Analytical Methods

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Journal:	*Analytical Methods*
Manuscript ID:	AY-CMT-10-2014-002352.R1
Article Type:	Comment
Date Submitted by the Author:	26-Jan-2015
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Reply to the 'Comment on "Rapid visual detection of blood cyanide"' by A. F. Kadjo, P. K. Dasgupta and G. R. Boss, *Analytical Methods*, 2015, 7, DOI: 10.1039/C4AY00190G

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Abstract:

Dasgupta, Boss and coworkers comment with regard to our paper 'Rapid Visual Detection of Blood Cyanide'. In this communication we described a new two step procedure for detecting cyanide in cyanide spiked blood. In this proof-of-concept study we did, however, not discuss whether the method detects free, transition-metal ion bound or total cyanide. We appreciate the comments and agree completely with the assumptions suggesting that the method detects free cyanide, but not cyanide bound to cyanomethemoglobin (CNMetHb). This hypothesis is now supported by us experimentally. To the best of our knowledge, our method represents therefore the first strategy for the rapid detection of free cyanide in blood. In contrast to the opinion of Boss and Dasgupta, we believe that this new form of blood analysis is of much scientific interest and most probably also of medicinal impact in the future diagnosis of cyanide intoxications in emergency situations.

In 2012 Croisé and Zelder reported on a new two-step protocol for detecting cyanide in cyanide spiked blood.[1] In this approach, a corrin-based chemosensor is added to the spiked blood sample and after short mixing (~ 1 min) extracted on the top of a hydrophobic solid phase. In contrast to the cyano-bound chemosensor, the blood sample is not retained on the solid-phase and residual blood can be washed off easily with water. For this reason, cyanide can be identified in form of the violet-colored cyano-chemosensor complex before or after eluting the metal-complex from the solid phase. Due to the characteristic color of the metal complex, naked-eye detection without sophisticated laboratory instrumentation is also principally possible. For more details, the reader is referred to the original publication.[1] A short movie describing the method can now also be found on youtube (https://www.youtube.com/watch?v=geSIQDjvFys). It is important to note that we performed -*as described in the paper*- all experiments at defined time points of 15 minutes after spiking. This defined point in time was selected because of two reasons. First, cyanide is a strong nucleophile that reacts with a range of organic functionalities and binds also strongly to many transition metal ions in high oxidation states. Most important in biological systems are probably ferri-containing cofactors such as methemoglobin and cytochrome C oxidase. As a consequence of this behavior, cyanide shows a very complex metabolism and recovery rates depend strongly on sample collection, storage and preparation.[2, 3] For example, literature

reports for established Conway diffusion assays recovery rates of only 30 % for spiked samples analyzed after one hour, compared to full recovery for immediate detection.[2, 3] Dasgupta, Boss and coworkers observed a similar effect for analyzing spiked samples over a period of one hour with our method (see Figure 2; *Anal. Methods* Doi). This behavior is also in agreement with our own observations.[4] More importantly, they stress on the question whether the new method detects either (i) free, (ii) transition-metal ion bound or (iii) total cyanide. This is indeed a very important question that was not discussed in our pioneering publication. Based on basic thermodynamic and kinetic considerations, Dasgupta, Boss and coworkers underline that the new method is only able to detect free cyanide, but not cyanide bound as cyanomethemoglobin (CNMetHb). We completely agree with these assumptions and give herein some experimental support. For this purpose, we investigated the reaction between CNMetHb ([heme] = 20 μM) and our chemosensor (42 μM) in solution. A time frame of two minutes was chosen, representing the approximate duration of the assay. Mixing the two components together led to only minor shifts of the absorption maxima in the UV-Vis spectra as shown in Figure 1.

