

Screening of Iron Fortificants

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Introduction

Iron deficiency (ID) affects an estimated 50% of children, 42% of women and 26% of men in developing countries.¹ ID heavily burdens children and women of child-bearing age in developing countries, where diets are cereal-based and low in bioavailable iron. It is associated with low neonatal weight and pre-term birth, compromised cognitive performance, behavior, and motor development in infants, preschool, and school-aged children, and limited work performance in adolescents and adults. Many of its effects on early development and cognition may be irreversible, creating lifelong impairments that add to the burden of already inadequate national health care capacities.

Iron enrichment of foods can help alleviate this problem if the products used are sufficiently bioavailable (effectively absorbed during digestion). In reality, elemental iron powders continue to be the dominant iron forms used in fortification programs in many countries due to the relatively low cost of such products and their lack of

undesirable interactions with food vehicles. However, a growing body of evidence indicates that some elemental iron powders in use today are ineffective in alleviating iron deficiency.

Recent laboratory research and a human efficacy study undertaken by SUSTAIN, in collaboration with research partners in industry, government and academia, revealed significant differences among different types of elemental iron powders in attributes correlated with bioavailability. SUSTAIN and its partners developed a simple, rapid in vitro screening procedure, based on dissolution rate in dilute hydrochloric acid, that accurately and reliably predicts the potential efficacy of iron products.² This test method holds great potential to serve as a means of estimating how well different iron forms are likely to be absorbed from foods during digestion. Once fully proven and validated, it can be used by industry to initially screen new products (and decide which of them merit further evaluation in human trials), and as a quality control tool to periodically evaluate product batches in manufacturing. Its use can also support innovation to improve product performance. Effective early screening of iron fortificants can lead to more informed choice on products for food fortification initiatives.

SUSTAIN's elemental iron project

In 2000, SUSTAIN invited an expert group of food scientists, hematologists, nutritionists, iron fortificant suppliers, and other stakeholders to a workshop in Monterrey, Mexico, to address questions and confusion on the bioavailability of elemental iron powders, and to recommend action. Research conducted over the previous 50 years had produced highly variable data on the bioavailability of these products, ranging from 5% to 145% relative to the standard, ferrous sulfate. Workshop participants noted that past research on elemental iron fortificants was not only contradictory and confusing, it was also largely out of date, having focused on samples that were not always representative of commercially-available products or even specifically identified. Screening

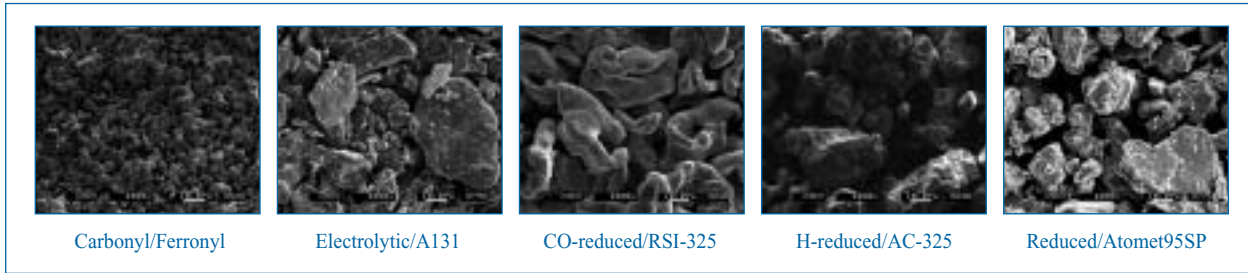


Figure 1: SEM (scanning electron microscopy) images of iron powders

methods used to evaluate the powders also varied widely. The inconsistent data, coupled with concerns about the higher cost of possibly more effective iron fortificants, posed a real dilemma for developing countries trying to establish effective fortification programs.

Findings and recommendations from this workshop were presented at food industry and scientific meetings and conferences, and subsequently published.³ Industry, government and scientific experts (including workshop participants) urged a rigorous review of these issues and a resolution to questions regarding the efficacy of elemental iron food fortificants.

In response, SUSTAIN formed a Task Force on Iron Powders to evaluate approaches to screening commercial iron powders for predicted bioavailability and to determine which products are most likely to be effective in improving iron status of consumers.

