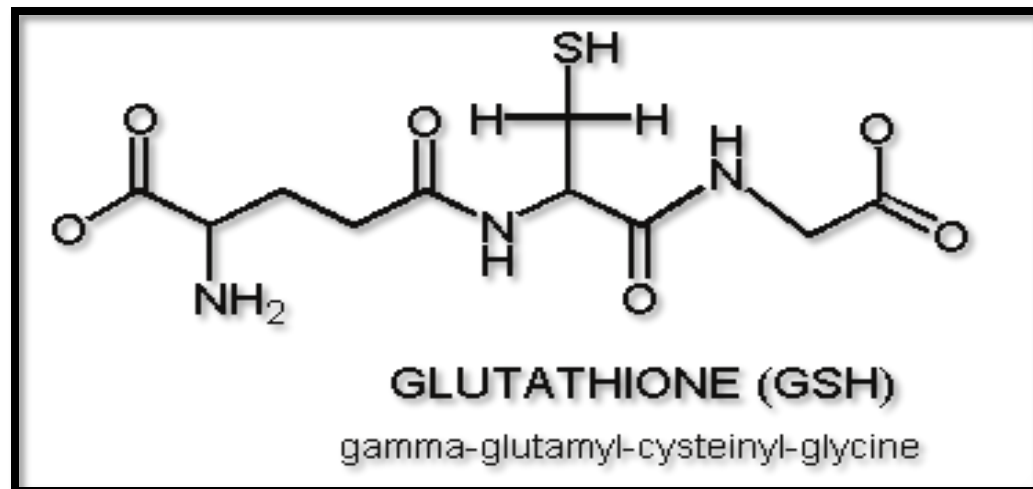




BCH 447

Estimation of Glutathione in Plasma



- Objectives:

- ✓ To draw the standard curve of glutathione by given known amount of glutathione assay procedure using spectrophotometric technique.
- ✓ To estimate the amount of glutathione in plasma sample.

Glutathione

- It occurs naturally as a **tripeptid** (gamma-**glutamylcysteinylglycine**)
- it has two form : Reduced form(GSH) and Oxidized form (GSSG)
- It has nucleophilic and reducing properties, those properties **due to the presence of Sulfahydryl group or thiol group (-SH).**

****Nucleophelic,** means GSH will loss the H ion then become nucleophilic (contain negative charge) can react with the compound which carrying positive charge.

****reducing ,** means it will give the other protein the H ion to maintain them in reducing form.

Functions of Glutathione

➤ It acts as anti-oxidant , blood booster , and cell detoxifier in the body.

GSH is crucial to a variety of life processes, including

**Amino acid
transport
across
membrane**

**Maintenance
of the SH
level of
proteins**

**detoxification
of xenobiotics
such as drugs**

**Removal of
hydroperoxide
and free
radicals.**

**Coenzyme with
glutathione
peroxidase,
glutathione S-
transferase and
thiol
transferase.**

- Physiological values of intracellular GSH generally range **from 1 to 10 mM**.
- Glutathione deficiencies have been linked to many forms of **cancer**.

