# The role of near infrared spectroscopy in maintaining food integrity

Grant A. Abernethy, Ling Feng, Stephen E. Holroyd* and Elizabeth Nickless

Fonterra Research Centre, Dairy Farm Road, Palmerston North 4442, New Zealand
*Corresponding author: steve.holroyd@fonterra.com

## Introduction

The adulteration of food for economic gain has historically been widespread. The risk of deliberate adulteration varies depending on a number of factors such as the availability of chemical adulterants, the compatibility of those compounds with food matrices and financial incentives. In 2008, the deliberate contamination of infant formula with melamine highlighted the risk posed by the adulteration of foodstuffs with chemically harmful compounds. Since then, in recognition that any type of food adulteration carries a significant food safety risk, the worldwide analytical community has devoted a significant amount of effort aimed at detecting adulteration and thus minimising the risk to consumers and brands. Melamine was added to liquid milk for economic gain; melamine increased the apparent protein content by increasing the amount of nitrogen measured during the standard protein reference Kjeldahl test. Figure 1 shows how addition of melamine can increase apparent protein content in liquid milk.
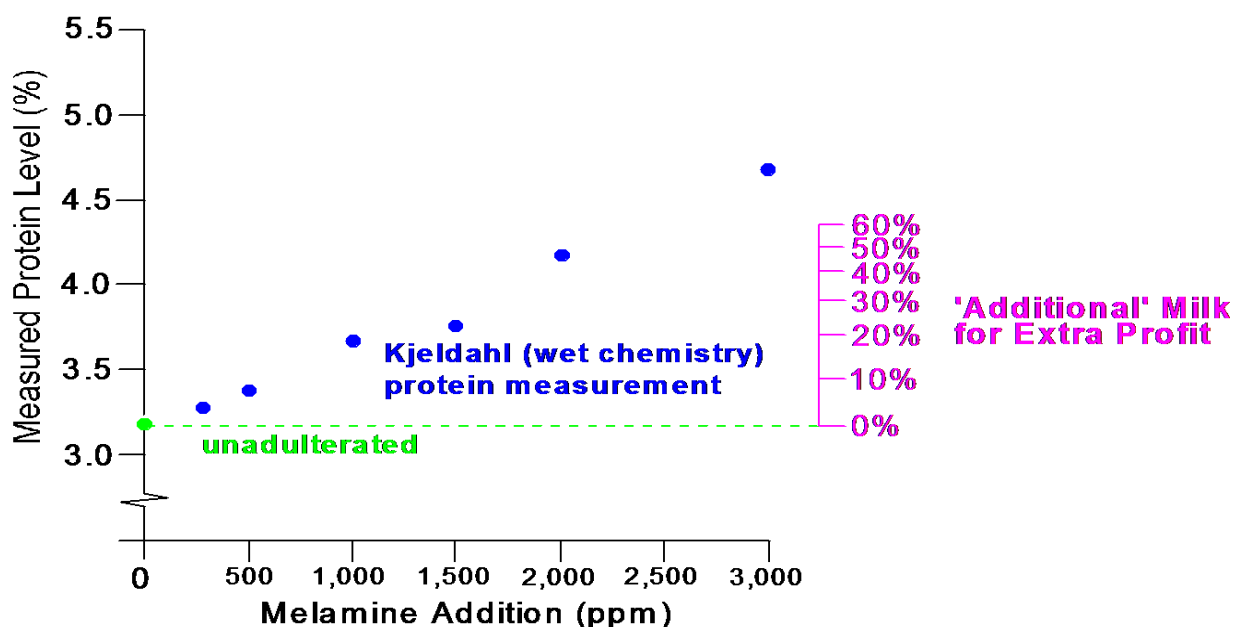