This rigorous evaluation of all commercially available elemental iron fortificants involved scanning electron microscopy images (**Figure 1**) and the full range of available *in vitro* and biological screens, as well as a human efficacy study. Significant differences were found among products with respect to characteristics likely to affect bioavailability; even powders of the same production type made by two different companies differed with respect to characteristics relevant to bioavailability. The screening methods utilized varied in cost, difficulty of execution, and predictive value. The prevalence of iron deficiency and anemia in individuals receiving snacks fortified by one of the two powders tested in the human efficacy study was not different from the control group at the end of the trial.⁴

Given the difficulty and expense of human efficacy studies, it became clear that a standardized and accurate screen was needed to rigorously evaluate the potential for iron fortificants to work in the ways they are intended. Without such a tool, there is no basis for distinguishing between products or for integrating minimum bioavailability criteria in product standards

and specifications in order to assure the use of effective products. SUSTAIN found that the dissolution rate of commercial iron fortificants in dilute HCl under carefully standardized conditions provides an accurate and reliable means of initially screening iron fortificants for bioavailability. The method can be used in three principle ways:

- As a tool for researchers interested in evaluating only the most promising of iron fortificants in costly human efficacy trials;
- during manufacturing of the powders for process quality control; and
- to ensure that only products likely to improve iron status in consumers are used in fortification initiatives.

The latter application will depend on the establishment of a minimum bioavailability-based standard for iron in micronutrient premixes. This could be based on a validated dissolution screen derived from the SUSTAIN protocol.

Dissolution method summary

Drawing on published research (Shah et al,⁵ Forbes et al,⁶ and findings from our own research initiatives⁷), we developed a prototype dissolution screen to predict the bioavailability of elemental iron powders. Once fully evaluated and validated by an accrediting body, this screen will provide a useful tool for eliminating inefficacious products, for screening newly developed products, and in quality control. It could also become the basis for new elemental iron bioavailability standards.

Iron powder dissolution rates were studied under different pH, time, and temperature conditions. Parameters that had the most consistent values and closest linear correlation with relative bioavailability, as measured in AOAC rat hemoglobin repletion bioassays, were identified. These preliminary experiments provided the basis for a standardized dissolution test protocol.⁸

A collaborative study was conducted to evaluate the repeatability and reproducibility of the method. Fourteen blinded duplicate samples of seven powders were submitted to nine laboratories, together with a detailed description of the method. The powders belonged to one of five groups based on the production method; carbonyl, electrolytic, hydrogen reduced, carbon monoxide reduced, and other reduced (**Figure 1**).

Results showed statistically significant differences in the dissolution rates for the seven elemental iron powders tested, with excellent repeatability within labs and reproducibility across labs (**Figure 2**). Small observed variations in results led to minor method refinements. This included an evaluation of how variations in time, temperature, and stirring speed affected dissolution rates. On the basis of correlation analyses between the iron powder dissolution data and the relative bioavailability values of our AOAC rat hemoglobin repletion bioassays, the parameters

for temperature, time and stirring speed were adjusted.

The current format of the method includes the scope, method summary, safety, terms, common sources of error, equipment, reagent preparation, equipment preparation, sample preparation, procedure, calculations, data reporting, method statistics, special notes, and references.

With support from SIGHT AND LIFE, several potential pathways to the method's official accreditation were explored; the results of this initiative have been shared in a number of scientific meetings. The protocol was presented to international standards officials at AACC International and ASTM International, with very positive feedback from ASTM about its potential for validation.

Potential for new industry standards

This project generated significant interest and support from compa-

nies that produce iron powders, even though over 99.8% of their products are manufactured not as food fortificants but for the automotive industry. SUSTAIN engaged industry as an active participant and supporter from the beginning, bringing company representatives to a briefing on the project, and a tutorial on iron absorption. Prior to their involvement in this project, industry representatives had virtually no involvement with the nutrition community or familiarity with food fortification issues. Manufacturers worked collaboratively with us both to evaluate commercial iron fortificants and to develop the dissolution method as a simple product screening tool.