Assay Principle

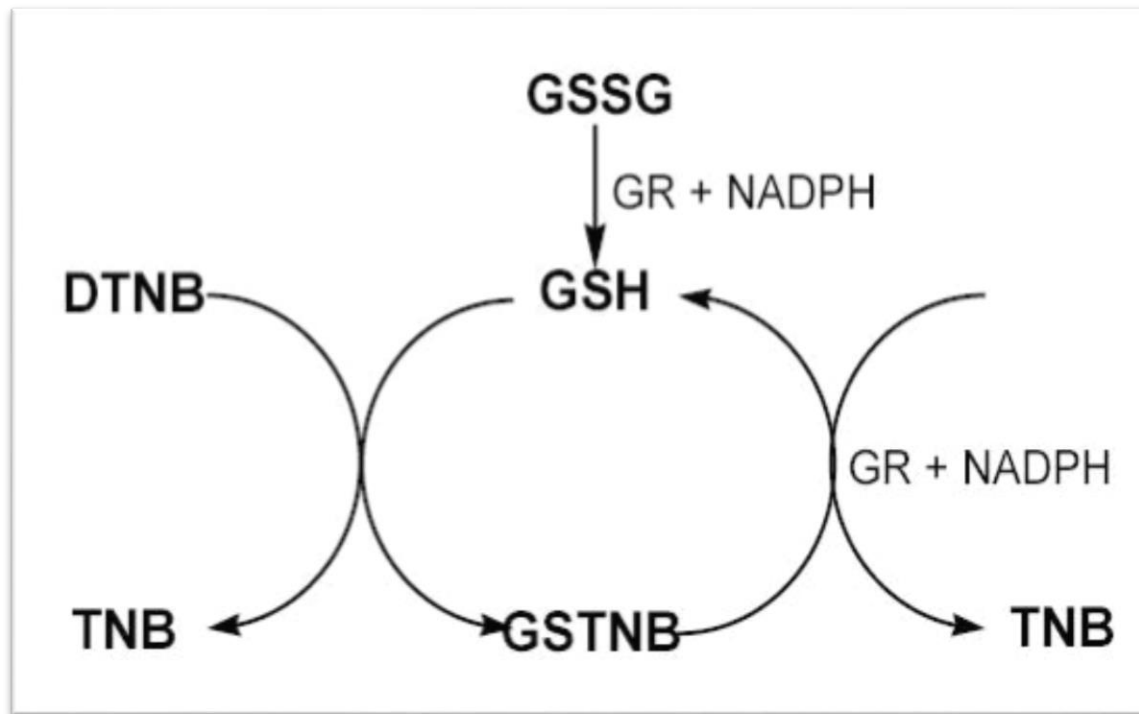
- The principle of the assay is based on **the oxidation of the reduced form of glutathione (GSH)** by the aromatic disulphide compound [5,5-dithiobis-2-nitrobenzoic acid (DTNB)] to form GS-TNB and the aromatic thiol, 5thio-2nitrobenzoic acid (TNB).



- Yellow color is formed.** It can be measured **at 412nm** and is proportional to the amount of glutathione present in the sample.

Note : This method is also useful to determine the GSSG.

First , GSSG will be converted to GSH by glutathione reductase (GR) and NADPH assay then it will react with DTNB reagent



- Method:

1- Prepare serial GSH concentration, as the following table:

Tube No.	GSH stock Solution (ml)	Phosphate Solution (ml)	Total Volume (ml)	GSH Conc (mg/dl)
Blank	0	3	3	0
1	0.6	2.4	3	2
2	1.2	1.8	3	4
3	1.8	1.2	3	6
4	2.4	0.6	3	8
5	3	0	3	10

2- For standard curve (Tubes from blank to 5)

In a separate test tube, take 0.5 ml of solution+ 2ml of Phosphate Solution + 0.25ml DTNB+ 0.25ml H₂O.

3- For Sample: Take 0.3 ml of sample +2ml of Phosphate Solution + 0.25ml DTNB+ 0.45ml H₂O.

****Incubate all tubes for 10min at 37°C→ Read absorbance at 412 nm ****

- Results:

Tube No.	GSH concentration ($\mu\text{g/ml}$)	Absorbance At 412 nm
1	20	
2	40	
3	60	
4	80	
5	100	
Blank	0	
Sample	From Sandard Curve	

- How to convert concentration unit from mg/dl to $\mu\text{g/ml}$?
- 2 mg/dl to $\mu\text{g/ml}$ \longrightarrow $2 \times 1000/100 = 20 \mu\text{g/ml}$

Calculations:

- **Plot the standard curve** and determine **glutathione concentration from the graph** expressed as $\mu\text{g/ml}$.
- **Calculate the glutathione concentration in Total volume of extracted plasma (= 3 ml).**
 - **Example:**
 $0.3 \text{ ml of sample} \rightarrow 0.2 \mu\text{g}$
 $3 \text{ ml of plasma} \rightarrow ?$
- **Calculate glutathione concentration in mg by converting μg to mg.**

Normal range of glutathione concentration = 3.344-4.84 mg