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RIBOFLAVIN AS AN INHIBITOR OF AQUATIC OXIDATIVE: SELF-PURIFICATION

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Introduction

Natural aquatic ecosystems possess an intrinsic capacity for self-purification, governed mainly by redox reactions that regulate the degradation and transformation of organic and inorganic pollutants. While considerable attention has been directed towards pharmaceuticals, pesticides, and industrial contaminants, the environmental role of naturally occurring redox-active biomolecules, such as vitamins, in modulating these processes remains comparatively unexplored.

Riboflavin (vitamin B_2) is a redox-active flavin compound that enters aquatic environments through domestic wastewater, food-processing effluents, and pharmaceutical residues. Owing to its reversible oxidation-reduction behavior and pronounced photochemical reactivity, riboflavin may interfere with the oxidative pathways responsible for pollutant attenuation. However, its specific influence on aquatic self-purification capacity has received limited experimental attention. Comparable quantitative data for redox-active B vitamins, such as riboflavin, remain scarce compared with established antioxidants such as ascorbic acid or Trolox.

The present study aimed to evaluate, using the ABTS radical cation method, the concentration-dependent inhibitory effect of riboflavin on model oxidative self-purification processes, and to assess the environmental relevance of the observed effects at both experimental and naturally occurring concentration ranges.

Materials and methods

Riboflavin was used as the model redox-active compound. ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) and potassium persulphate were used to generate the ABTS radical cation ($ABTS^{\bullet+}$). All solutions were prepared in double-distilled water at pH 6.5, and spectrophotometric measurements were performed at 734 nm and a controlled temperature of 20°C using an Agilent Cary 60 UV-Vis spectrophotometer equipped with quartz cuvettes. The stock $ABTS^{\bullet+}$ solution was prepared by dissolving ABTS (7.00 mM) and potassium persulphate (4.95 mM) in distilled water and standing in the dark for 16 h at room temperature. The working solution was obtained by dilution to an initial absorbance of 0.70 ± 0.03 at 734 nm.

Ten model water systems containing riboflavin at concentrations ranging from $0.5 \cdot 10^{-5}$ to $10.2 \cdot 10^{-5}$ M were prepared. For each system, 2.5 mL of riboflavin solution was mixed with 0.5 mL of the working $ABTS^{\bullet+}$ solution directly in the cuvette. The initial absorbance (A_0) was recorded, and the samples were incubated in the dark for 30 min before measuring the final absorbance (A_{30}). The inhibition percentage (I, %) was calculated as $I = [(A_0 - A_{30})/A_0] \cdot 100\%$. A blank sample (with double-distilled water) was analyzed under identical conditions, and its inhibition value (0.92 ± 0.13%) was

subtracted from each riboflavin sample result. Determinations were performed in triplicate, and results are expressed as mean ± SD. The concentration–inhibition relationship was evaluated by linear regression.

Results and conclusions

The inhibitory effect of riboflavin on the model oxidative self-purification system increased with concentration (Table 1): below $0.8 \cdot 10^{-5}$ M, inhibition stayed below 15% (unpolluted); moderate inhibition (14-55%) occurred between $1.8 \cdot 10^{-5}$ and $6.9 \cdot 10^{-5}$ M; above $8.4 \cdot 10^{-5}$ M, inhibition exceeded 65% (highly polluted). Linear regression confirmed excellent concentration-dependence ($R^2 = 0.999$), yielding an IC_{50} of $6.4 \cdot 10^{-5}$ M (≈24 μg/mL).

Table 1 - Inhibition (I, %) and water pollution classification vs. riboflavin concentration in model water systems. *Experimental conditions: pH=6.5; T=20°C*

$[B_2] \cdot 10^5$, M	I, %	Water pollution classification
0.50	4.00 ± 0.14	unpolluted water
0.80	6.56 ± 0.21	
1.80	14.28 ± 0.19	moderately polluted water
2.50	19.73 ± 0.26	
4.20	32.98 ± 0.66	
5.90	46.31 ± 1.34	
6.90	53.85 ± 1.36	
8.40	66.87 ± 2.02	polluted water
10.00	76.25 ± 2.36	
10.20	80.03 ± 2.55	highly polluted water

The relationship followed a well-defined linear trend, confirming assay sensitivity. Extrapolation to environmentally relevant concentrations, typically 10^{-9} to 10^{-11} M in natural waters, yielded inhibition values of only 0.1–0.2%, indicating that riboflavin at natural levels does not significantly compromise aquatic self-purification capacity. This IC_{50} (≈24 μg/mL) is of the same order of magnitude as ABTS-based IC_{50} values reported for ascorbic acid and Trolox (single-digit to tens of μg/mL), indicating a moderate but measurable radical-scavenging capacity for riboflavin.

However, point-source contamination from pharmaceutical or food-processing effluents – which can reach active-ingredient concentrations above 100 mg/L – could locally impair natural self-purification. These findings confirm the ABTS radical cation method as a sensitive, rapid, and reproducible tool for assessing redox interference by bioactive compounds in aquatic pollution assessment.

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