**Figure 1.** Measured protein vs. added melamine.

There are a number of strategies that can be used to protect against the economic adulteration of milk products. Factors that are important when making decisions about the most appropriate strategy to adopt include selection of the optimum sampling location, ability to sample, and impact on customers and business. Selection of individual analytical techniques must take into account factors such as speed, cost, accuracy, sensitivity and reliability. There are already numerous assays, with a range of performances, for detection of melamine across many food matrices.[1-3] A business or regulatory authority carrying out such testing must also formulate and implement an appropriate "response to detection" strategy with business decisions based on risk.[4-5] Infrared spectroscopy, as a widely applied analytical technique for the measurement of liquid milk and milk products, has a number of attributes that make it a potential tool for adoption as part of such a strategy.[6] Figure 2 is a plot of potential concentrations of an adulterant that has been added to liquid milk for economic gain. Typically mid-infrared spectroscopy is applied to quantitation in the milk powder production chain at the liquid milk stage when the actual adulteration concentration is at a maximum (1-5 hours), and near infrared (NIR) spectroscopy is used for quantitative analysis once the milk powder has been made (33+ hours). Previous work has shown that mid-infrared spectroscopy can be used to distinguish a number of nitrogen containing adulterants in liquid milk.[7] This paper focuses on the ability to NIR reflectance spectroscopy to detect melamine in whole milk powder (WMP).

**Figure 2.** Hypothetical adulterant concentration (ppm adulterant) with time for milk powder process.

## Materials and Methods

The effect of deliberate contamination with highly nitrogenated chemical contaminants on a typical dairy process was examined in terms of levels of adulteration during milk powder production. Samples of WMP deliberately adulterated with chemically pure melamine across a broad concentration range (0, 500, 1000, 2000, 5000 ppm) were prepared. Dry mixing of samples was achieved by weighing chemically pure melamine (Sigma-Aldrich) into instantised WMP (Fonterra Co-operative Group Ltd, Palmerston North, New Zealand) and then mixing via vigorous shaking in an appropriately sized container for 5 minutes. Wet mixed samples were prepared by weighing chemically pure melamine into liquid whole milk (Fonterra Co-operative Group Ltd, Palmerston North, New Zealand), stirring at 30°C for 30 minutes, then spray drying the milk (Pulvis GB22 laboratory spray drier, Yamato Scientific, Tokyo, Japan) and collecting the resulting powder. The wet mixed samples were manufactured at concentrations of 0, 500, 1000 and 2000 ppm melamine. A sample concentration of 5000 ppm melamine was not achievable due to the limited solubility of chemically pure melamine in milk. The samples were measured by a multi-purpose NIR analyser (Bruker Optic, Ettlingen, Germany) and using OPUS (Bruker Optic, Ettlingen, Germany) software calibrations for melamine concentration were prepared. Using multiplicative scatter correction and leave one out cross validation.

## Results and Discussion

An initial PLS based calibration using the dry mixed samples was created (Figure 3). This replicated and supports the work of Baeten et al.[8] While a correlation between added melamine and NIR spectral response is possible it does not prove that the spectral response is due to melamine concentration. A PLS calibration using only the wet mixed samples was also calculated, as shown in Figure 4. Even given the limited number of samples used the performance of these two calibrations is significantly different. The dry blended calibration could probably be useful when assessing melamine concentration below 1000 ppm, the wet blended calibration would clearly not provide any useful information in this concentration range. This suggests that at levels above 1000 ppm the wet mixed samples did exhibit some correlation with increasing melamine levels linked to a similar spectral response to the dry mixed samples.

