Applying UV-vis Spectrophotometry

Qualitative (as discussed)

I) The UV-vis absorption spectrum is related to molecular structure, therefore it provides qualitative information as to structure both from ε and λ. In order to take advantage of this it is desirable to have a scanning instrument to measure the entire spectrum (either a double beam, direct reading instrument or a single beam unit with computer interface.

By itself an electronic spectrum is not sufficient to identify a compound (while an IR spectrum is)

Quantitative

II) The UV-vis region of the spectrum is particularly well suited to quantitative analysis for several reasons (both chemical & spectroscopic reasons):

1) Large values for ε make it possible to measure low concentrations

2) Many compounds (both organic & inorganic) absorb in the UV-vis region, but most common solvents do not. This is a limiting factor in IR.

3) Absorption bands are broad making it easy to achieve conditions where there are no instrumental deviations from Beer's Law

4) Good instrumentation is available, i.e. good sources, detectors & monochromators

III) UV-vis can be used for studying chemical processes that involve conversion of a non-absorber to an absorber or vice versa

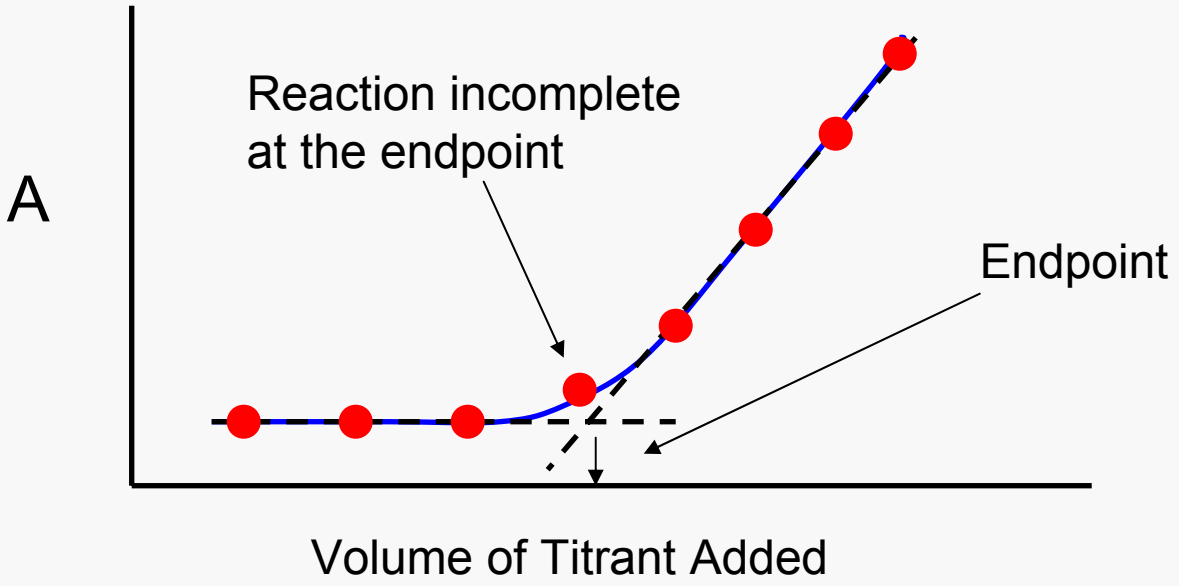
1) Spectrophotometric Titrations

Example where titrant absorbs

Sample + Titrant → Product

absorbs

MnO_4^- + Fe^{2+} → MnO_2 + Fe^{3+}



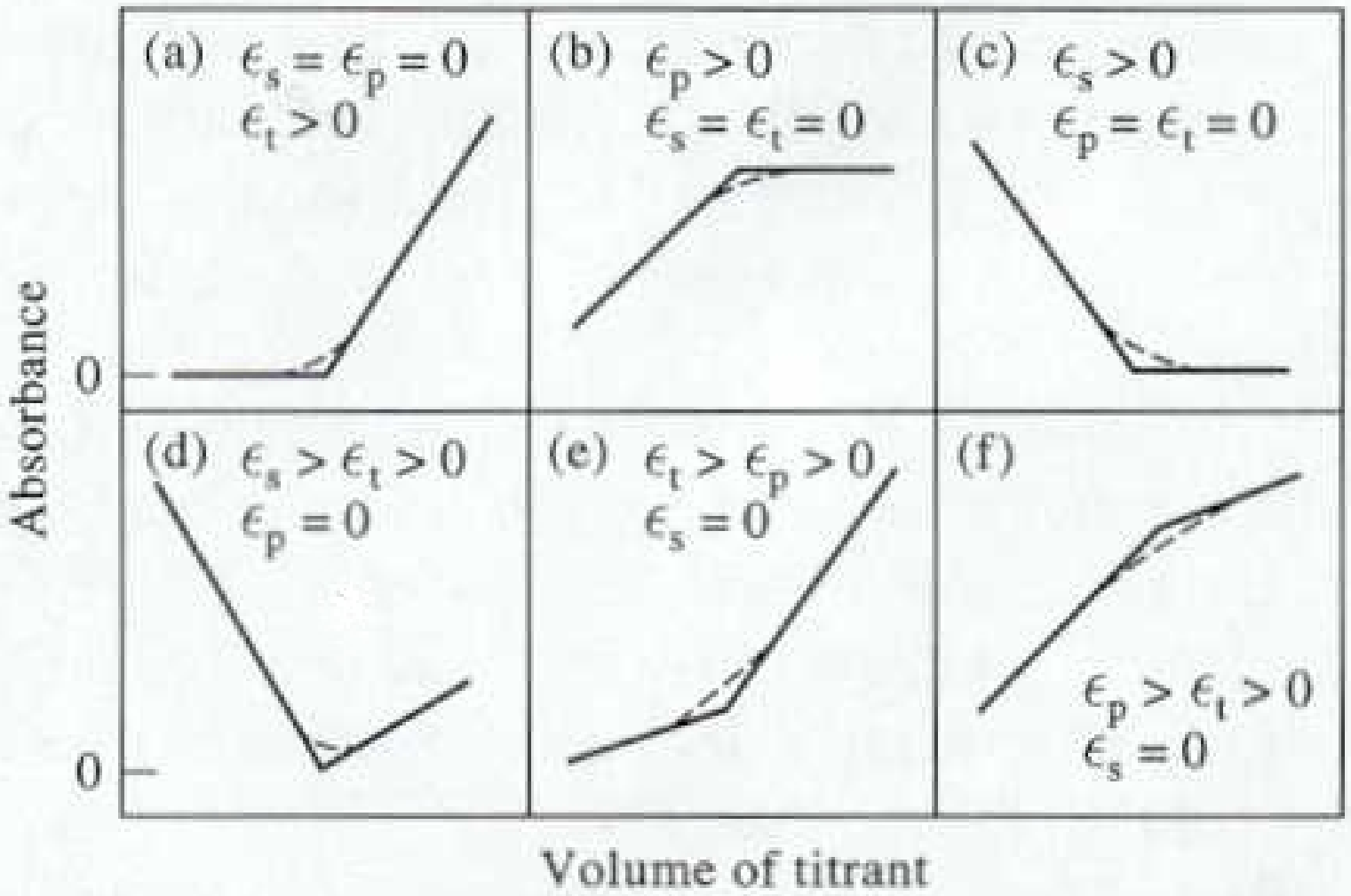


Figure 14-18 Typical photometric titration curves. Molar absorptivities of the substance titrated, the product, and the titrant are given by ϵ_s, ϵ_p, ϵ_t, respectively.

