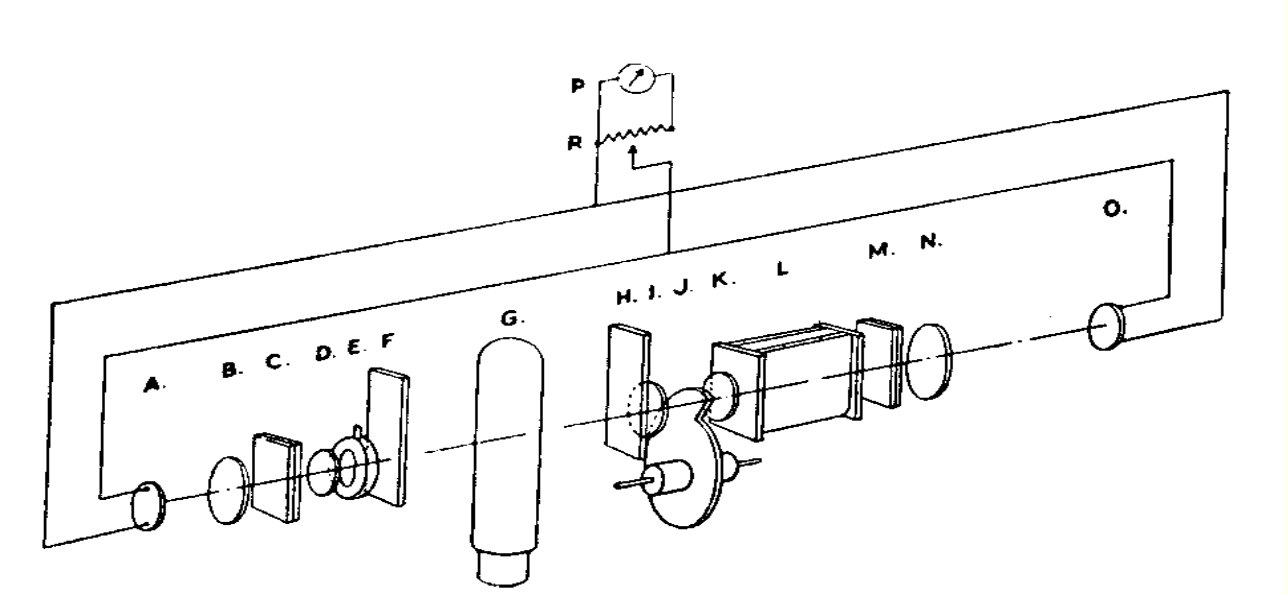
Hilger Spekker Absorptiometer

- 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



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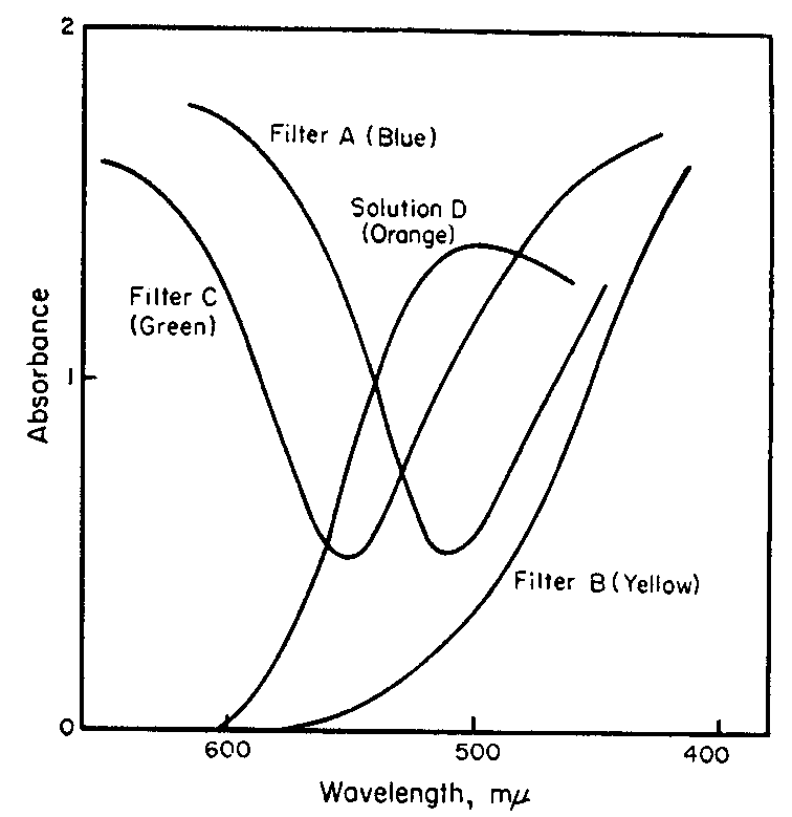
Band-pass filters for filter photometers



- **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**

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Loughborough University of Technology

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

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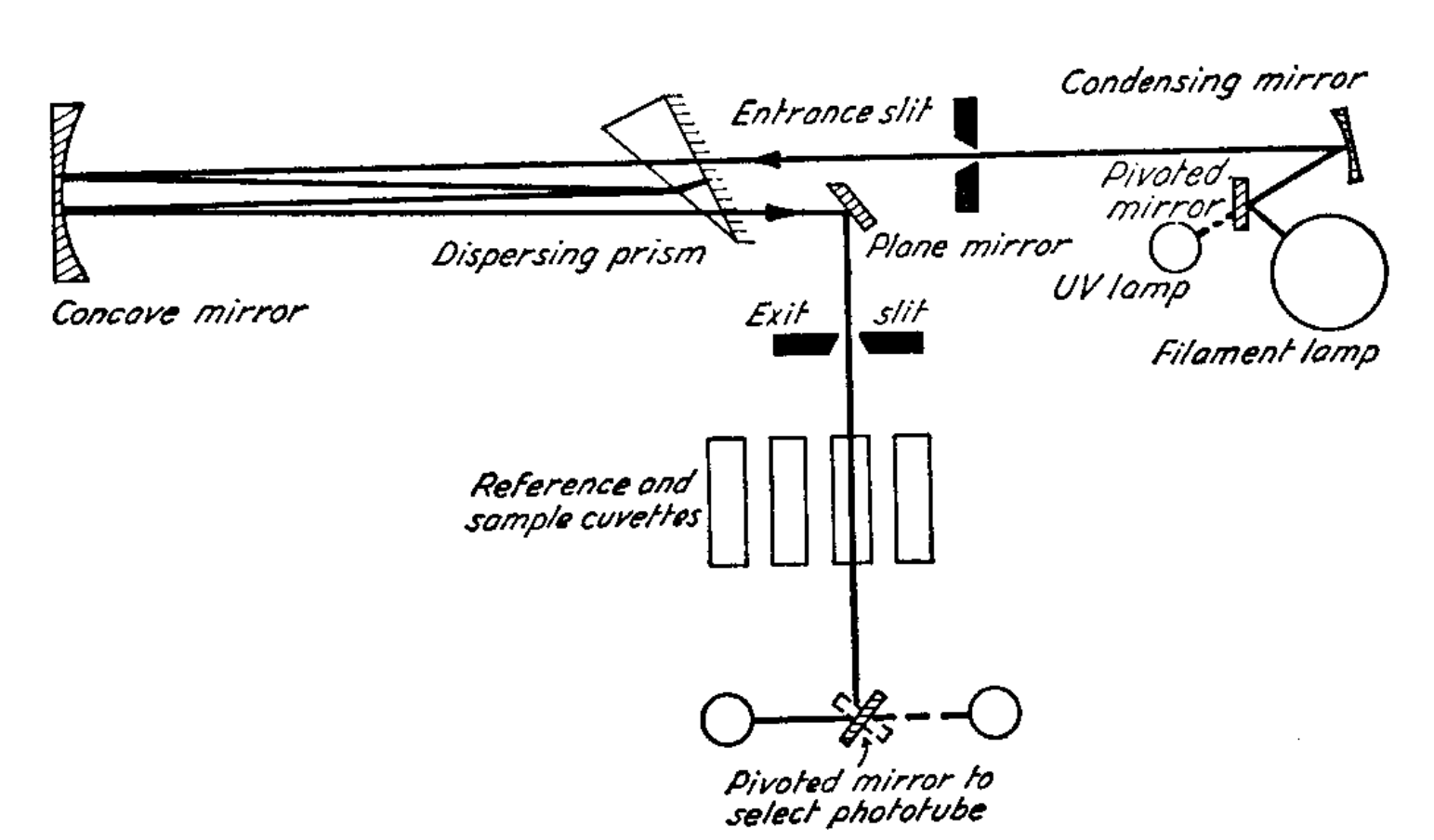
Hilger & Watts Uvispeck



