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Modern Aminos

Re: NAD Stability

Stability of NAD+ in Aqueous Solution: Effects of Shipping and Storage Conditions

Abstract

Nicotinamide adenine dinucleotide (NAD+) is a critical coenzyme involved in cellular redox reactions, but its stability in aqueous solutions is influenced by environmental factors such as temperature, pH, and time. This study evaluated the stability of NAD+ (nominal 200 mg/mL) during simulated shipping (short: ~12–15 hours; long: ~3 days) and subsequent storage (room temperature vs. refrigeration) using high-performance liquid chromatography with UV/Vis detection (HPLC-UV/Vis). Baseline concentration post-short transit was 214.77 mg/mL with 99.6% purity. No significant degradation occurred under refrigeration over 7 days, while room temperature storage resulted in minor losses (2.67% by Day 7). Long-transit sample showed 2.22% degradation upon arrival. Linear extrapolation predicts ~11.4% loss at 30 days and ~22.8% at 60 days at room temperature, with stability maintained under refrigeration. These findings align with literature indicating temperature-dependent hydrolysis to nicotinamide, recommending refrigerated storage for extended periods.

Introduction

Background

NAD+ plays a pivotal role in metabolic pathways, including glycolysis and the citric acid cycle, and has garnered interest as a therapeutic agent for age-related diseases due to its role in sirtuin activation and redox homeostasis. However, NAD+ is prone to degradation in aqueous solutions, primarily via hydrolysis at the nicotinamide-ribose bond, yielding nicotinamide and adenosine

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diphosphate ribose (ADPR). This process is accelerated by elevated temperatures, basic pH, and certain buffers, as evidenced by multiple studies. For instance, in phosphate buffers at 25°C, NAD+ exhibits significant degradation over weeks, with rates influenced by buffer composition and pH. Literature reports half-lives ranging from days at 37°C to months under refrigeration, highlighting the need for stability assessments in practical applications like shipping and storage.

This small-scale study aimed to quantify NAD+ degradation during typical shipping times and short-term storage, informing recommendations for handling. Given the absence of temperature loggers during transit, assumptions were made based on ambient conditions.

Materials and Methods

Samples and Shipping

Three 20 mL vials of NAD+ solution (nominal 200 mg/mL) were provided by Modern Aminos. Vials 1 and 2 were shipped via UPS Next Day Air Early (~12–15 hours transit), arriving July 18, 2025. Vial 3 was shipped via USPS (~3 days transit), arriving July 21, 2025. No temperature logger was sent with the samples, so transit temperatures are unknown but assumed ambient (20–25°C). Vials were inspected upon arrival (intact, no leaks) and protected from light.

Storage Conditions

Post-arrival:

- Vial 1: Stored refrigerated (2–8°C).
- Vial 2: Stored at room temperature (20–25°C).
- Vial 3: Tested immediately (no further storage).

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Analytical Method

HPLC-UV/Vis was performed per ISO 17025-validated method (adapted from USP <1225> and AOAC 2011.06). Equipment: Agilent 1200 HPLC, C18 column (150 mm × 4.6 mm, 5 µm), detector at 260 nm. Mobile phase: Isocratic 90:10 water/acetonitrile with 0.1% TFA; flow 1.0 mL/min; injection 10 µL. Samples diluted to 100 ppm, filtered (0.45 µm), injected in triplicate. Calibration: 5-point curve ($R^2 \geq 0.999$). Quantification: Peak area for concentration (mg/mL) and % area for purity. Degradation products (e.g., nicotinamide) identified by retention time.

Testing Schedule

- Day 0 (July 18, 2025): Vial 1 tested (baseline).
- Day 3 (July 21, 2025): Vials 1, 2, and 3 tested.
- Day 7 (July 25, 2025): Vials 1 and 2 tested.

Data Analysis

Concentrations reported as mean of triplicates (± SD <5% RSD). % Degradation = [(Baseline - Measured)/Baseline] × 100. Linear regression (SciPy linregress) fitted to room temperature data for extrapolation (slope -0.807 mg/mL/day, intercept 214.28 mg/mL, $R^2 = 0.935$).

Results

Table 1 summarizes the results. No changes were observed in refrigerated samples. Room temperature storage showed progressive decline in concentration and purity, with nicotinamide increasing as the primary degradation product. Vial 3 (long transit) exhibited slight degradation comparable to 3 days at room temperature.

Table 1: NAD+ Concentration, Purity, and Degradation Over Time

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Test Date	Vial ID	Condition	Concentration (mg/mL)	% Degradation from Baseline	NAD+ Purity (% Area)	Nicotinamide (% Area)
July 18, 2025	1	Post-Short Transit (Baseline)	214.77	0	99.6	~0.4
July 21, 2025	3	Post-Long Transit	210	2.22	99.56	N/A
July 21, 2025	1	Refrigerated (Day 3)	214.77	0	99.6	~0.4
July 21, 2025	2	Room Temp (Day 3)	211	1.75	99.1	0.9
July 25, 2025	1	Refrigerated (Day 7)	214.77	0	99.6	~0.4
July 25, 2025	2	Room Temp (Day 7)	209	2.67	98.869	1.223

Discussion

The results indicate minimal degradation during short transit and under refrigeration, but gradual loss at room temperature, consistent with hydrolysis kinetics. The observed rate (~0.8 mg/mL/day or 0.38%/day) is slower than reported at 37°C (half-life ~1.4 days), likely due to lower temperature (20–25°C) and solution conditions. In Tris buffer at 25°C, literature shows ~25% NADH loss over 43 days, aligning with our extrapolated ~16% at 43 days.

Expected Stability at 30 and 60 Days

Based on linear extrapolation:

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- Room Temperature: ~190 mg/mL (11.4% loss) at 30 days; ~166 mg/mL (22.8% loss) at 60 days. Purity may decline to ~97% at 30 days and ~94% at 60 days, assuming continued nicotinamide formation (~0.17%/day).
- Refrigerated: Stable at ~215 mg/mL and 99.6% purity, as no loss observed over 7 days; literature supports extended stability at 5°C.

These predictions assume constant conditions; actual rates may vary with pH or buffer, where phosphate accelerates degradation. For long-term, refrigerated storage is essential to minimize hydrolysis.

Conclusions

NAD+ solutions are stable during short shipping and refrigerated storage but degrade slowly at room temperature. Recommend refrigerated shipping for extended transit and storage once opened. Future studies should include temperature logging and pH monitoring.

References

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Prepared by Dustin Newman, Vanguard Laboratory, August 14, 2025.