CREATININE

→ Why creatinin is proportional to creatin?

↓

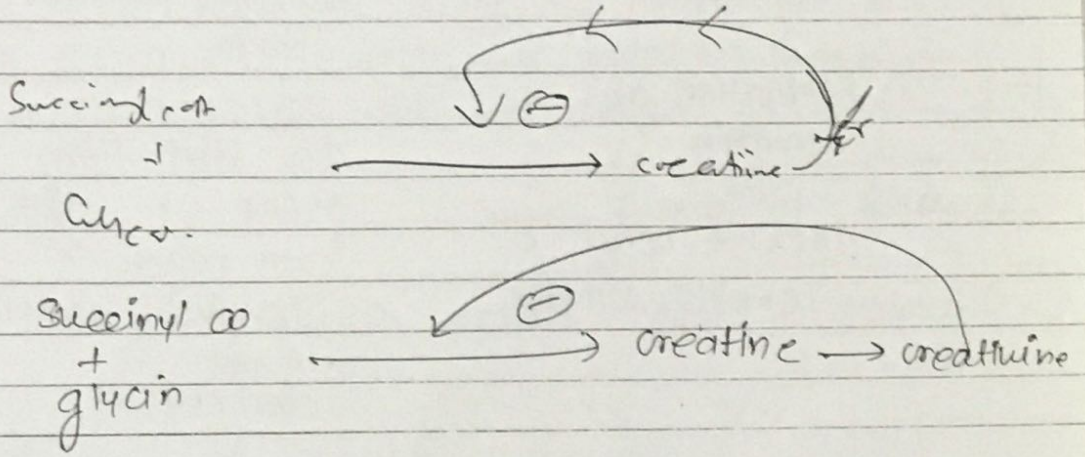
Because creatin ——(Spontaneously converted into)——→ creatinine

→ Creatinin

→ Creatinine conc ↑

↓

- reabsorption to creatinin → creatin
- feedback



* Interfering substance :-

→ Jaffe's reaction occurs in keto group of creatinine (C=O)

⟹ Protein → contain keto group in side chain (mainly but Asparagin, glutamin, Aspartate, glutamate)

⟹ Hb F → resistant to protein denaturation in alkalin pH.

⟹ Pyruvate → Contains C=O

→ keton body → contain C=O

Alkalin denaturation test :—

* differentiate b/w HbF & HbA.

↓

After addition of Alkalin solution

↓ denaturation of HbA

↓ Heam group remove

↓ formation of Hemachin

↓ doesn't react ē Jenovki complex

↓ denaturation of HbF will not occur

↓ Hematin will not form

↓ so ~~HbF~~ Hem group of HbF can react ē Jenovski complex

↓ destroy it

↓ -ve interference

* How will you remove hemolysis ?

↓ add NaOH to solution

↓ remain it for some time.

↓ then add R_2 of Cn.

* Uric
* Diabetic → acetoacetate → keton bodies → +ve interference

* Borate phosphate
Bilirubin → destroy jenovski complex.
-ve interference :→ Removal method :—

→ ① Borate
phosphate } in Buffer (2)

→ ② Incuba^{n} in NaOH → Oxida^{n} of Bil. → (3)
→ ③ Fuller's earth → isolate creatinin
→ ④ K^{a} ferricyanid → oxida^{n} of Bil. → (1)

Causes forma^{n} of Bilivesdin
↓
Bilivesdin doesn't react ē jenovski complex

* ~~⑤~~ Borate → can binds ē glucose → reduces interference of glucose

* ~~⑥~~ EDTA → reduce protein interference by binding ē protein

* ~~⑦~~ Acid Blanking :—

→ can be done By Rate B in ous machine
→

⑧ Cation exchange resine can be used

⑨ kinetic measure (fixed point kinetic)

1st 20 sec → acetoacetate

after 60 sec → Protein

So take b/w 20-80 sec mainly due to creatinin. mainly.

