Comment on the importance of using nitric oxide gas in the synthesis of nitrosylcobalamin and ICH-validated methods to assess purity and stability

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After a thorough read of this paper (1), we wish to clarify that the authors' anaerobic method of synthesis for the production of nitrocobalamin results in the transient formation of nitrosylcobalamin, an unstable intermediate upon exposure to air. We concur that the authors' method results in the production of nitrocobalamin based on the UV-visible data as shown. The authors' adapted anaerobic method consists of mixing hydroxocobalamin hydrochloride with diethylamine NONOate diethylammonium salt in aqueous solution. Of concern, the UV spectrum of nitric oxide overlaps that of all cobalamin species under anaerobic conditions, making any assignments of the binding of nitric oxide to hydroxocobalamin suspect (2). Additionally, the use of acetone to precipitate the authors' product causes precipitation of diethylamine NONOate, resulting in an impure product. As a result, its utility for drawing experimental conclusions is faulty.

The product from the authors' anaerobic synthetic method has not been assessed for purity, and the synthetic method itself has not been validated using a stability-indicating method as required by the International Conference on Harmonization (ICH) (ICH Q2B, Validation of Analytical Procedures) methodology, which is a hallmark for analytical characterization. Our nitrosylcobalamin synthesis involves reacting nitric oxide gas with hydroxocobalamin acetate as a heterogeneous mixture in a non-electron-donating solvent followed by rotary evaporation. Our nitrosylcobalamin product is stable in air, releases nitric oxide gas *in situ* (3), and meets ICH stability guidelines (4). Additionally, our nitrosylcobalamin product demonstrates biological activity, which has not been observed for nitrocobalamin (3, 5).

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Conflict of interest—The authors declare that they have no conflicts of interest with the contents of this article.

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