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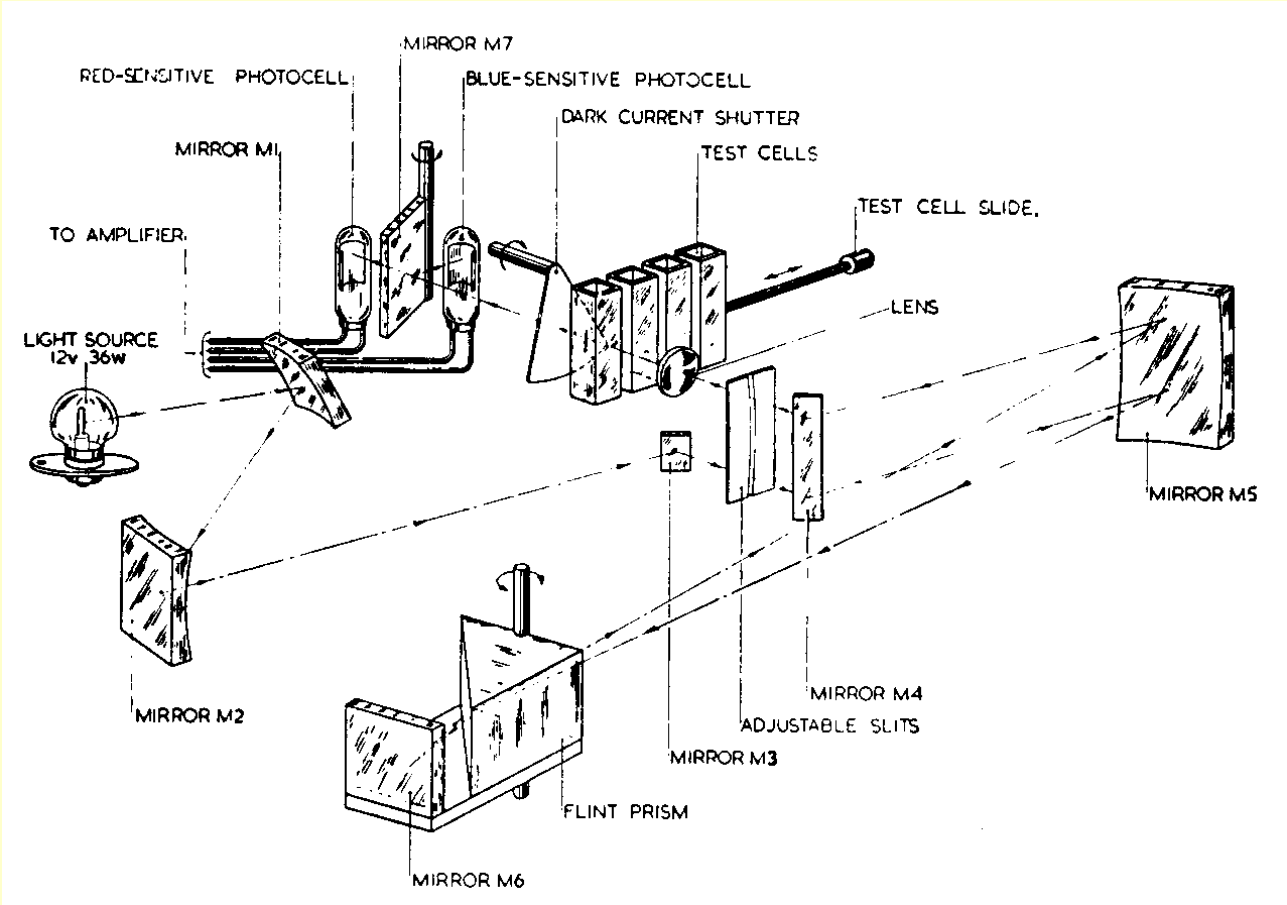
Unicam SP 600



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Basics of spectrophotometry

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

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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**

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Two More Assumptions

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

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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

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

Combining these two equations we get

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

and hence the measured absorbance A is

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

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

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Nuts and bolts

- Know your instrument
- Importance of optical design

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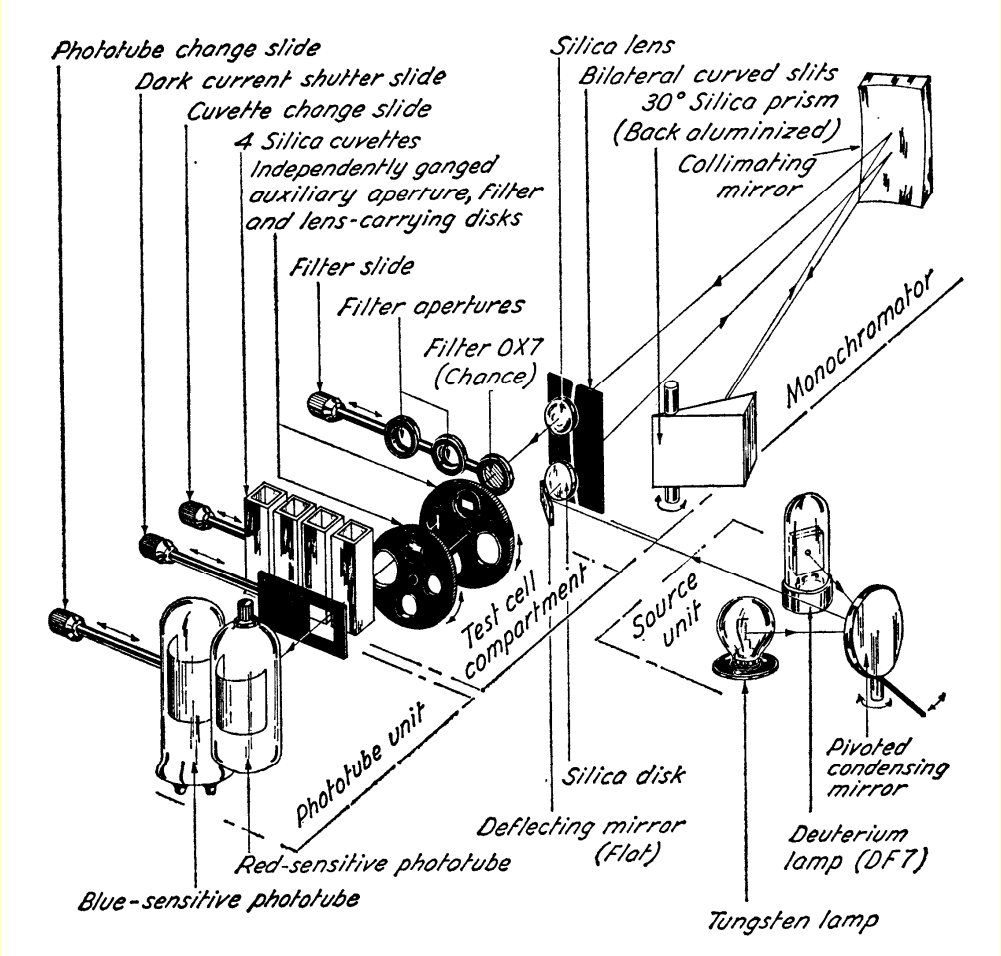
Pye Unicam SP500



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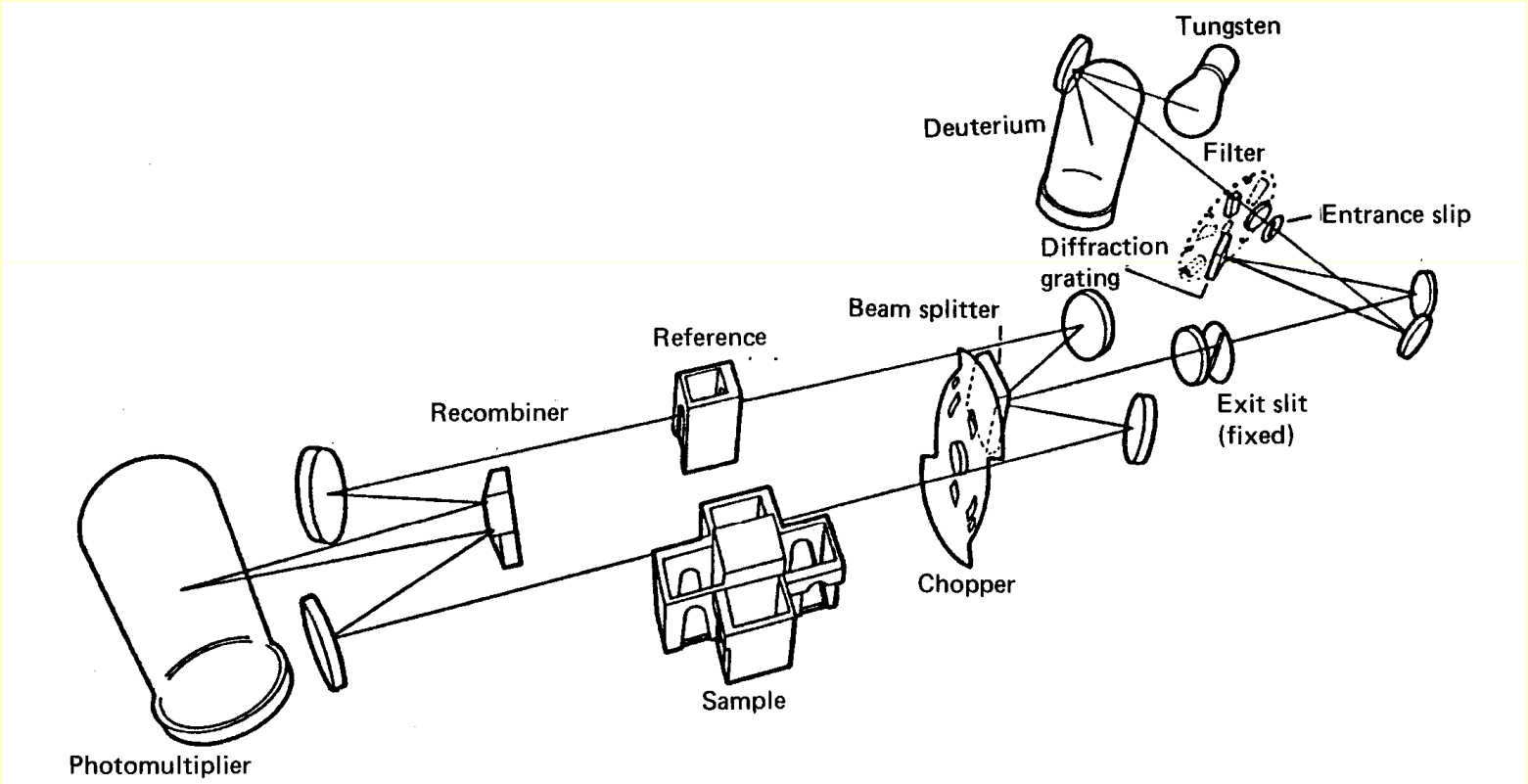
Double beam recording design