A PLS calibration using both the dry and wet mixed samples as a combined data set was not attempted as the particle sizes of the commercially available WMP used in the dry blending experiment and those produced via the lab scale drier in the wet blending experiments were very different. Prediction of the melamine content of the wet blended samples using the dry-blended model and vice versa was not successful. Of course the low number of samples used in both calibrations and lack of any independent validation set limit the drawing of strong conclusions.
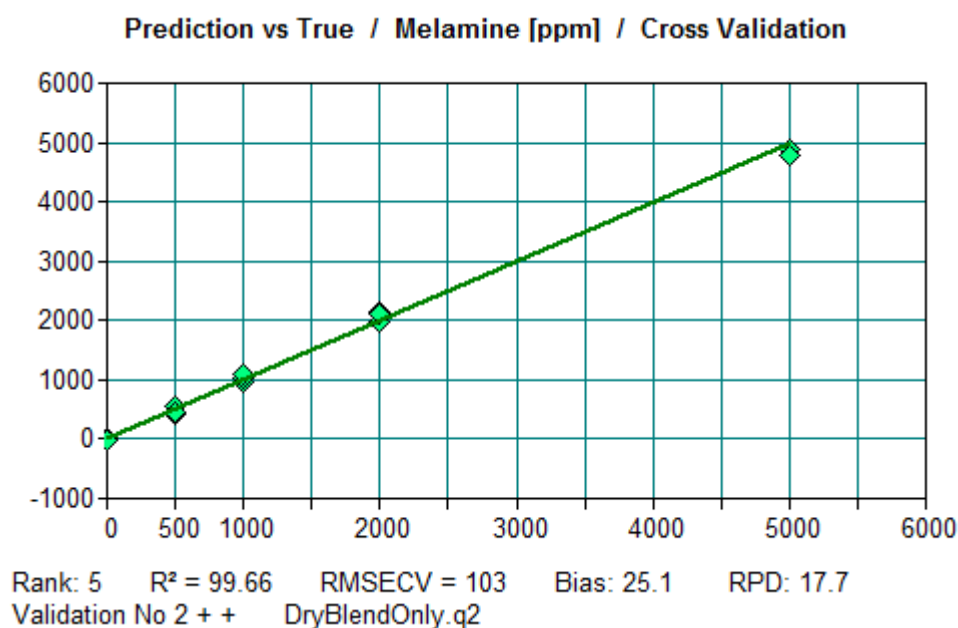
**Figure 3.** Cross validation predicted versus measured plot of dry blended whole milk powders and melamine.
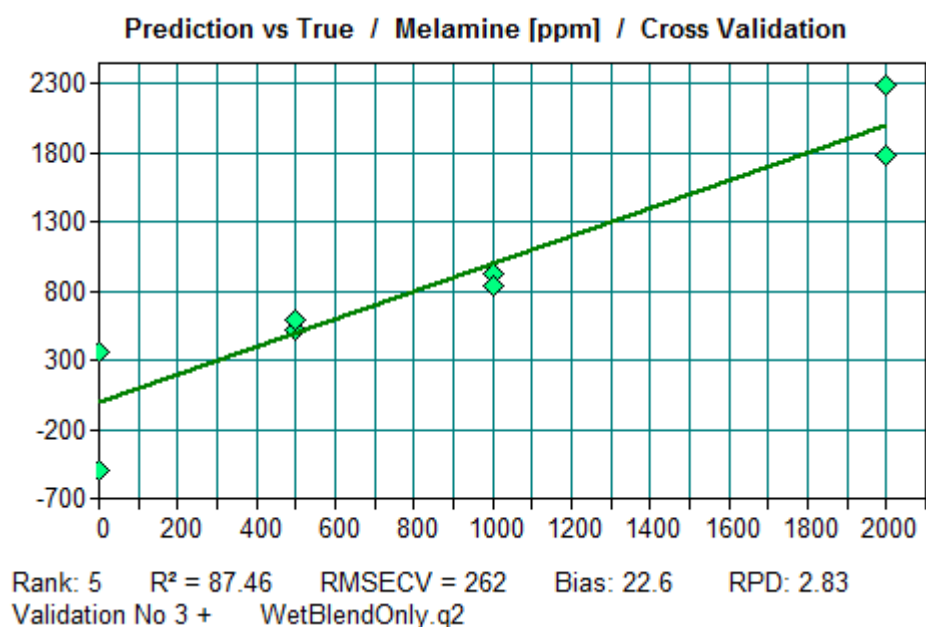