While industry's response to this project has been extremely gratifying, representatives have stressed that minimum standards for iron bioavailability (based on a reliable screen) are needed to drive the demand for improved products. Manufacturers have demonstrated the capability to make more soluble products and have endorsed the merits of the dissolution test as a rapid and reliable bioavailability screen. Many now feel it is essential for minimum regulatory standards to be established (based on this type of screen) to drive market demand.

Next steps

Additional funding and work is needed to confirm the protocol's robustness (capability of generating precise results when parameters vary slightly). In preparation for its accreditation, the finalized protocol would then be subjected to a collaborative study to again ensure that uniform results are achievable by different laboratories.

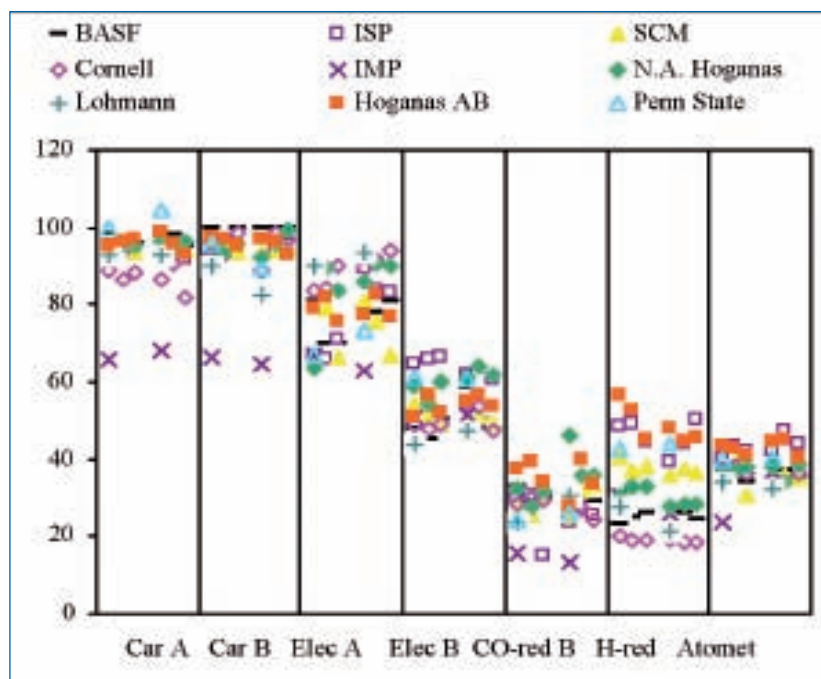


Figure 2: Percent iron dissolved based on the standard dissolution assay as tested by nine labs

SUSTAIN would then convene an expert panel to evaluate current recommendations on the use of iron fortificants, review recent research (including human efficacy studies in which products' effect on iron status is directly compared to their dissolution rates), and recommend protocols for practical applications of the validated screen in the manufacture and regulation of iron fortificants.

Concluding summary

At the onset of our work on this project, elemental iron powders were not well differentiated (leading to purchase of lowest-cost products for fortification initiatives). Through our work, we achieved insights into the important differences among elemental iron powders. By engaging iron powder manufacturers and premix suppliers in this project, we also effectively raised industry awareness of bioavailability issues. In addition to generating information critical to making informed choices among the wide array of available iron fortificants, we developed a prototype screening tool that, once fully evaluated, can be used in quality control and new product evaluations. This work has set the stage for longer-term impacts in the form of more effec-

tive iron fortification initiatives in developing countries, and potentially more rigorous bioavailability standards (based on powder dissolution rates) in product specifications.

Acknowledgments

We thank SUSTAIN for permission to publish this summary of their dissolution method. Work on the development and testing of the method was carried out with the generous support of the Bill & Melinda Gates Foundation, the Micronutrient Initiative, the Global Alliance for Improved Nutrition (GAIN), and SIGHT AND LIFE. For more information, contact SUSTAIN (www.sustaintech.org).

References and footnotes

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 8. An accurately weighed iron powder sample was placed in HCl (pH 1.0) and incubated with constant stirring. The solution was filtered to remove residual iron particles and diluted to the desired quantity. Iron concentrations were measured by inductively coupled plasma-atomic emission spectrometry (ICP-AES).



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