✱ Saturated picrate : 60 mmol/L

→ picrate is in alkaline pH is orange in colour

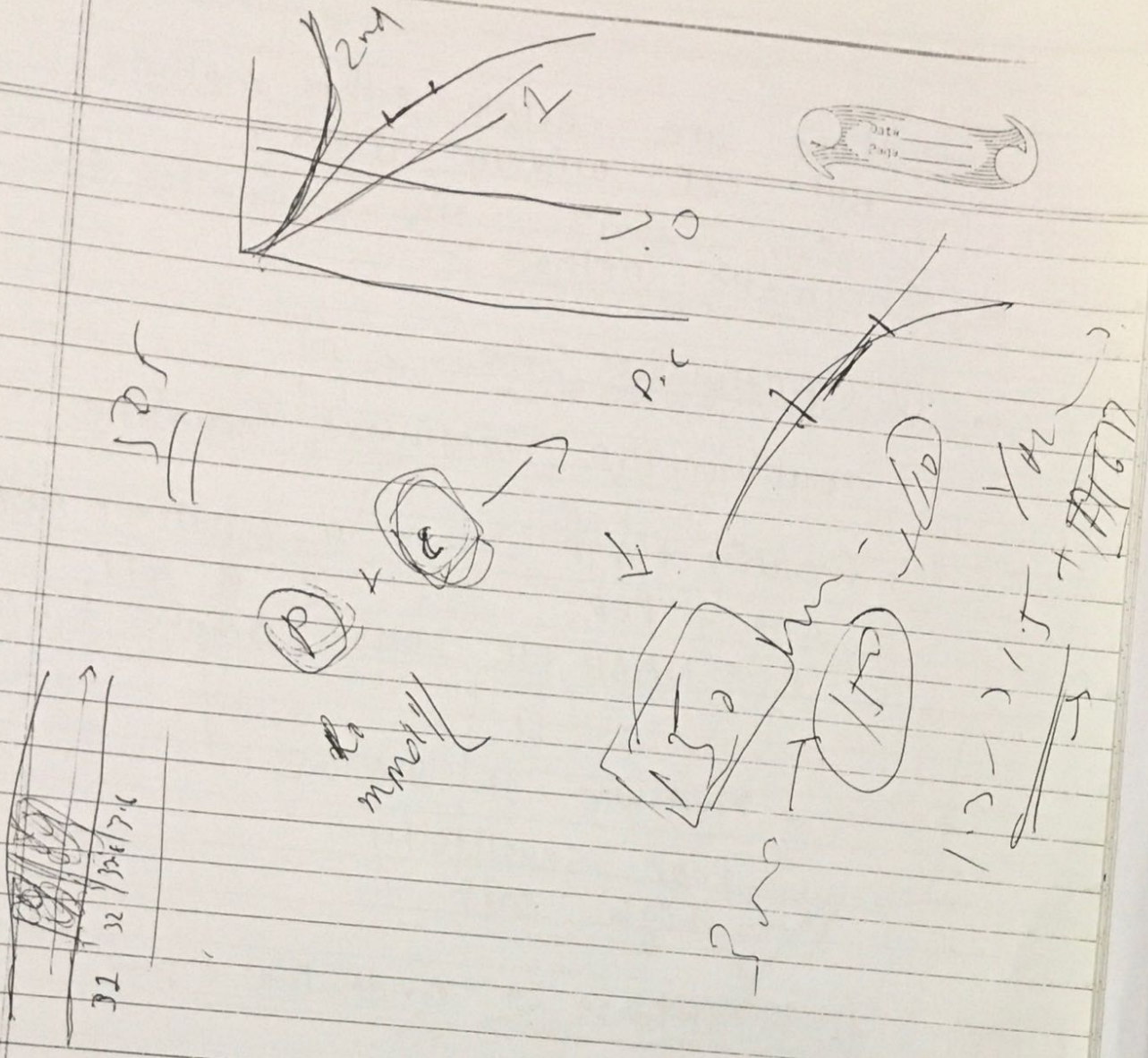
So Blank ↑ if NaOH is w'qu

→ Peak wavelength : 490 to 500 nm
we use → 505 nm

→ Temp :- 25°C & 30°C

→ Jenovskin complex is used to measure the temperature on cuvette

Jenovski complex's O.D s are already measure ī pertiular temp → then add complex sol. → ~~da~~, measure O.D → then can say tem of ~~the~~



* Compensation method :-

→ Fix. amount is deducted from result to improve the result

↓

make result neares to reference method

↓

But all time → all sample doesn't have same chromogen

→ these are certain other method But not widely used.