**Figure 4.** Cross validation predicted versus measured plot of wet blended whole milk powders and melamine.

Typically when highly scattering samples behave differently with respect to NIR spectral response it is due to differences in sample microstructure. Scanning electron microscopy was used to explore morphological differences between the dry and wet mixed samples. Figures 5, 6, 7 and 8 show respectively unadulterated whole milk powder, whole milk powder with melamine wet mixed at 2000 ppm and whole milk powder with melamine dry mixed at 2000 ppm and in a 1:1 ratio (used here to illustrate the effect). Figures 5 and 6 show that the bulk surfaces of the WMP are very similar. We postulate that the dissolved melamine is evenly distributed throughout the sample. Figure 7 shows significant irregularities on the surfaces of the WMP particles, with the effect greatly magnified in Figure 8. We suggest that these irregular areas are pure melamine particles adhering to the hydrophobic WMP particles. Given their size relative to the wavelength of NIR radiation these contribute significantly to the NIR spectral response seen in the dry mixed samples, especially when there is an absence of a spectral response from the chemical bonds within the melamine present due to low concentrations.
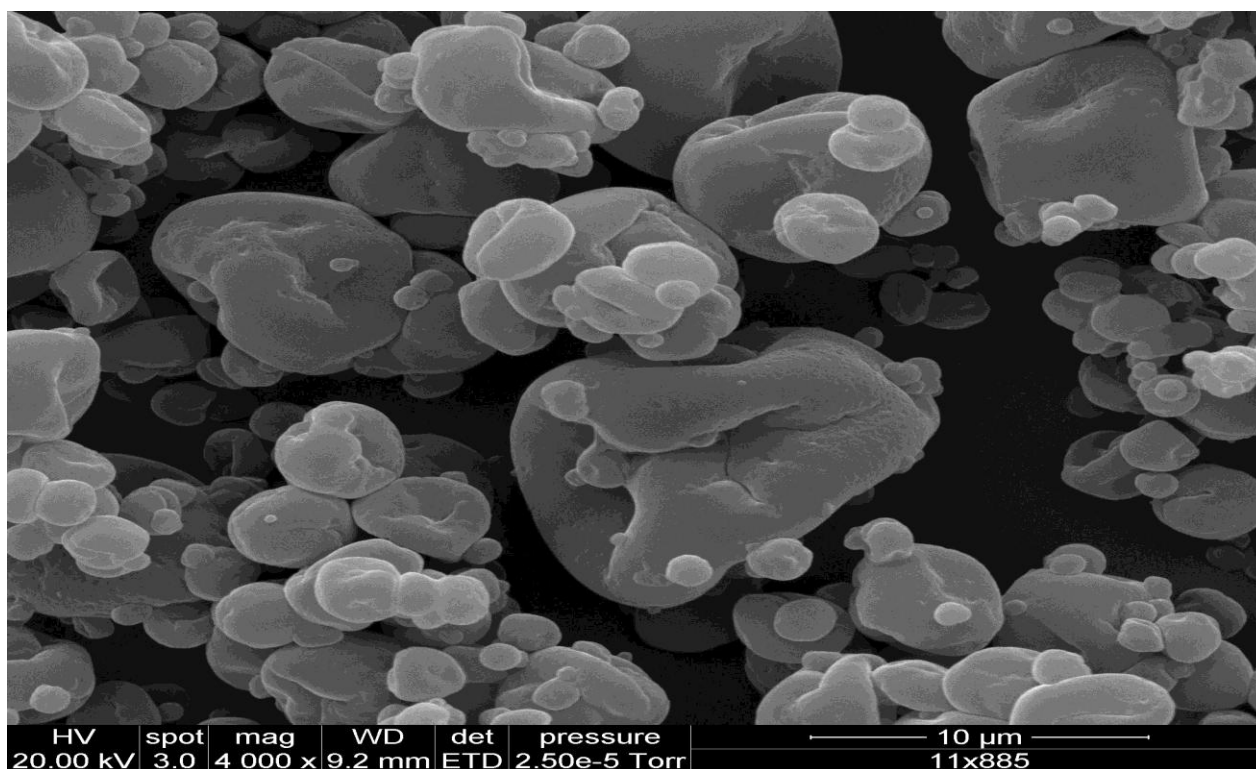
Reference paper as:
G.A. Abernethy, L. Feng, S.E. Holroyd and E. Nickless (2012).The role of near infrared spectroscopy in maintaining food integrity, in: Proceedings of the 15th International Conference on Near Infrared Spectroscopy, Edited by M. Manley, C.M. McGoverin, D.B. Thomas and G. Downey, Cape Town, South Africa, pp. 392-397.