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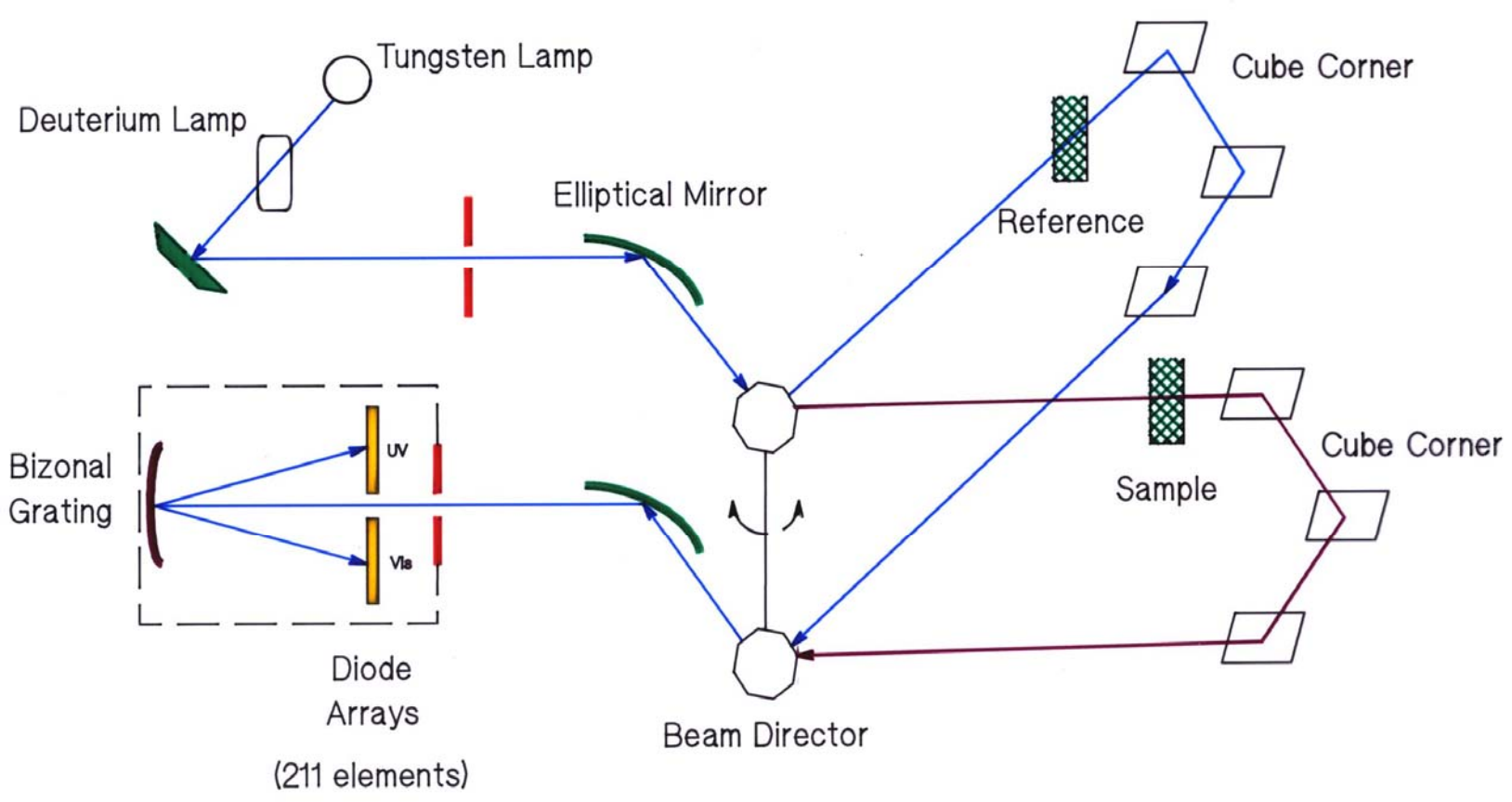
HP 8450 diode array spectrometer



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The rise of spectrophotometry

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

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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

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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 21st Century
   1998

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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

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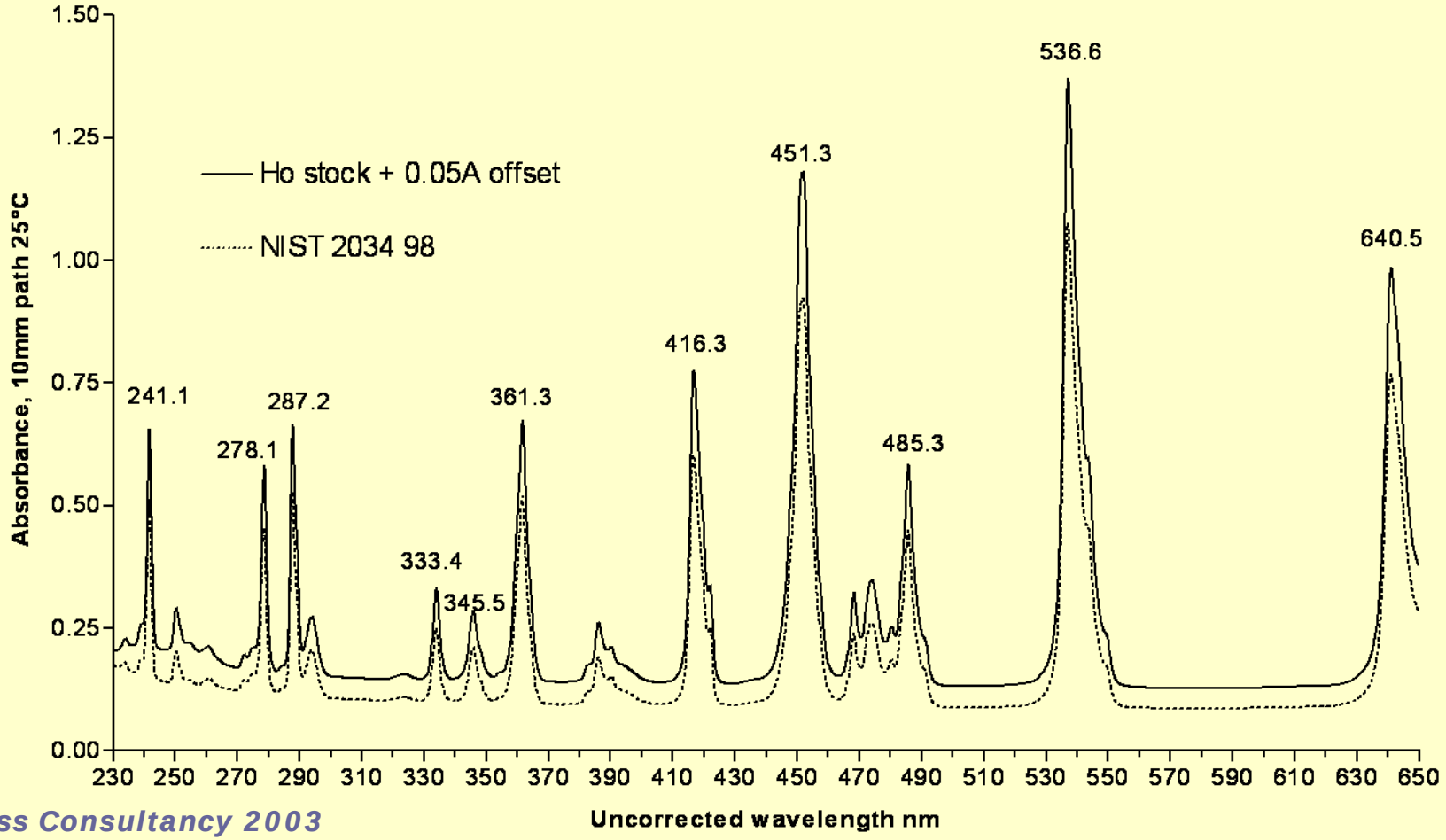
Ho(III) as a wavelength standard



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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**

