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UNCERTAINTY EVALUATION FOR THE QUANTIFICATION OF MELAMINE IN MILK DETECTED BY SURFACE ENHANCED RAMAN SCATTERING (SERS)

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ABSTRACT

- A **calibration procedure** is presented for the quantification of melamine in spiked milk samples, based on the application of **Weighted Total Least-Squares (WTLS)**, together with the evaluation of the associated **uncertainty**, carried out by applying the Law of Propagation of Uncertainty (LPU).
- This approach was applied to an experimental innovative method developed and validated at INRIM based on the **Surface Enhanced Raman Scattering** (SERS).
- SERS was exploited for the screening and the quantification of melamine in milk due to its specific fingerprint in the Raman spectrum and to the huge enhancement of the melamine Raman signals promoted by its interaction with gold nanoparticles (AuNPs).

1. MELAMINE AS FOOD ADULTERANT

- With the widespread growth of food adulteration, **food safety** has emerged as a fundamental issue.
- **Melamine** (2,4,6-triamino-1,3,5-triazine) is an organic compound that is commercially synthesized from urea and used for the production of melamine-formaldehyde resins and for polymer manufacturing.
- Melamine is known for many cases of food adulteration, occurred around 2008: being a high rich-nitrogen molecule, melamine was intentionally added into food ingredients to apparently boost the protein content, determined by total nitrogen determination (as by the Kjeldhal or Dumas methods).

2. QUANTIFICATION OF MELAMINE BY RAMAN SERS

- The INRIM method in fig. 2 [1] was developed in accordance with the European law limits for melamine in foodstuffs [2].
- **Samples**: Aliquots of a 100 mg L^{-1} melamine solution were added to the semi-skimmed milk. After the extraction procedure, which results in a 10-fold dilution of the samples, the amount of substance concentrations of melamine in the milk samples are 0.099 mg L^{-1} and 0.291 mg L^{-1}.
- **Calibration**: The Raman spectrophotometer was calibrated by means of melamine standard solutions in a non-spiked milk extract, in the concentration range (0.016-0.50) mg L^{-1}. The software used for determining the calibration curve of the Raman spectrophotometer is the **Calibration Curves Computing (CCC) software** [3], developed at INRIM.

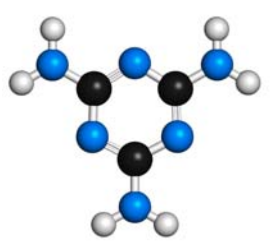
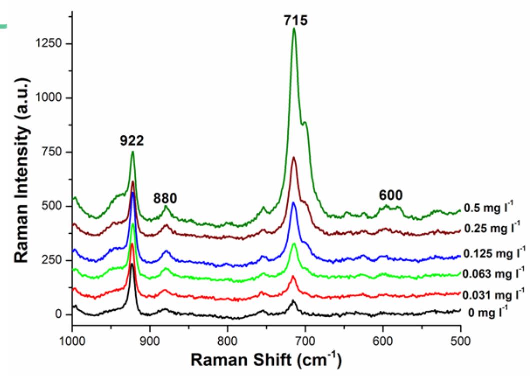


Fig 1: SERS spectra of 5 levels of melamine concentration with 40 nm AuNPs (melamine peak at 715 cm^{-1}) [1]



3. MODEL EQUATION

- The measurand, C_{mel}, is the melamine concentration (in mg L^{-1}) in milk samples, as defined by the **model equation** (eq. 1):

$$C_{mel} = \frac{C_a \cdot f_d}{R} \qquad (1)$$

where:
C_a = concentration of melamine determined by SERS (mg L^{-1})
f_d = dilution factor (due to the extraction procedure)
R = recovery efficiency

- This work focuses on the evaluation of the **recovery efficiency *R*** and the uncertainty associated with this parameter. *R* can be defined as the ratio (eq. 2):

$$R = \frac{C_{calc}}{C_{theor}} \qquad (2)$$

where:
C_{calc} = concentration of melamine determined by SERS in the spiked extracts (mg L^{-1})
C_{theor} = theoretical concentration of melamine in each spiked extract (mg L^{-1})