| HV | spot | mag | WD | det | pressure | 10 μm |
|---|---|---|---|---|---|---|
| 20.00 kV | 3.0 | 4 000 x | 9.2 mm | ETD | 2.50e-5 Torr | 11x885 |

**Figure 5.** Scanning electron micrograph of unadulterated whole milk powder.



| HV | spot | mag | WD | det | pressure | 10 μm |
|---|---|---|---|---|---|---|
| 20.00 kV | 3.0 | 4 000 x | 9.3 mm | ETD | 1.86e-5 Torr | 11x886 |
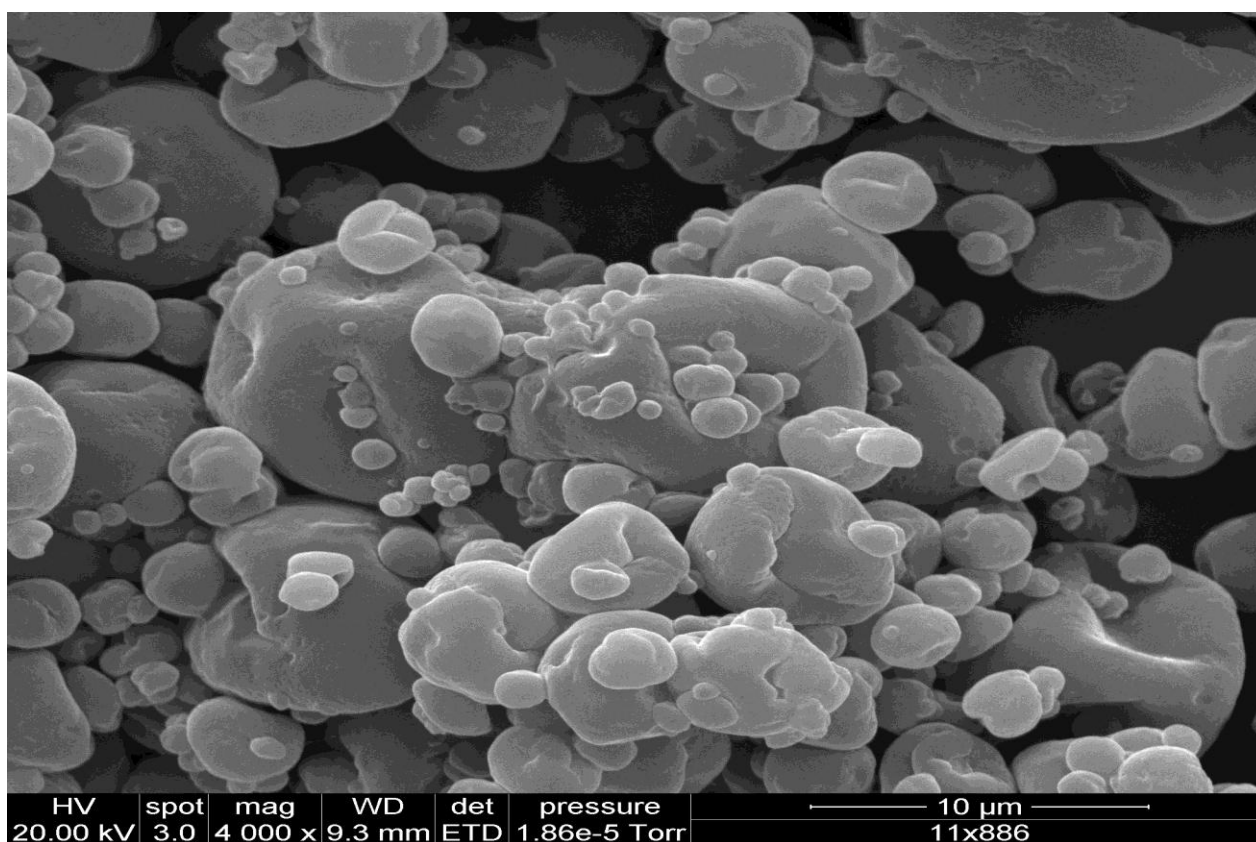
**Figure 6.** Scanning electron micrograph of whole milk powder with melamine wet mixed at 2000 ppm.