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Traceable standards

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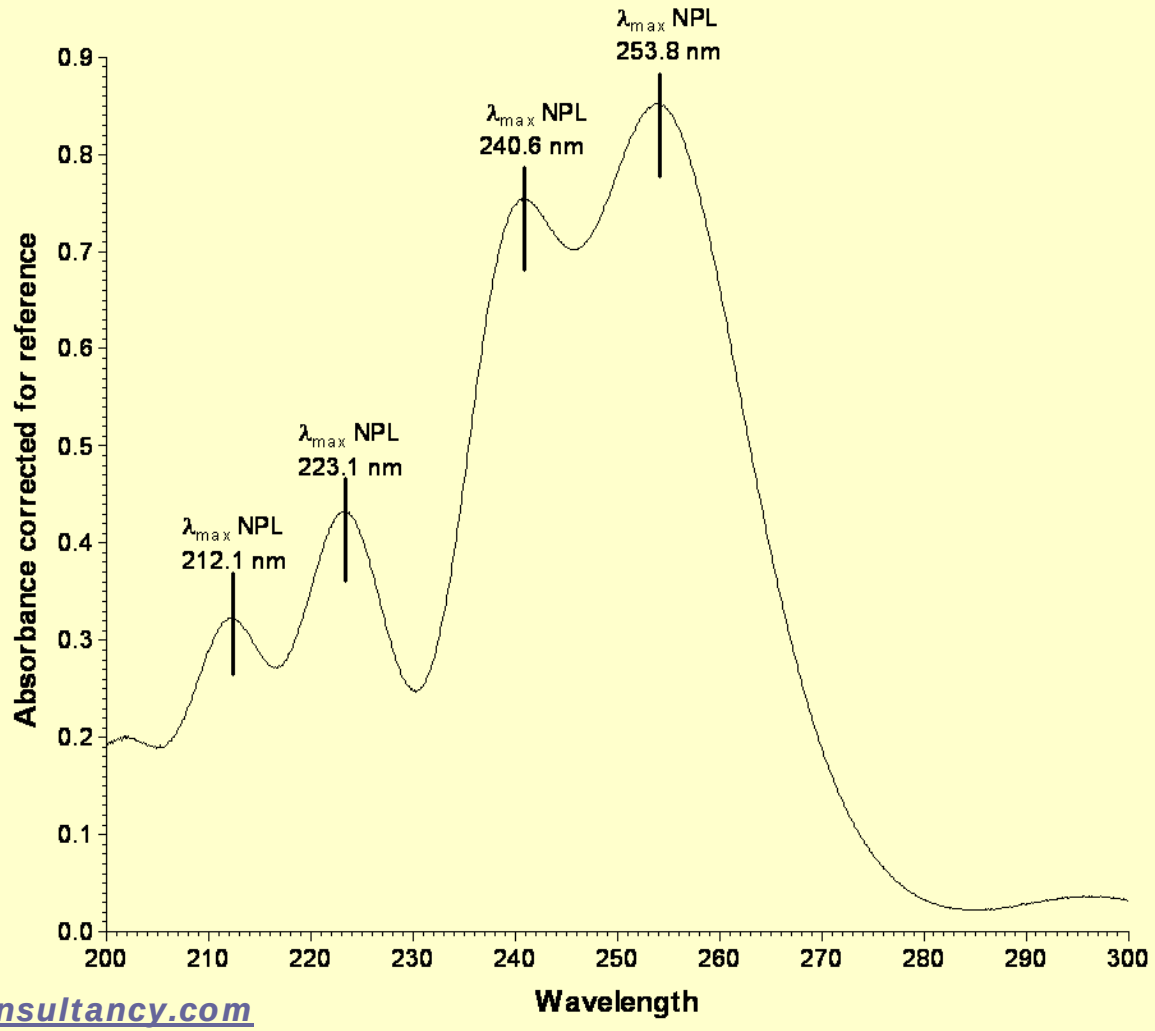
Starna RM-RE



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Long term photometric stability

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

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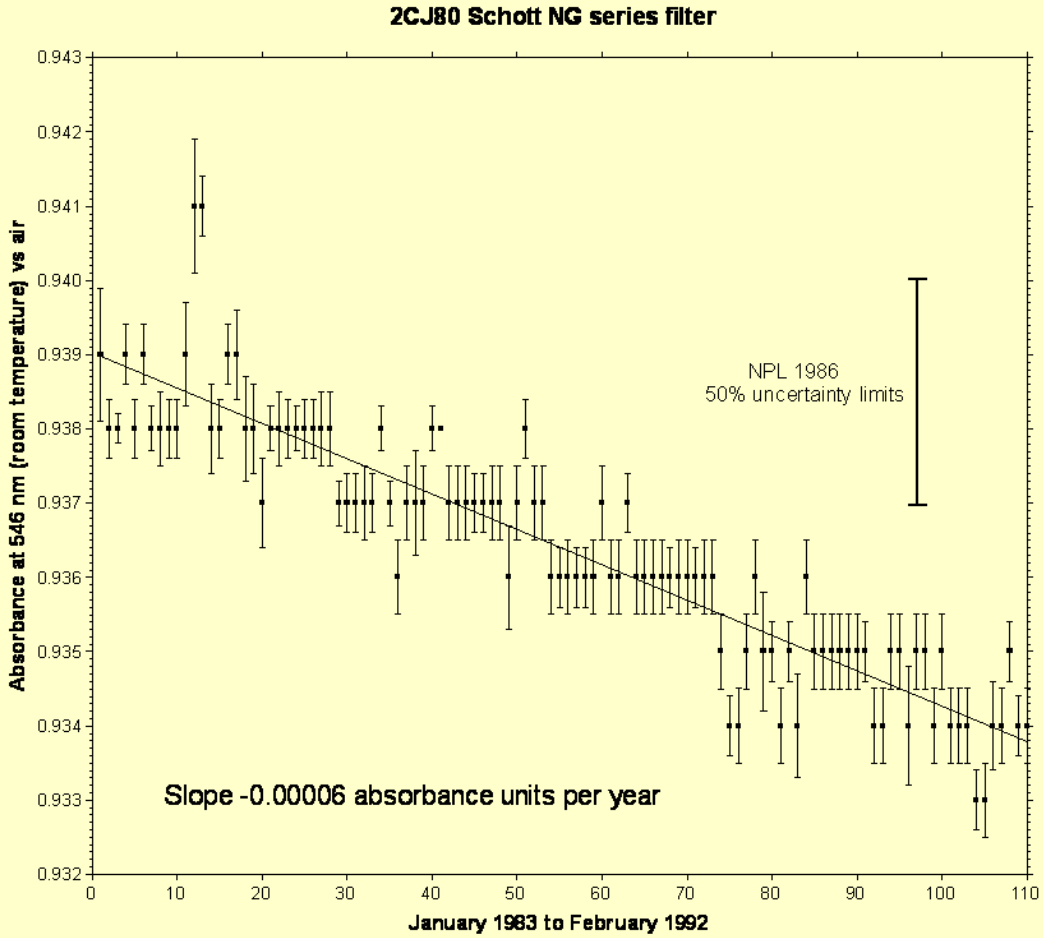
HP 8450 life time photometric accuracy

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



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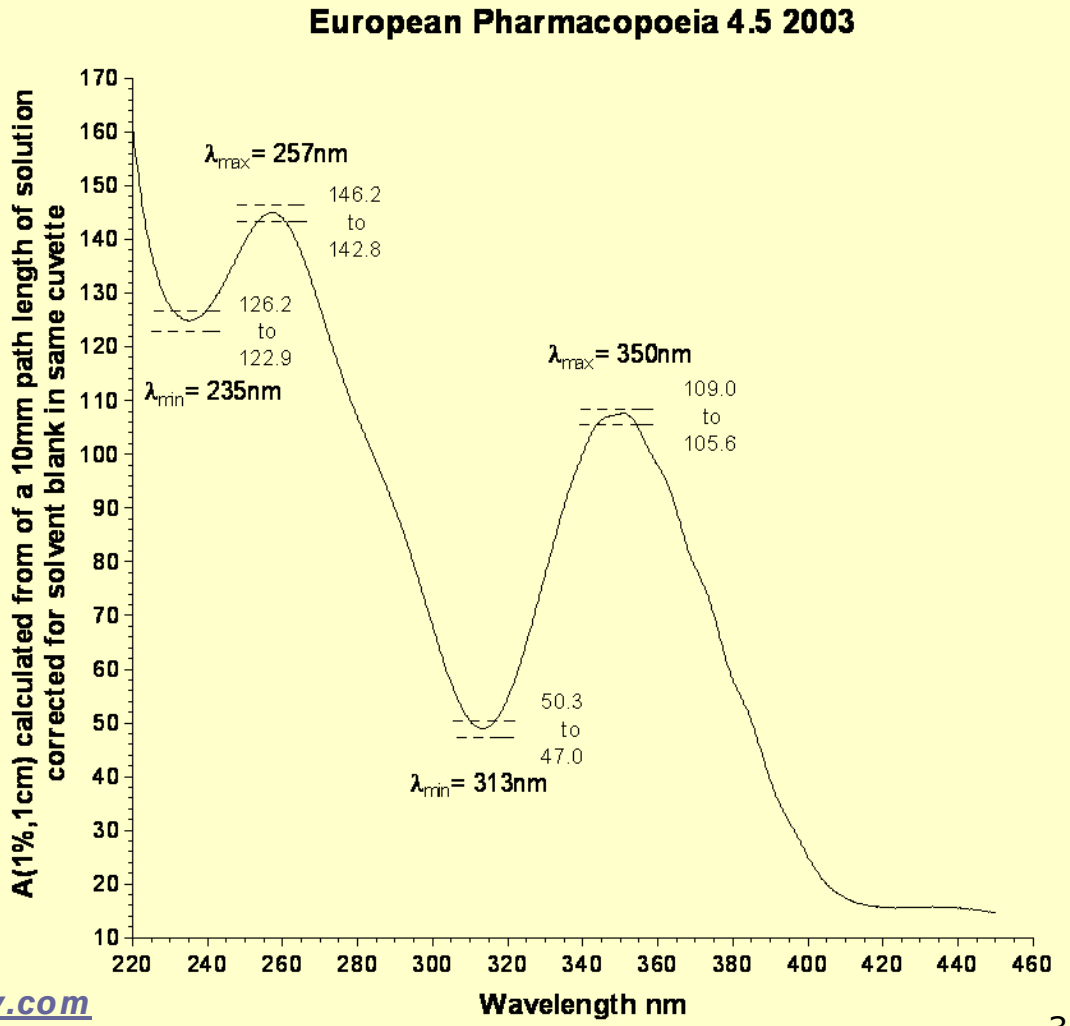
Potassium dichromate

- 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)