4. UNCERTAINTY EVALUATION

- For the evaluation of $u(C_{calc})$, the inverse of the calibration curve $y = a + bx$, i.e. the analysis curve, obtained via WTLS regression [4] was used. The analysis curve (eq. 3) gives the values of the unknown amount of substance concentration of melamine C_{calc} in each spiked extract analysed by SERS:

$$C_{calc} = \frac{(y-a)}{b} \qquad (3)$$

- The theoretical amount of substance concentration of melamine C_{theor} in each spiked extract was obtained by a dilution process. The uncertainty $u(C_{theor})$ was evaluated by applying the LPU to the model equation (eq. 4):

$$C_{theor} = C_i \cdot \frac{V_i}{(V_i + V_s)} \qquad (4)$$

where:
C_i = initial amount of substance concentration of the melamine solution to be added to the milk sample (in mg L^{-1})
V_i = initial volume of the melamine solution (in L)
V_s = volume of the dilution solvent (in L)

- The main advantage of the WTLS is the possibility to deal with regression problems involving uncertain and correlated input and output variables - e.g. the determination of calibration curves when the uncertainties on the independent variables x_i (i.e. the concentrations of the calibration solutions) cannot be considered negligible with respect to those associated with the dependent variables y_i (i.e. the analytical response) and when correlation exists among x_i and among y_i

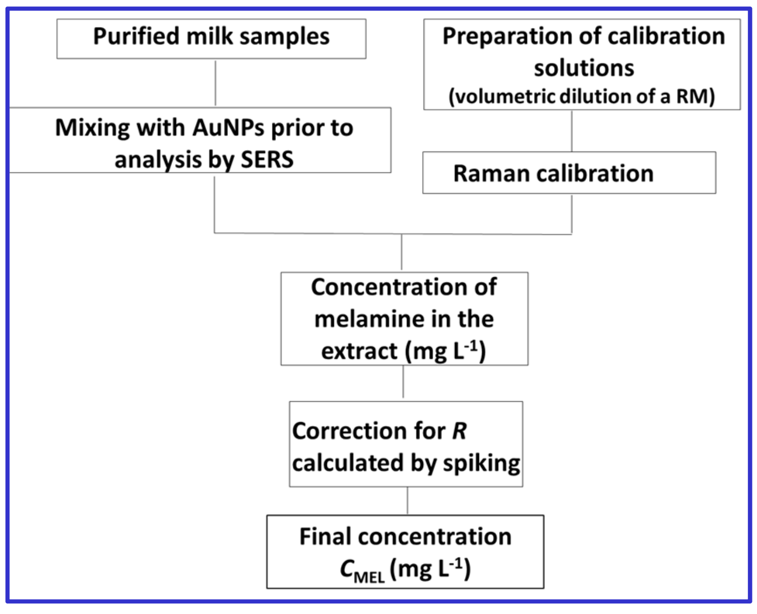


Fig 2: INRIM method for the quantification of melamine by SERS

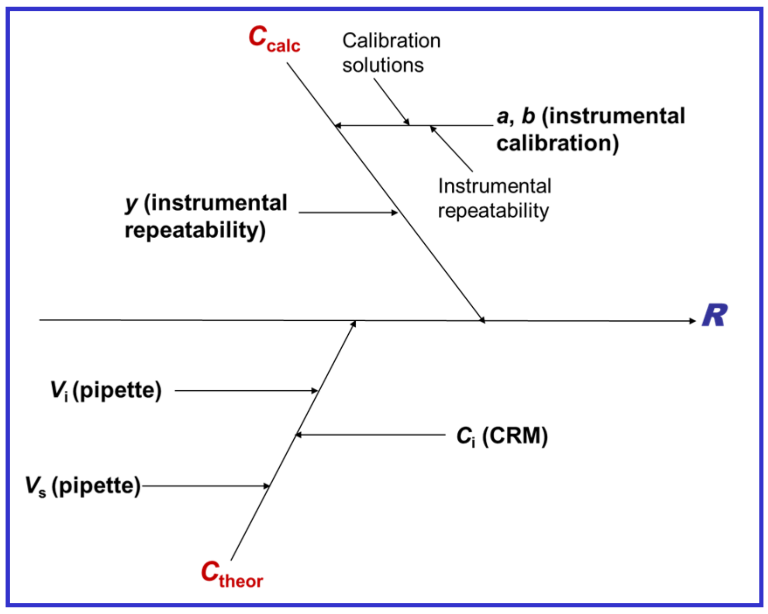


Fig. 3: Ishikawa diagram showing the contributions of the input quantities to the final uncertainty of R, $u_c(R)$

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