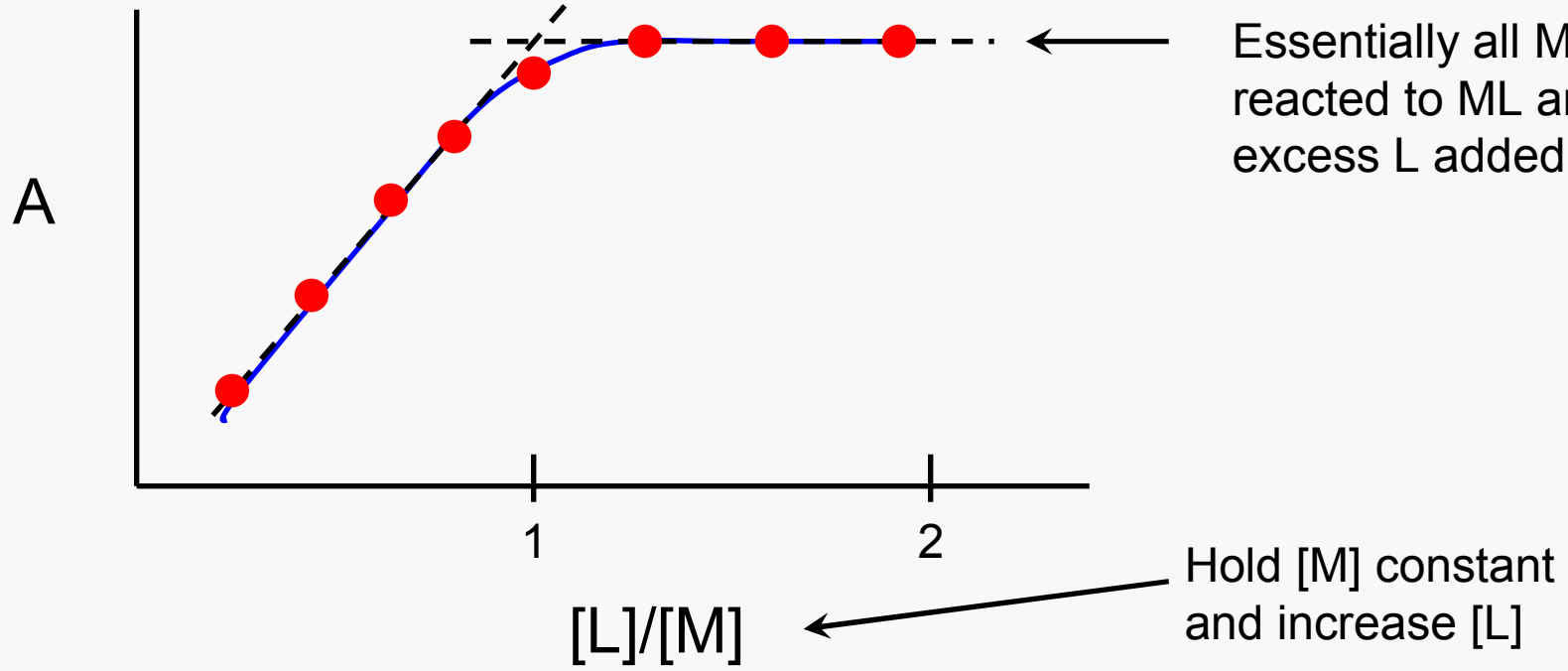
2) Study Equilibria (acid-base, complex formation, redox, etc.)

Reactants don't absorb

$$M + nL \leftrightarrow ML_n$$

Products absorb

$$Fe^{3+} + SCN^{-} \leftrightarrow FeSCN^{2+}$$



Can calculate ε_{ML} from A at excess [L]

Get n from extrapolated curve

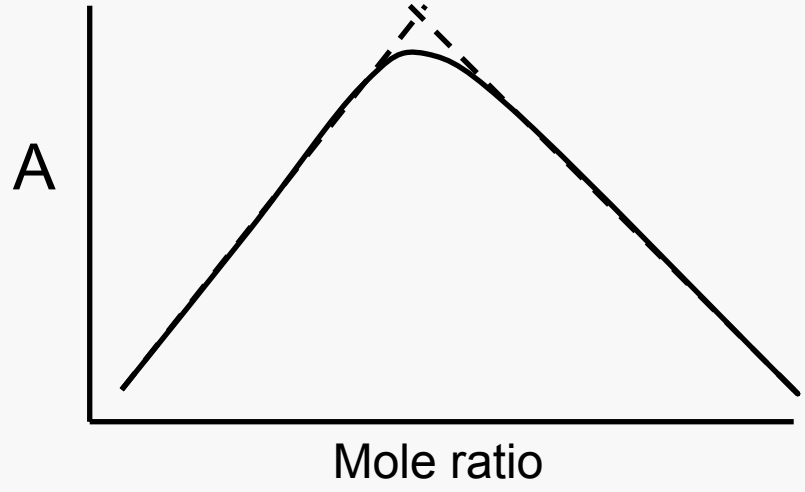
Can calculate equilibrium constant (K) from

