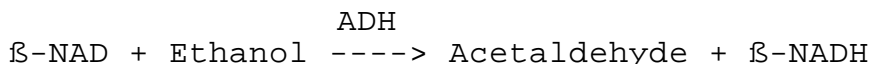


**Determination of Concentration and Molecular Weight of
β-NICOTINAMIDE ADENINE DINUCLEOTIDE**

PRINCIPLE:



Abbreviations used:

β-NAD = β-Nicotinamide Adenine Dinucleotide, Oxidized Form

ADH = Alcohol Dehydrogenase

β-NADH = β-Nicotinamide Adenine Dinucleotide, Reduced Form

CONDITIONS: T = 25°C, pH 8.8, A_{340nm}, Light path = 1 cm

METHOD: Spectrophotometric

REAGENTS:

- A. 100 mM Pyrophosphate Buffer with 45 mM Semicarbazide HCl pH 8.8 at 25°C
(Prepare 100 ml in deionized water using Tetrasodium Pyrophosphate, Decahydrate, Sigma Prod. No. P-9146 and Semicarbazide, Hydrochloride, Sigma Prod. No. S-4125. Adjust to pH 8.8 at 25°C with 1 M HCl or 1 M NaOH.)
- B. 100% (v/v) Ethanol (EtOH)
(Use 200 Proof USP Ethyl Alcohol, available from Quantum Chemical Company.)
- C. 0.25 mM β-Nicotinamide Adenine Dinucleotide, Oxidized Form (NAD)
(Immediately before use, prepare 10 ml in cold deionized water with β-Nicotinamide Adenine Dinucleotide using a Class A, volumetric flasks.¹)
- D. Alcohol Dehydrogenase Enzyme Solution (ADH)
(Immediately before use, prepare a solution containing 300 - 400 units/ml of Alcohol Dehydrogenase, Sigma Prod. No. A-7011, in cold Reagent A.)

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PROCEDURE:

Pipette (in milliliters) the following reagents into suitable cuvettes:

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	1.50	1.50
Reagent B (EtOH)	0.05	0.05
Reagent C (NAD)	1.00	-----
Deionized Water	0.40	1.40

Mix by inversion and equilibrate for 5 minutes at 25°C. Record the initial absorbance, $A_i, 340\text{nm}$, using a suitably thermostatted spectrophotometer. Then add:

Reagent D (ADH)	0.10	0.10
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Mix by inversion and allow the reaction to proceed for 5 - 10 minutes until the reaction is complete.² Record the final absorbance, $A_f, 340\text{nm}$ for both the Test and Blank.

CALCULATIONS:

$$r A = (A_f \text{ Test} - A_i \text{ Test}) - (A_f \text{ Blank} - A_i \text{ Blank})$$

A_f = Final Absorbance

A_i = Initial Absorbance

$$\text{Micromoles NAD} = \frac{(r A)(3.05)(df)}{(6.22)(1)}$$

3.05 = Total volume (in milliliters) of assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of NADH

1 = Volume (in milliliters) of NAD sample

$$\text{Apparent MW} = \frac{\text{mg sample weighed} \times 1000}{\mu\text{moles NAD/sample}}$$

FINAL ASSAY CONCENTRATION:

In a 3.05 ml reaction mix, the final concentrations are 53 mM pyrophosphate, 24 mM semicarbazide, 2% (v/v) ethanol, and 30 - 40 units alcohol dehydrogenase and 0.08 mM of β-nicotinamide adenine dinucleotide.

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REFERENCE:

Klingenberg, M. (1974) in *Methods of Enzymatic Analysis*,
(Bergmeyer, H.U., ed) 2nd ed., Volume 4, pp 2048-2050,
Academic Press, Inc., New York, NY

NOTES:

1. Correct for purity, water content, and salts when determining concentration.
2. The reaction is complete when the $r A_{340nm}$ of the Test-Blank is less than $0.002 r A_{340nm}/min$.
3. This assay is based on the cited reference.
4. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.