* Enzymatic method :-

① Creatininase :-

Creatinine $+H_2O$ $\xrightarrow{\text{creatinase}}$ creatine

Creatin + ATP $\longrightarrow$ P_ creatin + ADP

ADP + PEP $\longrightarrow$ P + ATP

P + NADH + H $\xrightarrow{LDH}$ Lactate + NAD^+

↓

- measure at 340 nm
- poor sensitivity
- high cost

② Creatinase + creatininase :-

→ Trinder's reaction

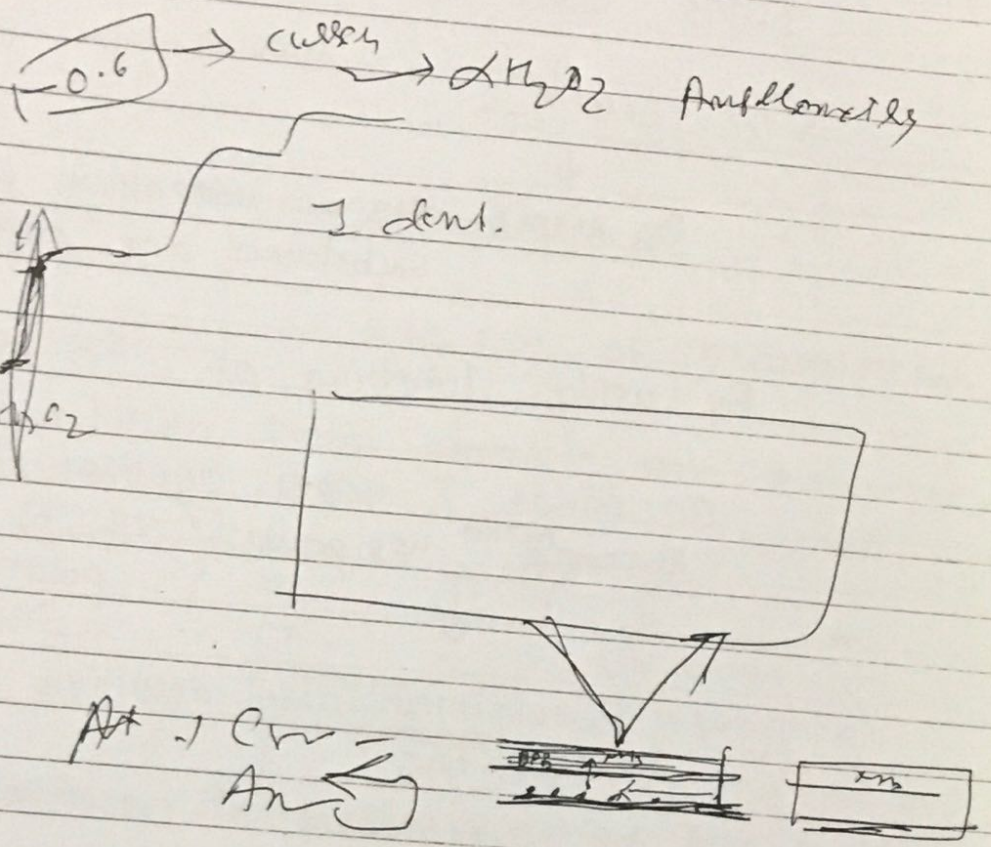
→ Interference of Bilirubin & H_2O_2

↓

overcome by pot. ferricyanide.

→ Ascorbate also interfere & its removed by ascorbate oxidase

→ Bilirubin & Vita. C Binds ∑ H_2O_2



(3) (c) Creatinine deaminase
↓
Causes formation methylhydantoin → several step →
Produce H_2O_2 → By Trinders reac^n → H_2O_2 ~~formation~~
utilized

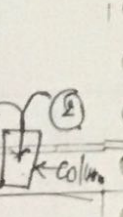
(4) (d) dry chemistry.

* definative method :- IDMS

Internal calibrator :- ~~C14~~ Cr in C C^{14} is added to the sample itself.

Isocratic :- only 1 mobile phase

Bicratic :- Both mobile phase injected Seposately → reqwires 2 pumps



↓

Mass spectrometry

↓

ratio b/w sample cr & cr c̄ C^{14}.

IDms used for small molecules. — cr, URE

→ GC-IDMS :→

↓

By vaporization → creatinin & Non creatinine substances are seperated

By using labelling of ^{13}C or creatinine

↓

cr Binds c̄ some cinothes compounds & make nonpolar (as (h) cr is polar)

↓

Butyldimethylsilyl desvitive is used for GC-MS

↓

attached c̄ cr. (for desivatization)

↔ Sample → cation exchange

↓

GC

↓

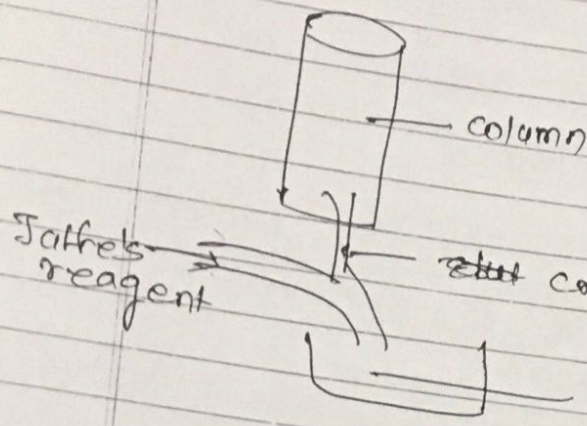
ms

→ GC can be replaced By LC

↓

protein precipitation on sample done before LC

→ HPLC → On stream Jaffe's method.



Elution from sample can be measured By

- Enzymatic
- On-stream Jaffe's
- UV rays absorbance.

→ designated method for manufacture

↓

HPLC

→ 2° Ref. method → given to manufacture

Quality issue for creatinine estimation

→ In Jaffe's method → nonchromogen interference

↓

alternative → enzymatic method

↓

costly

→ interfering compound

↓

glucose, Bilirubin, protein, keton Bdy

→ some also interferes in enzymetic

↓

Bilirubin → oxidase H_2O_2 → produce Bilivesdin

→ Till now enzymatic method → not that much useful.

→ delayed sepeation of serum from cells

↓

release of noncr. chromogen from RBC → interfere in jaffe's reac^n

→ ↑ cr after feeding → if cooked meat is eaten → B'cz contain creatine → converted creatinine → interfere in Jaffe's reaction,

→ Jaffe's method → obtain more 20% of than (n) creatinine → false high result.

→ Jaffe's gives upto 0.3 mg/dl (more) in (n) pesson.

→ Interlab. comparision variation → due to diff. method used by lab.

↓

manufactures diff. b'cz diff. in calibration

↳ see ufah