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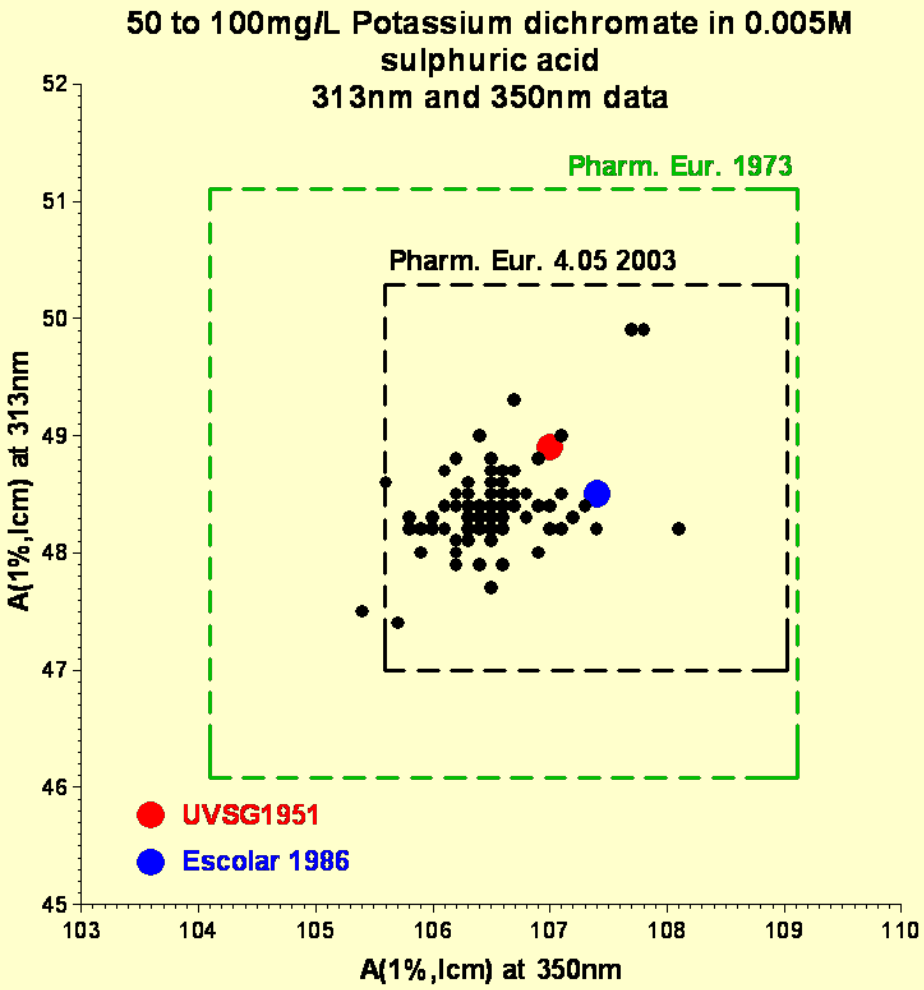
HP 8450 photometric accuracy

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



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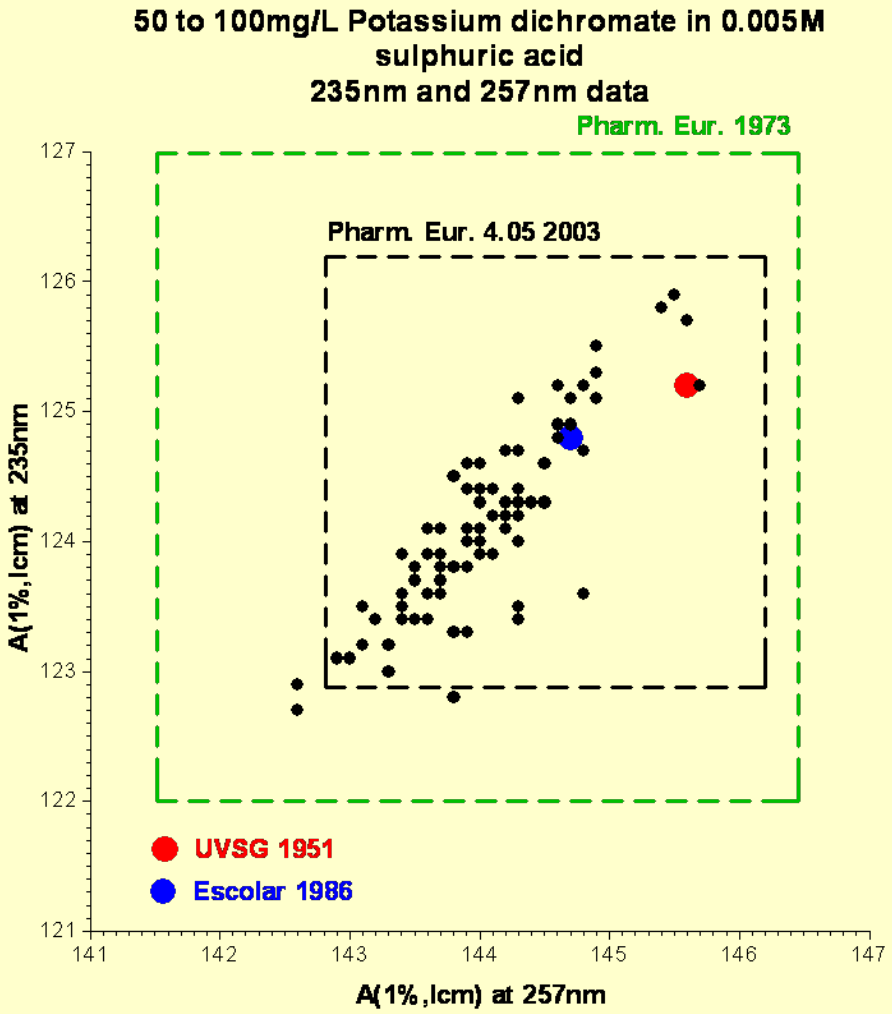
HP 8450 photometric accuracy

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



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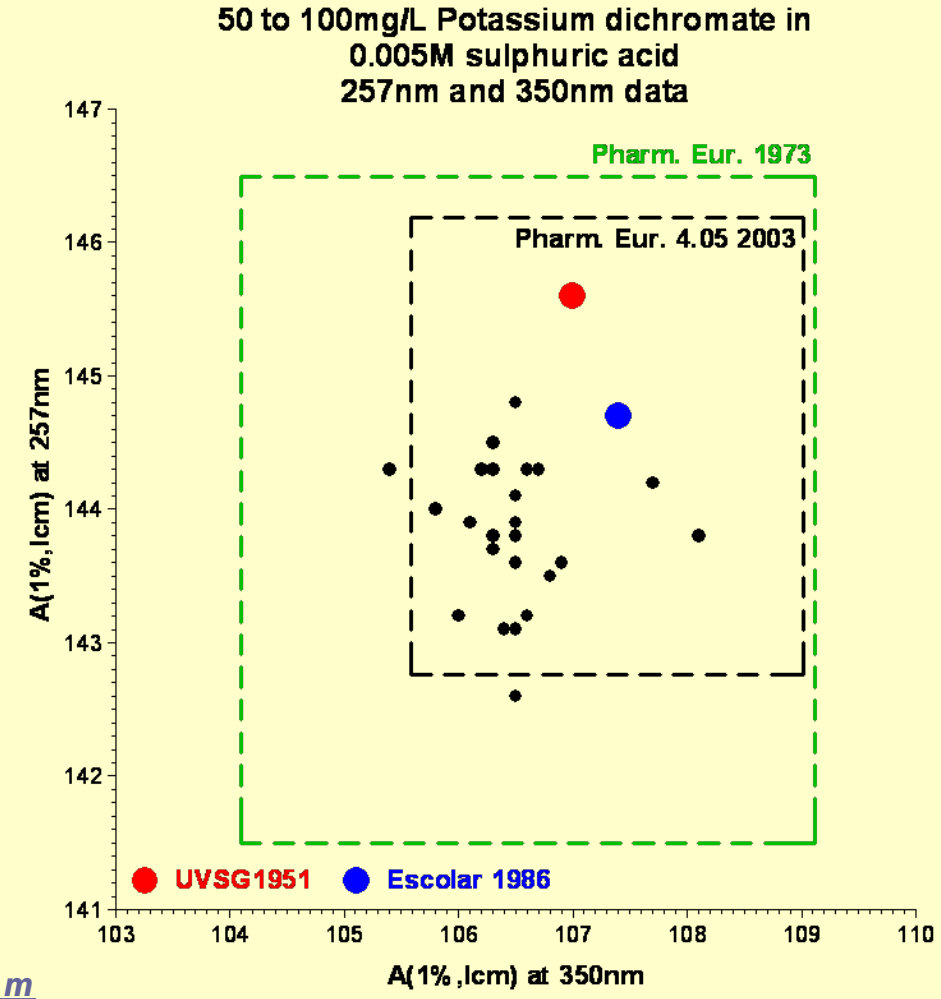
HP 8450 photometric accuracy

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



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Technology transfer standards