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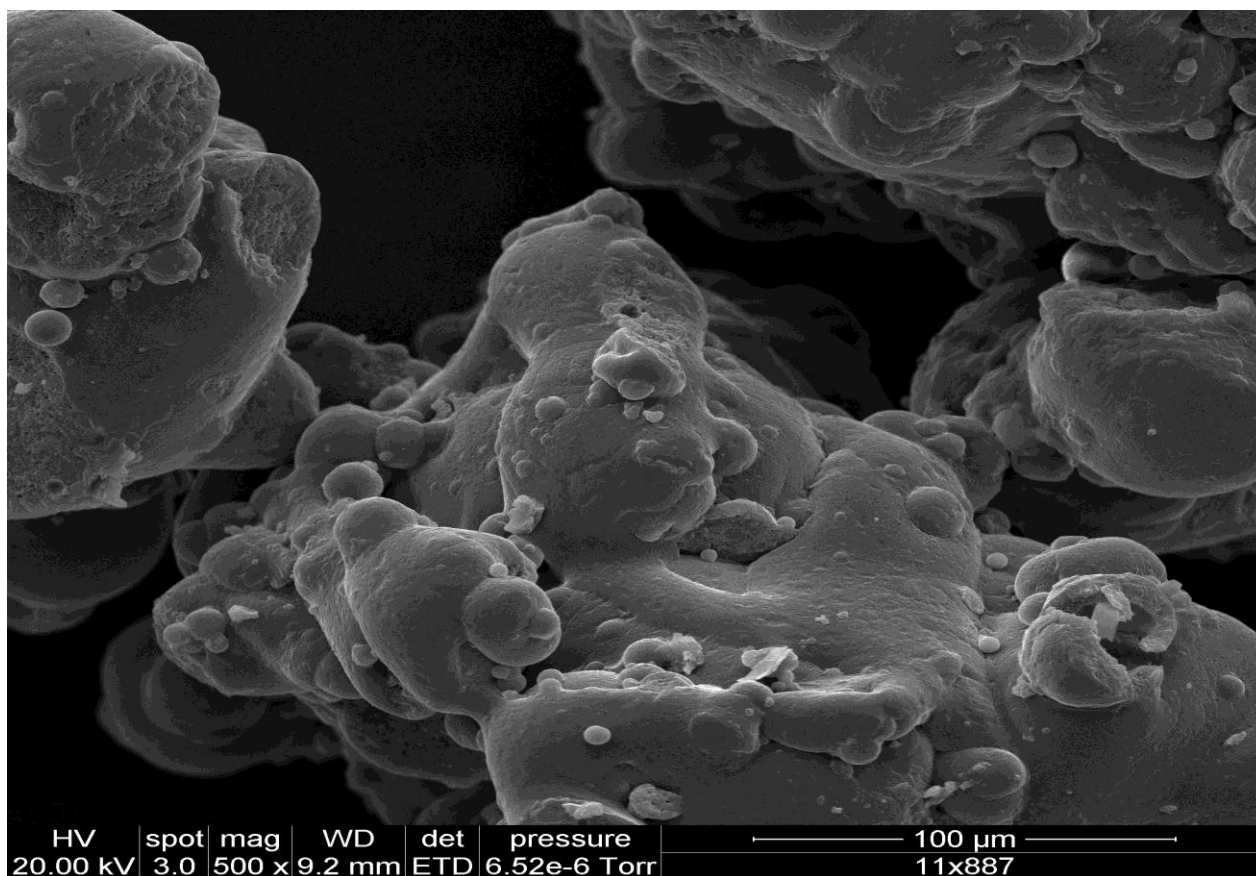
**Figure 7.** Scanning electron micrograph of whole milk powder with melamine dry mixed at 2000 ppm.