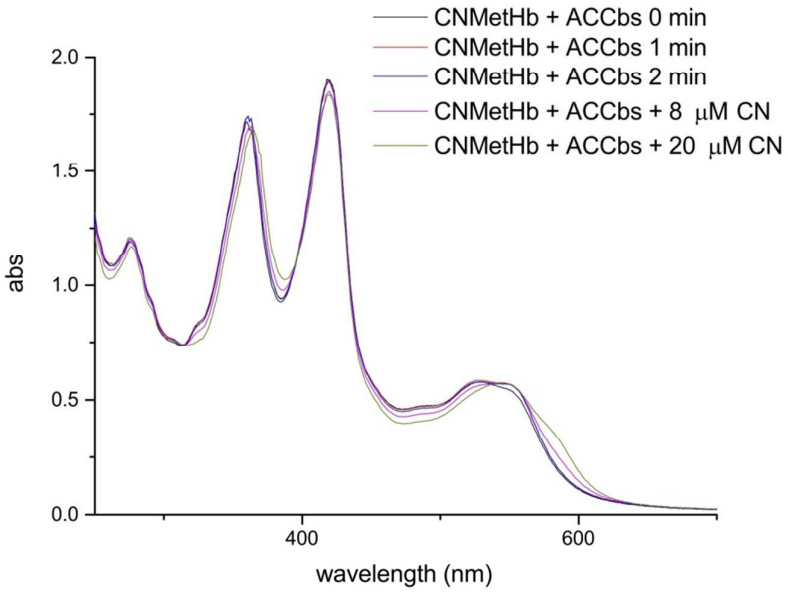

Figure 1 UV-vis spectra of a mixture of the chemosensor (ACCbs; 42 μM) and cyanomethemoglobin (CNMetHb; [heme] = 20 μM) after 0, 1 and 2 minutes after mixing as well as immediately after the addition of CN^- (8 and 20 μM), respectively.

This is probably due to some interactions between the protein and the chemosensor. More importantly, we did not observe any change in the spectra at around 580 nm. However, such a change would be expected for the formation of the cyano-chemosensor complex (λ_{max} (α-band) = 580 nm) by cyanide transfer from CNMetHb to the chemosensor. Indeed, this characteristic shift was only observed in the spectrum, immediately after titrating (free) cyanide to this reaction mixture (Figure 1). A limit of detection (LOD) of 0.5 μM was determined for this assay.[5] Based on the suggestions of Dasgupta, Boss and coworkers and the results of our own additional

experiments, it is now demonstrated that the proposed new two-step protocol detects `free cyanide`, but not transition metal-bound cyanide in spiked blood.

This new information on our detection method is indeed of much importance. To the best of our knowledge, the rapid detection of free cyanide in blood is momentarily not possible with any other method.

In contrast to the opinions of Dasgupta and Boss, we believe that rapid detection of free cyanide in blood may help to improve diagnosis and treatment of cyanide intoxications in the future. Cyanide`s toxicity is believed to be mostly caused by strong binding to cytochrome C oxidase, a process that inhibits cellular respiration. On the other hand, MetHb acts as natural detoxification agent that scavenges cyanide before it can reach more critical biological targets. In situations of acute cyanide intoxications the natural concentrations of MetHb (~1% of hemoglobin) are unfortunately not sufficient for complete protection. For this reason, the removal of `free` and therefore still potentially toxic cyanide is probably of primary importance in an emergency situation. The method developed by our group is capable of detecting the presence of this life-threatening agent, almost in real time.

In agreement with the statements of Lindsay and coworkers, we therefore believe that "a rapid, accurate bedside assay of BCC [BCC: blood cyanide concentrations] differentiating between bound and free cyanide would represent a leap forward in the clinical management of cyanide poisoning."[2] In accordance with this statement, we propose that our method can significantly contribute to such developments in the future.

1. C. Männel-Croise and F. Zelder, *Anal. Methods*, 2012, **4**, 2632-2634.
2. A. E. Lindsay, A. R. Greenbaum and D. O'Hare, *Anal. Chim. Acta*, 2004, **511**, 185-195.
3. M. Feldstein and N. C. Klendshoj, *J. Lab. Clinic. Med.*, 1954, **44**, 166-170.
4. Unpublished results.
5. The limit of detection was determined as three times the standard deviation of the blank divided by the slope of the calibration curve.