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

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Sap Green 1979

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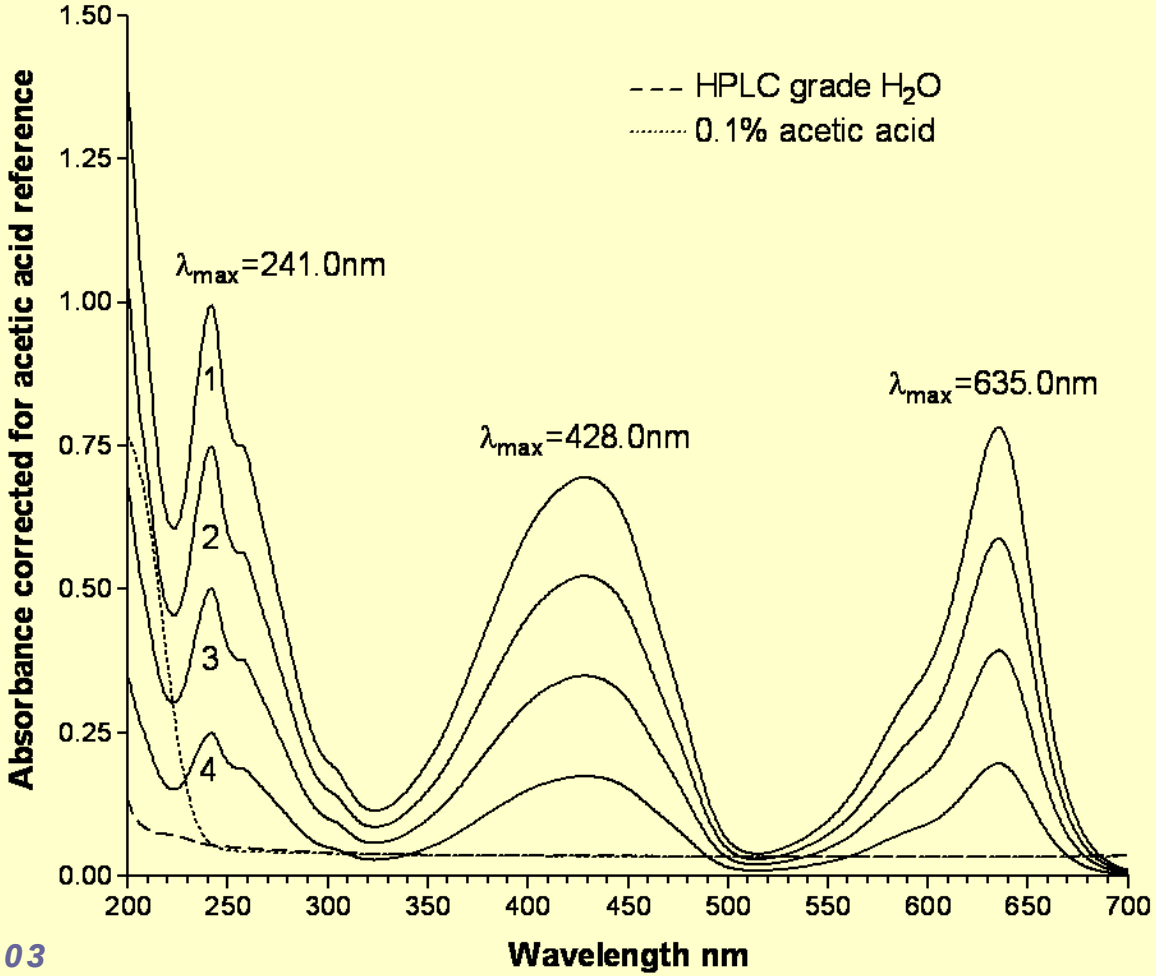
Sap Green Linearity



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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

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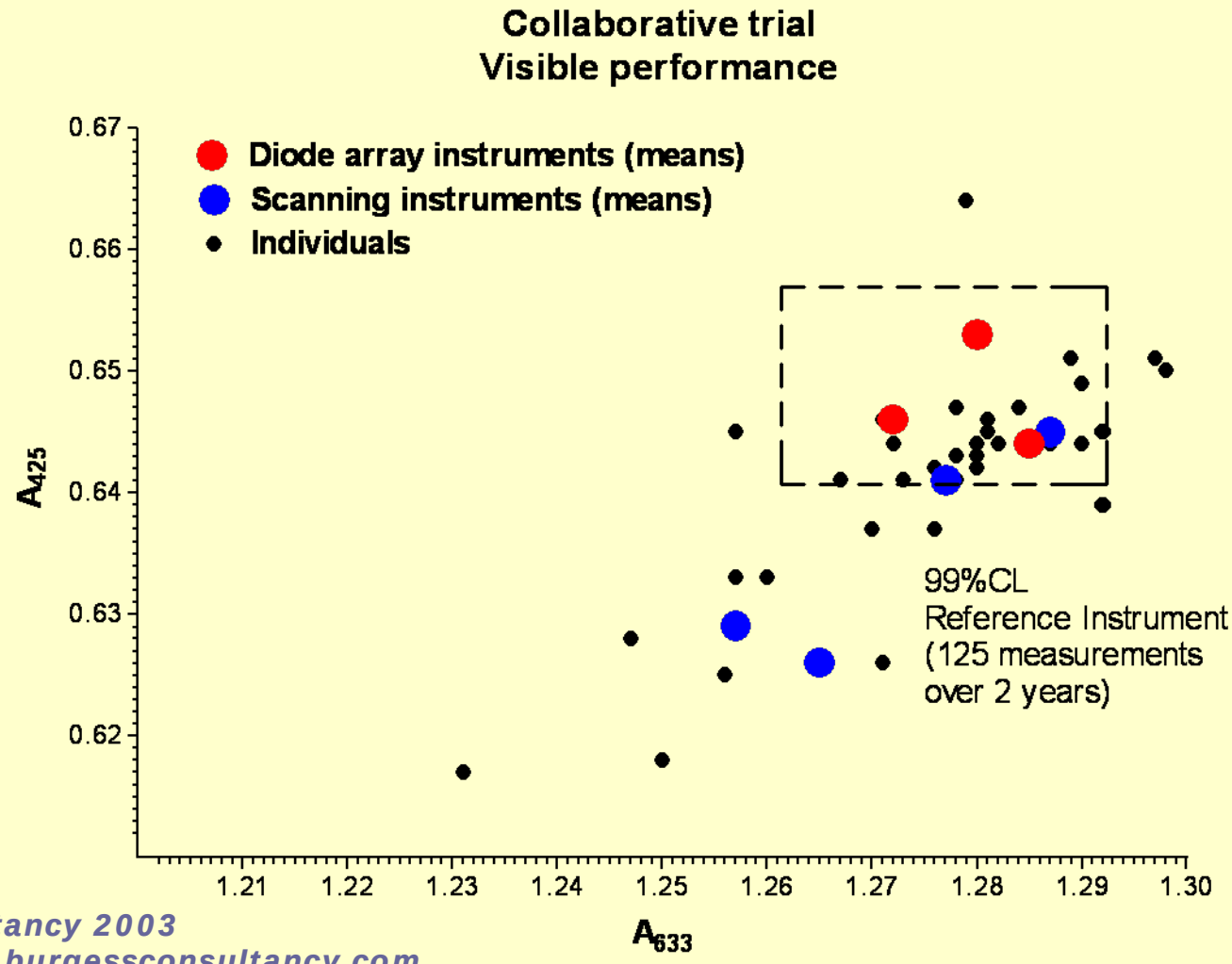
Collaborative trials with Sap Green



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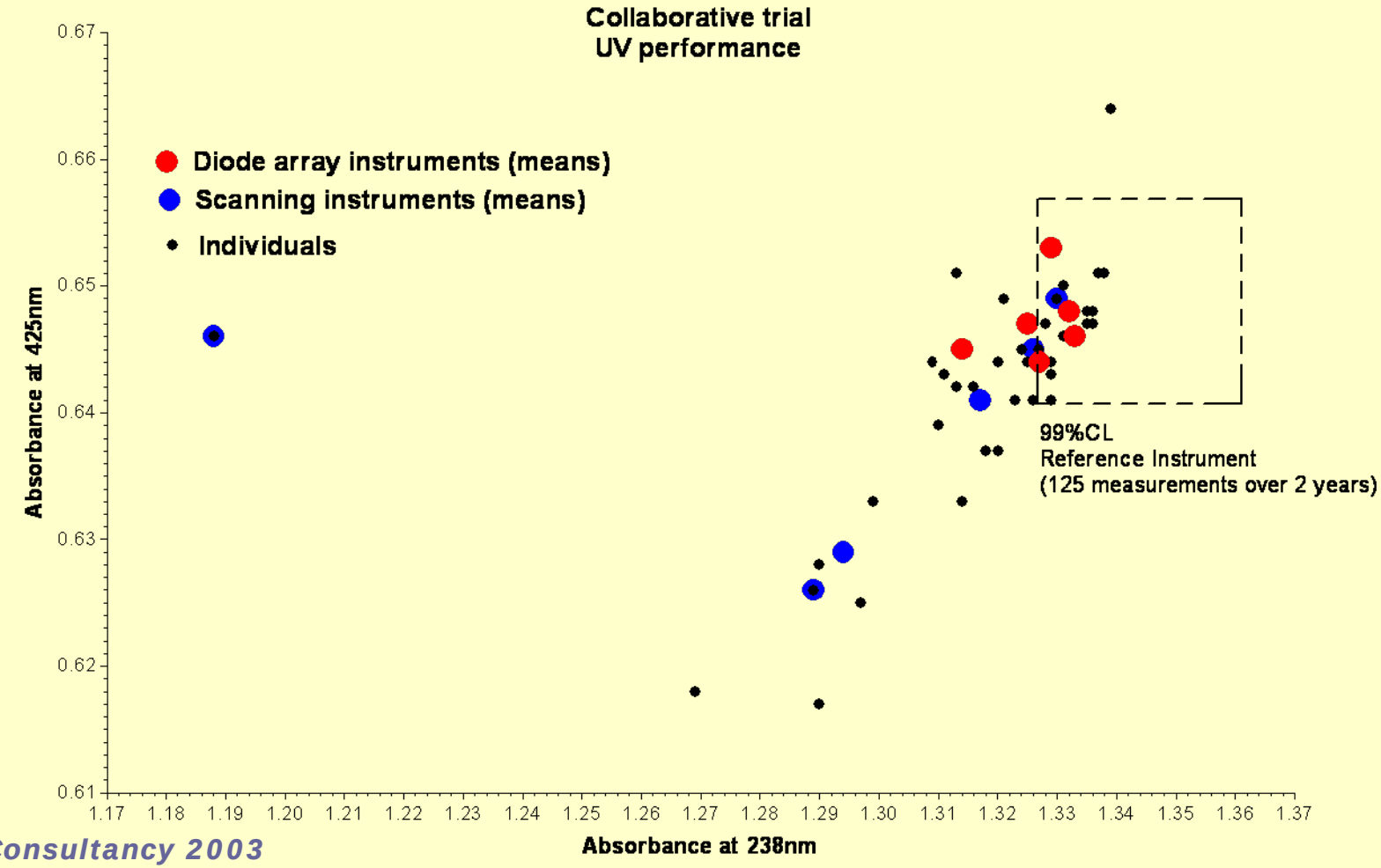
Collaborative trials with Sap Green



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Spectrophotometric methods

The instrument is OK but what about the method?

