

Molar absorptivity

$A = abc$

when $b = 1$ cm and c is expressed in moles/lit ex: 1 mol/lit.

a is called as molar absorptivity constant for given compound at given wavelength, condition temp, pH

ex: molar absorptivity of NADH at 340 nm wavelength = 6220 $\Delta A / \text{min}$ for 1 mole

applications of molar absorptivity

(1) for characterizing compounds

ex: molar abs. of ^{pure} bilirubin at 480 nm is 80,700

absorbance of "soln" containing 5mg/dl (0.005 g/ml) can be calculated mathematically ($c_{\text{molar}} = 884$)

$A = 80,700 \times \frac{0.005}{884} = 0.5$

(2) Comparison of sensitivity of measurements obtained

ex: molar abs. of ferric ion and s-tripyridyl triazine ^{complex} is higher than that of iron o phenanthroline

s-tripyridyl triazine is more sensitive reagent

⑤ Calculatⁿ of calibration constant where calibrator are not available

→ calibration factor of crMB:-

ex molar absorptivity of NADH = 6220 for 1000000 U of crMB enzyme.

$$6220 \Delta A_{\min} = 1000000 U$$

$$1 \Delta A_{\min} = (1) U$$

$$= \frac{1000000}{6220} = 160.7 U$$

$$\text{Now } c = F \cdot \Delta A$$

when $\Delta A = 1$

$$\therefore F = \frac{c}{\Delta A} \times \text{dilut}^n \text{ factor} \quad c \downarrow = \text{Factor}$$

($\Delta A = 1$)

$$\therefore F = c$$

$$\therefore F = 160.7$$

AS 6220 Abs. is not measured by instrⁿ → dilⁿ factor

Now dilutⁿ factor for crMB = 11

$$\therefore \text{final calibrat}^n \text{ factor}^{\text{crMB}} = 160.7 \times 11 = 1768$$

$$\text{crMB} = 2(1768) = 3536$$

From pyruvate
phenanthroline

6220

160.7 x 11

1768 x 2 = 3536

* Applicasⁿ of Beer's law :-

- Calibration relationship b/w A and C for given instrument
- ↓
- using series of reference solution
- ↓
- linear relationship exists upto certain C or A
- ↓
- they obey beer's law at this point
- ↓
- Calibration constant k is derived to calculate C of unknown solⁿ

$$C \propto A$$
$$C = kA \quad (k = \text{calibration constant})$$

→ Precautions to be taken :-

- ① Calibration constant can not be used beyond linear portion of relationship
- ② ≥ 2 calibrations / reference should be included.
- ③ Constant provided in literature can be used when ref. materials are not available
- ↓
- but only if method is followed in details.

ex
NADH absorptivity = 6220 at 340 nm.
can be used as ref. only ↓ controlled

law is followed only in following conditions

- ① Incident radiation \rightarrow monochromatic
- ② Solvent absorption is insignificant compared to solvent absorption
- ③ Solution concentration \rightarrow within limit
- ④ Optical interference \rightarrow absent