$$K = \frac{[ML_n]}{[M]\,[L]^n}$$

and mass balances

e.g. $C_M = [M] + [ML_n]$

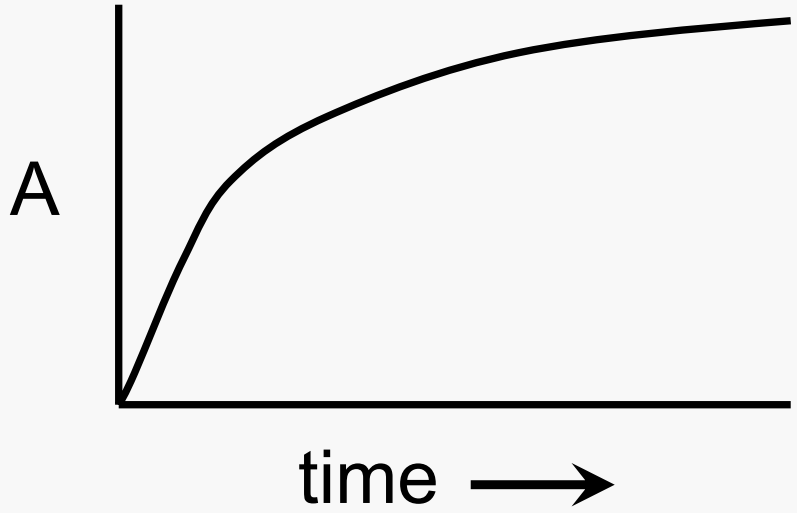
Other calculation methods can be used involving graphical or computer curve fitting procedures, e.g. Job's Method or the Method of Continuous Variation



3) Study Kinetics

absorbs

$X + Y \rightarrow Z$



Measure A vs time after mixing

For quantitative applications, we usually want to measure at one particular λ. Must first scan spectrum to determine the most suitable λ for measurements.

Errors in Photometric Analysis

1) Deviations from Beer's Law - either chemical or instrumental can be handled, in general, by preparing a calibration curve

   Important – the calibration curve should include standards both at higher & lower concentrations than the sample

2) Technique errors – cells must be carefully cleaned & kept free of scratches, fingerprints, etc. Clean in concentrated HNO_3. Rinse several times with water. Rinse with sample before use.

Round cells, e.g. Spectronic 20 cells should be positioned the same way for each measurement because of imperfections in glass (square cells too)

3) Source drift – in single beam instruments.
   Must: a) Allow source sufficient time to warm up
   b) Check 0 and 100 % T settings frequently

4) Stray Radiation – strikes detector without going through sample

$$A_{true} = \log \frac{I_o}{I}$$

$$A_{measured} = \log \frac{I_o + I_s}{I + I_s}$$

where I_s is intensity of stray radiation

Stray Light Specification – important characteristic of monochromators

Stray Light Specification

e.g. Cary 14 $I_s = < 10^{-6} I_o$ at all λ's

Spectronic 505 $I_s = < 10^{-3} I_o$

Stray light more serious at high concentrations (i.e. high A) → makes it difficult to measure high A (i.e. A = 3 or 4) Some instruments are capable of measuring large absorbance

Spec 505

for $A_{true} = 2$

$$A_{measured} = \log \frac{100 + 0.1}{1 + 0.1} = 1.96$$

$$= \log \frac{I_o}{I} = \log \frac{100}{1} = 2$$

$I_s = 10^{-3} \times 100 = 0.1$

5) Reading Error – random error involved in reading A value; today with digital readouts this usually involves:

   1) Not allowing instrument to stabilize before taking the reading
   2) Assuming greater precision (or accuracy) than the instrument is capable of
   3) Biasing the result

   e.g. 0.019 ± 0.002 and 0.021 ± 0.002

   Are these 2 readings different from one another?

   Answer is NO! What about if s.d. is is 0.001?

Scale Expansion – sometimes reading error can be improved upon by expanding scale

Normally use a blank solution to set 100% T & set 0% T with shutter closed (i.e. no light)

Expand scale by using a standard that has (for example) 50% T & set instrument to 100% T → this expands lower end of scale by a factor of 2 & allows readings in the 0% T to 50% T range to be made with greater accuracy & precision

There is a limit to scale expansion imposed by instrument stability, so you can only expand scale to a point, then source and detector noise become limiting factors

Can expand both ends of the scale although it is usually preferable to dilute

Often reading error is not significant so it doesn’t help to expand the scale