Optimum conditions

- Best precision
- Best accuracy

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Precision and noise

The relative standard deviation of concentration

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

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
   $s_T = k_3 T$

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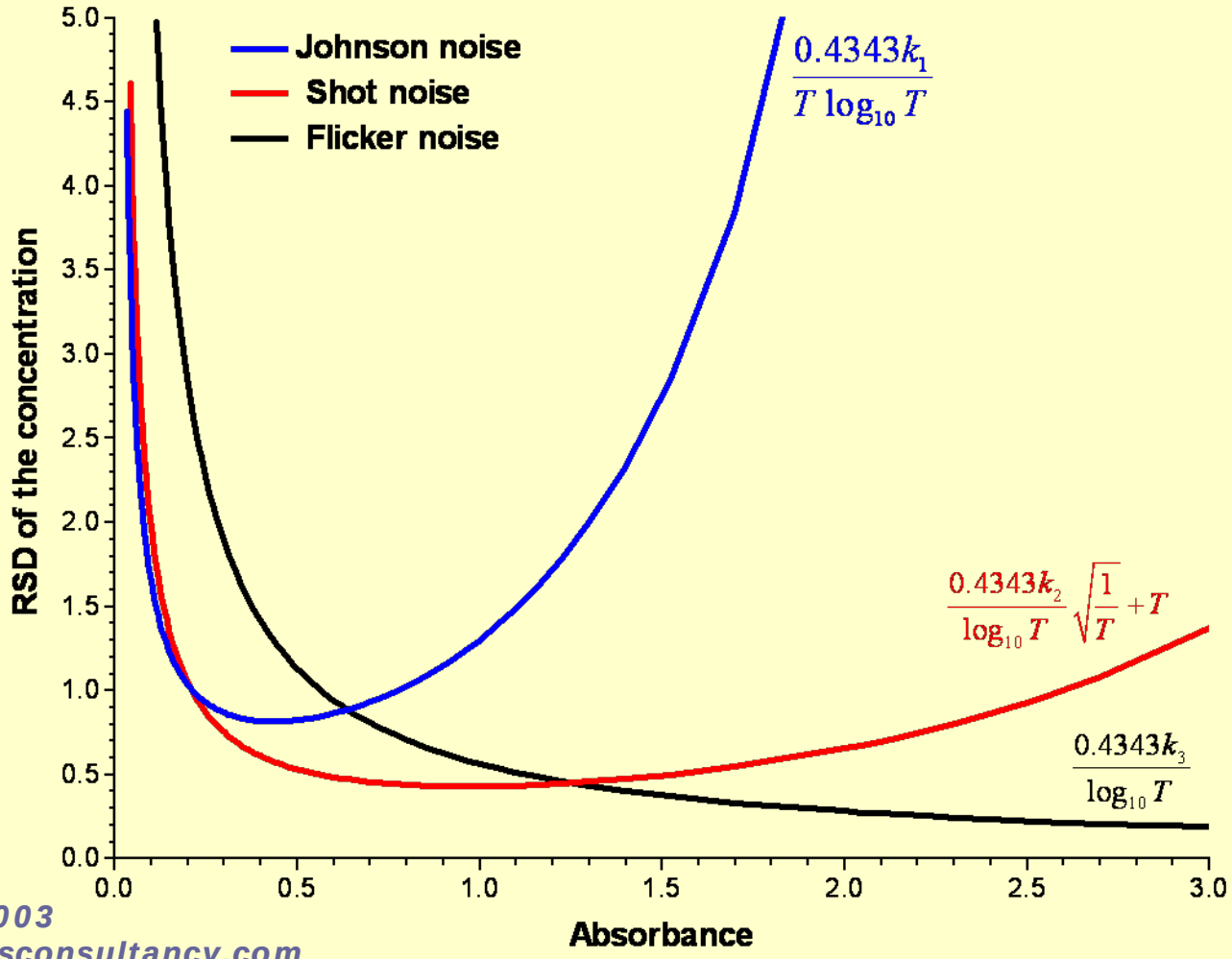
Precision and noise



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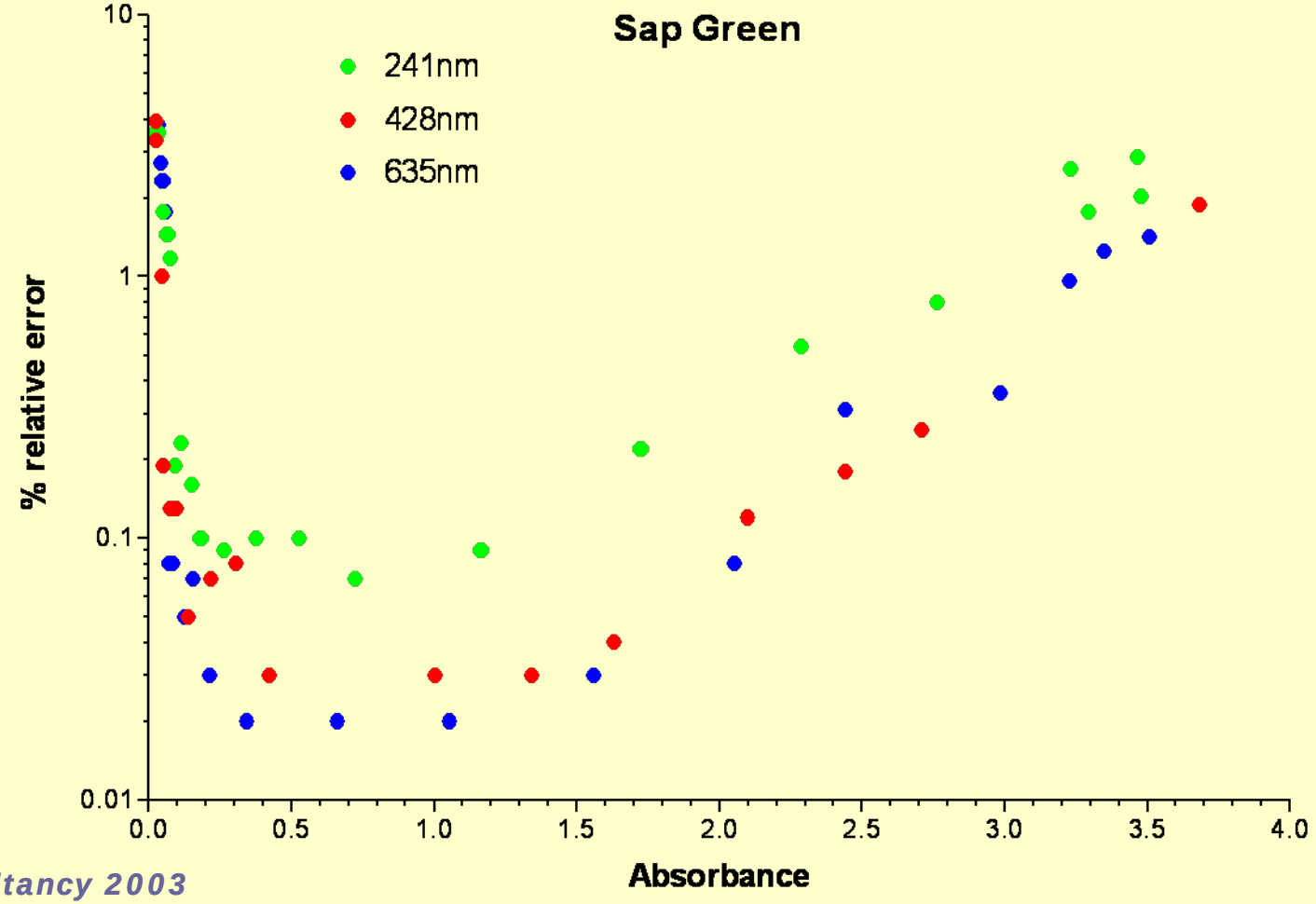
Relative errors;
HP 8450 diode array spectrometer



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Accurate values of absorbance

