

Reply to Sysel *et al.*: 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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In their comment (1) on our publication (2), the authors make two points: (i) they raise concerns about the possible effect of residual NONOate in our study, and (ii) they promote nitrosylcobalamin (NO₂Cbl) supplied by their own company. Both points lack merit for the following reasons. The authors make the astonishing claim that the spectra of nitric oxide (NO[•]) and cobalamins overlap. Unlike NO[•], cobalamin absorbs in the visible region, permitting unequivocal spectral assignment of NO₂Cbl as reported (3). We demonstrated that whereas NO₂Cbl is highly unstable in solution, it is stabilized by the B₁₂ trafficking protein CblC. So even if present, residual NONOate (which is unstable at neutral pH and is removed during the work-up (3)) could not account for the observed difference.

The authors then misrepresent our synthetic method, claiming that anaerobic conditions were used to generate nitrocobalamin (NO₂Cbl), which results in the transient formation of NO₂Cbl. We synthesized NO₂Cbl *aerobically* using nitrite as described (4); NO₂Cbl is *not* an intermediate in this ligand exchange reaction. The aerobic instability of NO₂Cbl has been rigorously described by inorganic chemists (3, 5) and raises obvious questions about its purported biological effects as exemplified by the authors' own 2003 JBC publication, which was later withdrawn.

As to promoting NO₂Cbl from their company, the authors refer to a synthetic route from a mixture of NO[•] gas and aquocobalamin. The authors' method (6) has been described as "dubious" by chemists (5). Whereas DEAE NONOate used in our method is widely known as an NO[•] donor, it reacts via the

direct transfer of a nitroxyl group (not NO[•]) to aquocobalamin, generating NO₂Cbl (7).

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

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