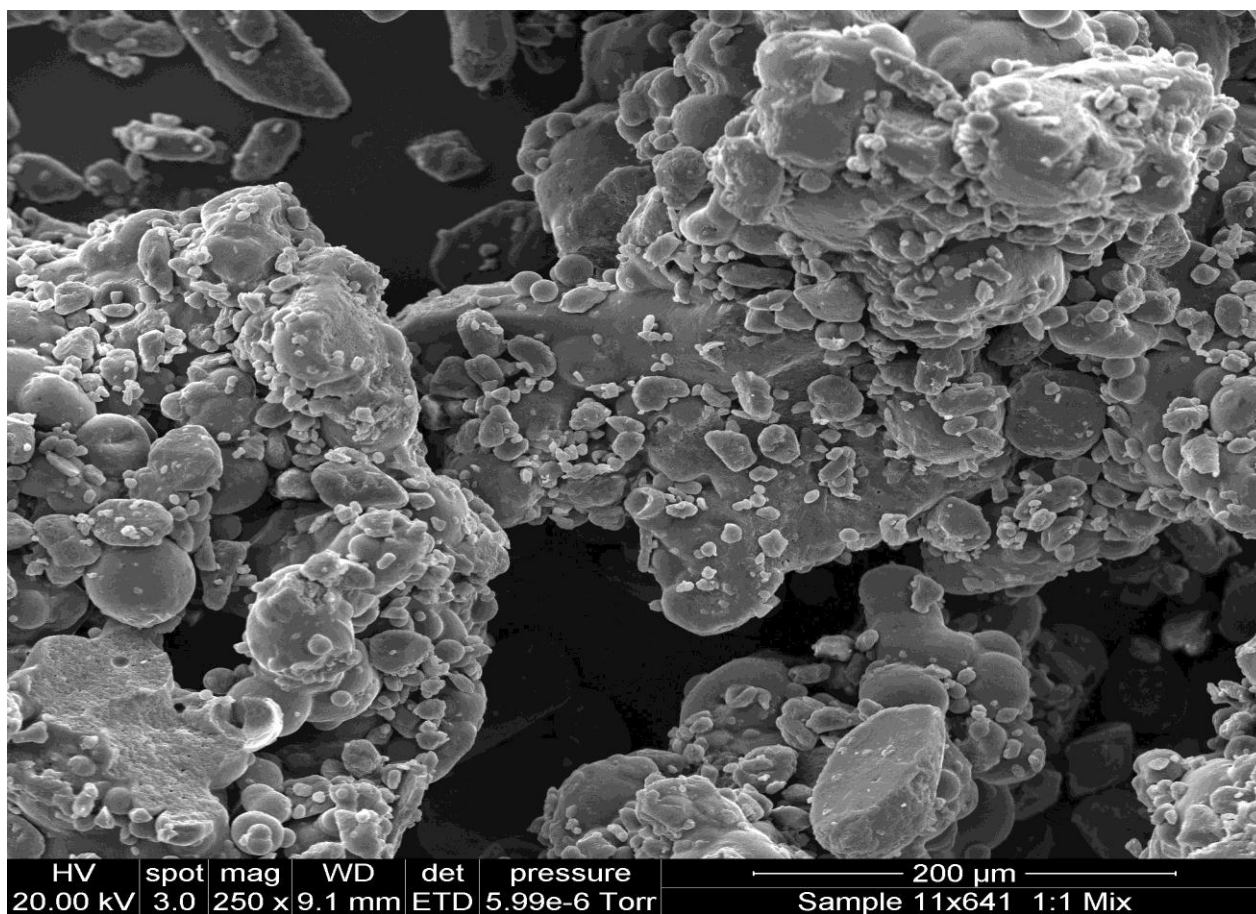**Figure 8.** Scanning electron micrograph of whole milk powder with melamine dry mixed at 1:1 ratio.

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## Conclusion

The dry mixing of chemically pure adulterants in milk powders was shown to be a poor method of achieving samples that are representative of "real-world" adulteration in milk powders where an adulterant would be wet mixed and more thoroughly blended into the sample and thus not detected at lower concentrations. Dissolution of adulterant into liquid milk prior to drying would be a favourable and more representative approach. Scanning electron microscopy was shown to be a useful tool for probing comparative whole milk powder microstructures. While NIR can be used to measure melamine in milk powder at levels over 1000 ppm care must be taken to ensure representative samples are used for both calibration and validation.

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