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

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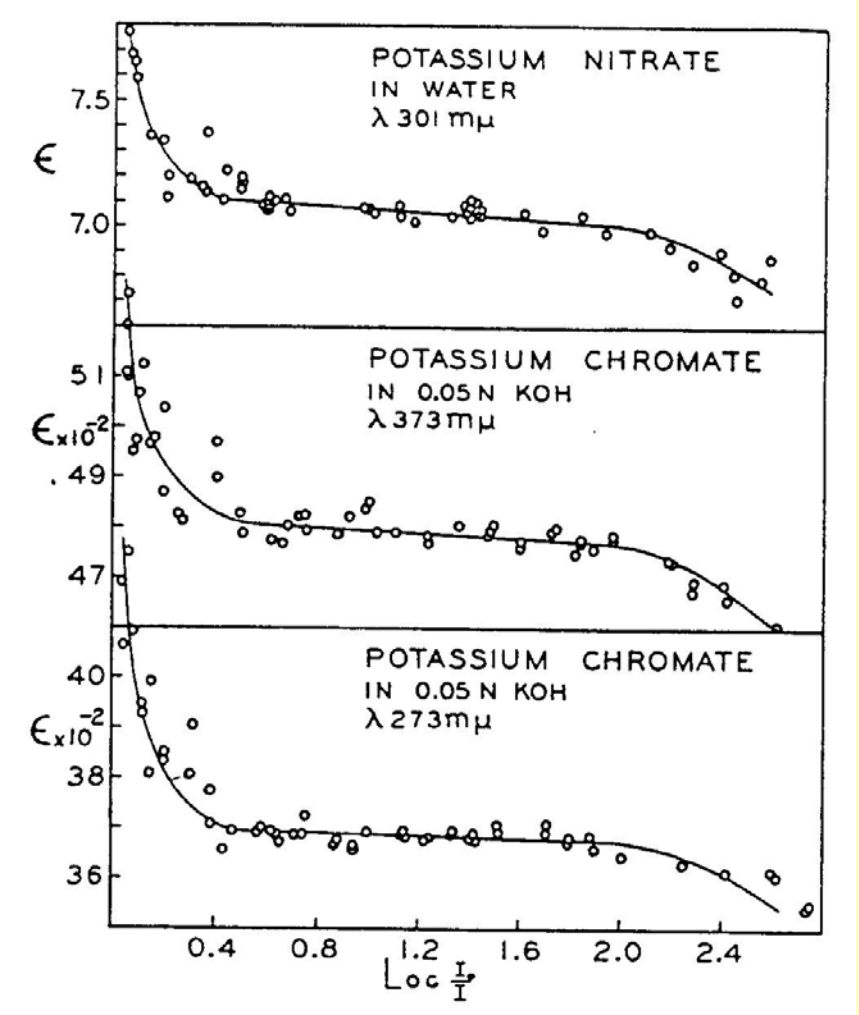
Vandenbelt, Forsyth and Garrett
Ind. Eng. Chem. (Analytical Edition) 1945

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



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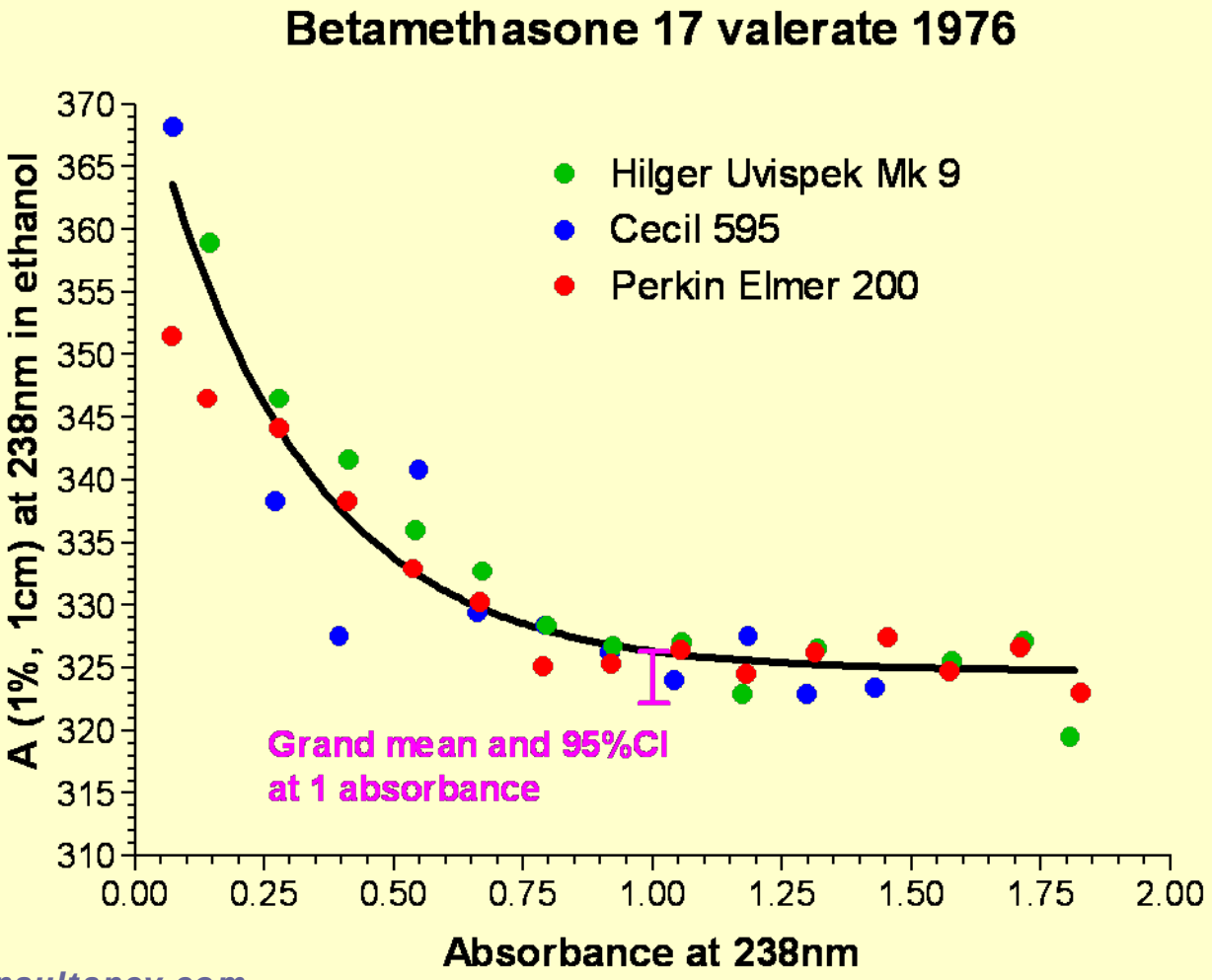
VFG plot



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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…

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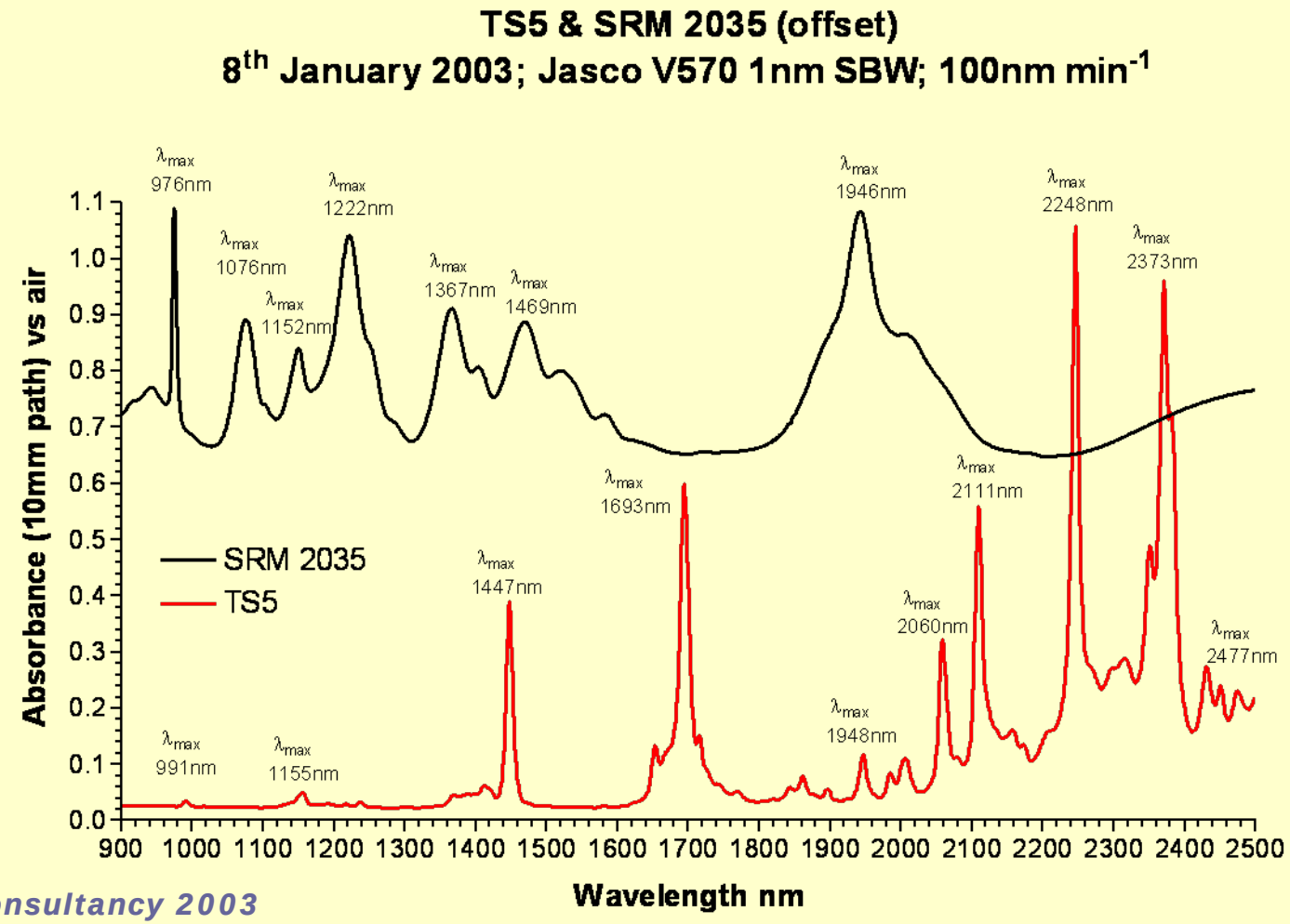
NIR wavelength for the future?



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