Assay of Urease activity

Aim: To assay the urease activity

Reaction: The enzyme splits urea liberating NH_3 and CO_2 as per the following reaction.

Principle:

The reaction is stoichiometric the enzyme activity is easily determined by measuring the amount formed this can be observed colorimetrically. Since NH_3 forms a brown complex in presence of Neissler's reagent (K_2 Hg I).

Reagents:

1. 0.66N H2SO4 (N=36) [calculated value is 1.83ml / 100ml distilled water]
2. 3% Urea
3. 1M Na tungastate [16.493 gms/50ml]
4. 0.2M Phosphate buffer (pH = 7)
5. **Stock standard:** Dissolve 20mgs of NH_4 $(SO_4)_2$ in 100ml of distilled water
6. **Nessler's reagent**

Standard Graph:

A pure ammonium sulphate solution (20mg/100ml) is prepared and different aliquots of it are taken and the volume is made up to 3ml with distilled water. To this 1ml of Nessler's reagent is added. After mixing the color intensity is measured at 500nm. A standard graph is drowning in the usual way.

Enzyme source:

Horse gram seeds contain high concentration of the enzyme. The seeds are powdered in pestle and motor and about 1gm of finely grinded powder is suspended in 100ml of distilled water and stirred well. This suspension is filtered through a coarse cloth and filtrate is used as the enzyme source.

Enzyme Assay:

Pipette out 1ml of substrate solution i.e., 3% Urea solution buffered with 1ml of 0.2M phosphate-buffer (pH 7). Add 1ml of enzyme extract and incubate at 55^0C for 15 minutes. At the end of incubation time quickly place the tubes in the ice. Add 1ml of 0.66N H_2SO_4 to stop the reaction and 1ml of 1M sodium tungastate solution to precipitate the protein. Filter or centrifuge to remove the precipitate and aliquots of supernant are assayed for NH_3 and the enzyme activity is calculated.

Result:

The amount of Ammonia present in the given unknown sample is _____mg of Ammonim (NH_3) formed / 1 ml of enzyme / 15 minutes.

S.No.	Volume of Ammonium Sulphate (ml)	Concentration (µg)	Volume of Distilled water (ml)	Nessler's reagent (ml)	500
1	BLANK	0	3	1.0	
2	0.2	40	2.8	1.0	
3	0.4	80	2.6	1.0	
4.	0.6	120	2.4	1.0	
5	0.8	160	2.2	1.0	
6	1.0	200	2.0	1.0	
7	TEST	-	-	1.0	

Calculation:

Value of supernatant = _____ml

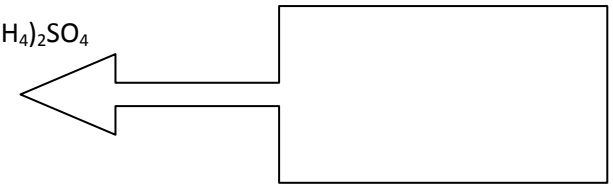
Concentration of Test = ___ µg

OD of Test – OD of Blank = _____

1ml of filtrate 5ml of filtrate

____ml of filtrate → _____ µg of $(NH_4)_2SO_4$

_____ml of filtrate → ?



_____µg of ammonia formed / 1ml enzyme / 15minutes

_____mg of NH3 formed / 1ml of enzyme / 15 minutes