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# QUANTITATIVE DETECTION OF MELAMINE ADULTERATION IN MILK POWDER USING γ-RAY SPECTROSCOPIC TECHNIQUE

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## ABSTRACT

We have used a non-destructive γ-ray spectroscopic technique for the detection of melamine contamination in solid milk powder. The attenuation characteristics of melamine, milk powder and the mixtures of melamine and milk powder were examined. The results indicate that the linear attenuation coefficient increases with increase in the quantity of melamine contamination in milk powder. The results demonstrate that the above technique can be used for the non-destructive qualitative and quantitative detection of melamine in adulterated milk powder.

**KEYWORDS:** Melamine, Milk Powder, Linear Attenuation Coefficient, γ - Ray Spectroscopy

## INTRODUCTION

Expert committees from World health Organization (WHO) and Food and Drug Administration (FDA) investigations revealed that the cause of deaths was due to the crystal formation and blockage of renal tubes leading to failure in the functioning of kidney. [1- 5] The analyses of the crystals formed in the kidney revealed to consist of meal mine and cyanuric acid (in pet animals) and melamine-uric acid (in humans). [6, 7] Melamine is not a dietary constituent and hence might be a contaminant/adulterant in food chain. Melamine and its derivatives are industrial chemicals which are routinely used in the synthesis of melamine-formaldehyde resins, adhesives, hand sanitizers, white boards, laminates, dish wares, tanning of leather, synthesis of organic compounds etc. To overcome the limitations of the above technique, high performance liquid chromatography (HPLC), gas chromatography (GC), mass spectroscopy (MS), Raman spectroscopy, Infrared spectrometry, ionization by laser desorption method, HPLC with UV detection, enzyme immunoassay, ultrasound-assisted extractive electro spray ionization, capillary zone electrophoresis were developed to detect melamine in samples at ppm (parts per million) level.[8- 15] The major limitation is the cost of the instruments, demand high skills to carry out the measurements, withering the sampled being analyzed and is also economically and practically not feasible for routine analyses.

As an alternative to the above methods, visual detection methods have been developed for the detection of melamine. Coloimetric methods have been reported to use gold and silver based nano particles as probes i.e crown ether assembled gold nano particles, cysteamine modified gold nano particles, citrate capped gold nano particles, astamer-gold nano particles, para-nitrobenzene sulfonic grafted silver nano particles etc.[16] In the above visual methods, the colour change on binding nano particles to melamine was monitored. The γ-ray spectroscopy is extensively used to carry out research in nuclear science, defense and medicine. Interaction of γ-ray radiation with materials can provide significant information about the nature of the sample and has also been used to characterize the samples. The photon attenuation

coefficient and mass absorption coefficient are dependent on the energy of the incident γ-radiation and the chemical composition of the shielding material. The linear attenuation and mass attenuation coefficients of dilute solutions of ferrous sulfate, potassium chloride and ammonium chloride, cobalt sulphate at different concentrations using different gamma energies are reported.[17- 21] The concentrations of organic compounds i.e phenol and p-nitroaniline in ethanolic solutions have also be determined based on the linear and mass attenuation coefficients.[22] Adulteration of milk samples with urea, lactose, water in solution phase have been reported using γ-ray spectroscopy.[23] To the best of our knowledge there are no reports on the application of γ-ray spectroscopy to determine the melamine in solid milk powder. The linear attenuation coefficient increases with increase in the melamine content in milk powder. We have compared the quantity of melamine taken to that estimated using γ-ray spectroscopy and matches well thereby reporting the efficiency and effectiveness of the method.

## EXPERIMENTAL WORK

### Materials Used

Melamine (SD Fine Chemicals, India) and milk powder were procured from commercial sources and used as such.

The intensity of gamma ray after transmitting through the material of thickness 'x' is:

$$I = I_0 e^{-\mu x}$$

Where '$I_0$' and 'I' are the initial and final intensities of γ-ray radiation, 'μ' is linear attenuation coefficient of material for γ-ray.

