

Urea Determination

Reagents:

1. PMA (Phenylmetric acetate): Dissolve 50 g of Phenylmetric acetate in 1000mL of deionized water.
2. KCl-PMA: Dissolve 1500 g KCl in 9000 ml of DT water and then add 1 L of PMA.
3. TSC (Thiosemicarbazide): 0.25 g of TSC in 100 ml DI water
4. DAM (Diacetyl monoxime): 2.5 g of DAM in 100 ml DI water.
5. Acid reagent: 300 ml of 85% of phosphoric acid and 10 ml of H₂SO₄ dilute to 500 ml with DI water.
6. Color reagent: pipette 25 ml DAM and 10 ml TSC and dilute to 500 ml with acid reagent.
7. Standard Urea-N solution (100 ug/ml): dissolve 0.4288 g of pure, dye urea in 2M KCL-PMA solution, dilute the solution to 2000 ml volumetric flask with 2 M KCl-PMA and mix, store in refrigerator.

Procedure:

1. Pipette 2 ml solution in a 50 ml volumetric flask.
2. Add 8 ml 2 M KCl-PMA, bring volume to 10 ml totally.
3. Add 30 ml color reagent and mix
4. Place them in boiling water, after 27 min, cool it immediately for 15 min in running water.
5. Make the solution to 50 ml.
6. Measure absorbance at 527 nm.

Standard curve:

Pipette 0, 0.25, 0.75, 1.0, 1.25 ml of 100 pm urea-N to 50 ml volumetric flask, make the solution to 10 ml with KCl-PMA, add 30 ml color reagent. Then proceed as with the samples.