Total protein

① Biuret method :-

→ Biuret is a same compound ≅ give same colour. (Biuret also chelates Cu).

→ In strong alkalin cond^n → Cu^+ Binds ~~c~~ 4 peptide Bond
↓
changes on absorp^n from Blue to pusple.
↳ at 540 nm.
powdes

→ Biuret^n forms from Urea.
↓
dry powdes heated into 150° C form biuret

→ interesence By some compound such as Serine, Asparagin, } due to Binding ~~c~~ Cu^{+2}
Tris

→ Cu^{+2} does n't reac^n ~~c~~ A.A & dipeptide.
↓
But some A.A who has min. ~~to~~ 2 NH_2 group → Cu^{+2} reacts.

→ peptide ~~with~~ which have proline.
↓
Bind less to Cu^{+2}

→ some side chain can contribute with Binding ~~c~~ Cu^{+2}

→ diff. protein has same reaction,
↓
Only high conc. of some A.A interfere

→ All End point have interference

Reh.

→ Serum Blank : Reagen cont $CuSO_2$

Only that interference due to which occur alkaline pH ← { addition + serum ↓ absorbance

→ then

do (n) test & then do subtraction.

→ Interference due to λ - light chain

→ Alkaline

↓

Unfolding of protein

method

End point ↙ ↘ Kinatic

↓

→ fast, small reacting molecule interference - remove

disadv: diff. protein denatured at diff. rate

(2) Direct Optical method :-

↓

for chromatographic detection of protein

↓

absorbance at 280nm → due to tryptophan, tyrosin

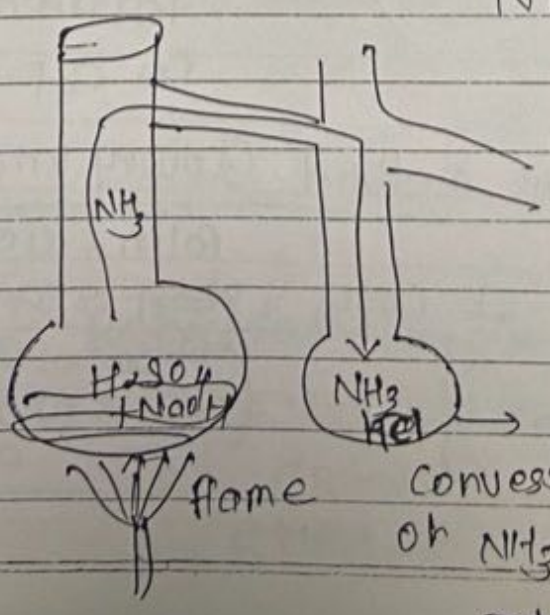
→ More useful for purified protein.

→ Complex mixture → ~~Accuracy~~ & not good method.

→ At 220 to 225 nm
↓
mainly peptide Bond.
↓
10 to 20 times greater sensitivity.
↓
→ 1000 mol. wt protein should be excluded 1st to ↓ interference.

③ kjeldahl method :-
↓
ref. method.

→ Protein $\xrightarrow{H_2SO_4}$ Ammonia
↓
By Nesseles reagent (K Besthelote)
↓
NH_4^+ — Alkanilation → NH_3
↓
Heat
↓
evaporation
↓
dissolved
↓
acid titration



Conversion of $NH_3 \rightarrow NH_4^+$
↓
~~acid~~ titration

↓
When NH_3 added into HCl
↓
colour will change
↓
Then titrate By using HCl
(to take that original colour)
↓
How much HCl is required.
is noted.

→ TP is not Homogeneous
↓ NO SI unit
(Bcz it is compound) Not any ref. standard.

④ Dye Binding method :-
↓
Based on protein error (due to Buffering Capacity or protein)

dye : CBB → Immunoglobulin gives 60%.
pyrogallol red → one of m. common method
↓
to measure protein in CSF & Urine.

⑤ Folin. Ciocalteu method (Lowry method) :-

Folin. ciocalteu
Serum + $CuSO_4$ + ~~Biuret~~ reagent — alkaline medium
(Peptides & protein containing tyrosine & Tryptophan)
(phosphotungustic acid & phosphomolybdic acid)

Cu – peptide contain Tyrosin & tryptophan complex + Tungstun Blue & molybdate Blue

↓

measures at 650 to 750 nm.

↘ forms due to reduction reaction.

⟶ Interference :– due to LMW compounds like tryptophan & Tyrosin

– salicylate } drugs
sulfa }

– phenolic compounds

⟶ If urine containing high compound on phenol ↓

removal of phenolic compound should do before protein estimation.

⑥ Refractometry :

↓

rapid estimate of high conc. of TP. (> 35 g/L)

↓

if conc. ↓ < 35 g/L ⟶ accuracy ↓

⟶ Refractometry is mainly used in lab

↓

To determin conc. of solute in urin

(7) Turbidimetric & Nephalometric method:

use for CSF & urine

↓

protein are aggregated by using various agents like TCA, sulfosalicylic acid

↓

formaⁿ of suspension of uniform Insoluble protein particle

↓

Scattering of incident light.

* Refrence Interval :—

↓

5.5 to 7.5 g/dL

→ plasma contain 0.3 g/dl higher protein than serum

↓

plasma contain fibrinogen & clotting factors.

→ ↑ Protein :— in ~~hemoconcentraⁿ~~
in vomiting,
diarrhoea,
addison's d'se
DKA

→ Hemodilution : water intoxication
Salt retention syndrome
massive IV infusion
recumbent position.