M.6: Detection of urea adulteration in packaged milk samples using spatially offset Raman spectroscopy

Milk is a nutritional food item that is frequently adulterated with urea. Water is routinely added to maximise milk volume for commercial gain. In order to account for density reduction in diluted milk, urea is added to compensate the lactometer reading. Further, urea is added to mimic the nitrogenous content of protein in diluted milk, which is generally estimated using the Kjeldahl method. However, consumption of urea-adulterated milk samples can pose several health hazards.

Over the years, many techniques have been used to detect urea in milk (conductometric, potentiometric, electrochemical, MS, GC, and chemical assays), but they often require tedious sample preparation, multiple extraction steps, have utility to the limited concentration ranges, and are unstable. Optical methods such as diffuse reflectance, ATR-FTIR and Raman spectroscopy have emerged as attractive alternatives, with Raman offering molecular specificity and robust quantitative performance. However, there is an urgent need for a non-invasive method for detecting adulteration in packaged milk, especially in India and other South Asian countries, where even packaged milk is often found to be adulterated with urea. This motivates to explore the depth-sensitive Raman technique like spatially offset Raman spectroscopy (SORS) for detection of urea adulteration in packaged milk. The technique is well suited for measuring depth sensitive Raman signal beyond the probing depth of confocal technique.

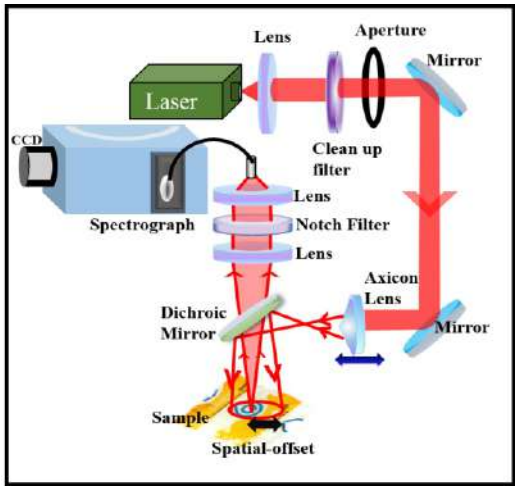

Fig. M.6.1: Optical setup of the inverse SORS system.

We report here, the development of a setup which employed inverse configuration of SORS measurement. The setup uses a 785 nm multimode diode laser for excitation, an axicon lens for ring illumination, and backscattered Raman photons are collected from the centre of the ring, thereby providing a spatial offset between illumination and collection. This configuration helps in preferentially recovering sub-surface Raman signals from milk beneath low-density polyethene (LDPE) packaging. The Raman spectra from urea-spiked cow milk samples (0.25–5.0 g/L) in 60 μm LDPE were measured at an optimised spatial offset of 6.5 mm, which maximises the relative contribution of milk compared to the surface polythene.

Various processing steps were performed on the measured spectra such as Savitzky–Golay smoothing, range-independent background subtraction and SNV normalization. These processed data are then used as input for the chemometric analysis, such as least squares regression and partial least squares regression to quantify the adulteration. It was found that PLSR with the optimized number of variables performed best in estimation of the adulterant concentrations. The root mean square error (RMSE) in predicting adulterant concentration was estimated to be 0.243 g/L for previous unseen data used to build the regression model. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.22 g/L and 0.67 g/L, respectively. The method reliably detects all urea concentrations above the 0.70 g/L, a limit set by FSSAI for safe use of the milk. The Fig. M.6.2a shows the correlation plots between reference and predicted urea concentrations, which show $R^2 = 0.99$ and RMSE=0.243 g/L.

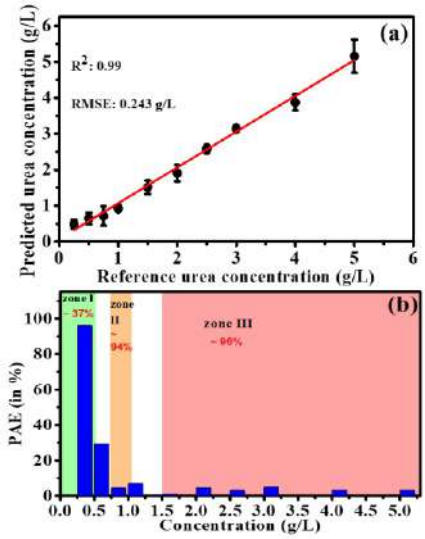

Fig. M.6.2: (a) Correlation plots between the reference and predicted concentrations. (b) Plot of the percentage absolute error at each adulteration.

The percentage absolute error (PAE) at each measured concentration is shown in Fig. M.6.2b and are organized into three zones: a safe green zone (⩽ 0.50 g/L) with low regulatory concern but higher relative error, an orange zone (0.75–1.0 g/L) and a red zone (1.5–5.0 g/L) where prediction accuracies are more than 94% and 96% respectively. To conclude, the developed inverse spatially offset Raman spectroscopic technique demonstrates the ability of quantitative detection of urea concentration in packaged milk samples without opening the packet and without the need of tedious sample preparation procedure.

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