In analogy with the above equation the intensity of gamma radiation after traversing the height 'h' of chemical mixture is given by $I = I_0 e^{-\mu h}$

Using the values of density (ρ) and linear attenuation coefficient (μ), the values of mass attenuation coefficient $\left(\frac{\mu}{\rho}\right)$ for different energies can be calculated. For the mixture or compounds, the total mass attenuation coefficient $\left(\frac{\mu}{\rho}\right)$ is related to the $\left(\frac{\mu}{\rho}\right)$ values of each chemical constituent. The total mass attenuation coefficient of the admixture of compounds can be calculated by the following mixture rule:

$$\left(\frac{\mu}{\rho}\right) = w_i \left(\frac{\mu}{\rho}\right)_i$$

Where $w_i$ is the fraction by weight of the $i^{th}$ constituent and $\left(\frac{\mu}{\rho}\right)_i$ is the mass attenuation coefficient of the $i^{th}$ constituent. For homogeneous mixture of melamine powder and milk powder, linear attenuation coefficient:

$$\mu_{mix} = w_{melamine}\, \mu_{melamine} + w_{milk}\, \mu_{milk}$$

Here $w_{melamine}$ is the fraction by weight of melamine powder, $\mu_{melamine}$ the linear attenuation coefficient of melamine powder, $w_{milk}$ the fraction by weight of milk powder and $\mu_{milk}$ the linear attenuation coefficient of milk powder. GSpec-Gamma ray spectroscopy consisting of NaI (Tl) crystal detector of size 2″× 2″ and MCA was used for the measurement linear attenuation coefficients of melamine, milk powder and admixture of melamine and milk powder.

**Calibration and Method of Measurement Using γ-Ray Spectroscopy**

Data acquisition and control is through PC based software, SAAS (Spectrum Acquisition and Analysis Software). The multi-channel analyzer displays the number of counts corresponding to the particular voltage on the vertical axis v/s the amplitude of the voltage pulse on the x-axis. Each position on the x-axis is known as a channel. Energy calibration was carried out by selecting two or three energy peaks of known radioisotopes. In the present work calibration was done using Co-60 (1.17 MeV, 1.333 MeV) and Cs-137 (0.662 MeV) source.

The gamma spectrometry system was initially tested for resolution, constancy and linear characteristics to fix the best operating conditions by performing groundwork experiments. The detector resolution was found to be 6% for 662 KeV gamma rays at operating voltage 750V. The materials used were melamine, milk powder and admixture of melamine and milk powder. The experimental set up for the determination of linear attenuation coefficient of the melamine, milk powder and the mixture of melamine and milk powder is shown in the figure 1. The experimental set up provides narrow beam geometry.

The Cs-137 radioactive source was kept in line with the gamma spectrometry assembly. The area under the peak was used for the attenuation measurements. Empty cylindrical glass container was introduced between the detector and the Cs-137 radioactive source. When the acquisition started, spectrum was collected and the control menu will make the stop acquisition button active. Acquisition will continue till the acquisition time matches the preset time. The gamma spectrum from the Cs-137 source was recorded for 101 seconds. For spectral analysis, it is necessary to select the peak regions. This was done by selecting the Region of Interest (ROI). The 662 keV photopeak has been identified and ROI was fixed.

The SAAS provides initial integral counts, background counts and background subtracted counts under ROI. The number of counts under ROI with empty container was recorded for 101 seconds. Next, melamine powder was filled in the glass container to a height of 0.5 cm and placed in between the source and the detector. The gamma spectrum was acquired for 101 seconds and number of counts under ROI was noted.

The experiment was repeated with increasing height of the melamine powder in the glass container. For measurement of linear attenuation coefficient of milk powder same procedure was employed. For different weighted ratios of melamine and milk powders, initially, the 2g of melamine powder was added to the 20g milk powder in the glass cylinder. This mixture contains 9.09% w/w of melamine and 90.91%w/w of milk powder.

The top of the glass cylinder was gently sealed by stretching and tightly wrapping a piece of Para film around it. The glass cylinder was hold securely and gently invert several times to obtain homogeneous mixture. The same procedure was used to prepare mixture containing16.67%w/w, 23.08%w/w and 28.57w/w of melamine. The mixture containing 9.09% w/w of melamine and 90.91%w/w of milk powder was taken in cylindrical metal vessel of height 3cm and the vessel was sealed by outer vessel.

The system was placed in line with the detector and the Cs- 137 source. The gamma spectrum was acquired for 101 seconds and number of counts under ROI has been recorded. The experiment was repeated with mixture containing16.67%w/w, 23.08%w/w and 28.57w/w of melamine. All the attenuation measurements were carried out with fixed height of mixture, 3cm in the cylindrical metal vessel.

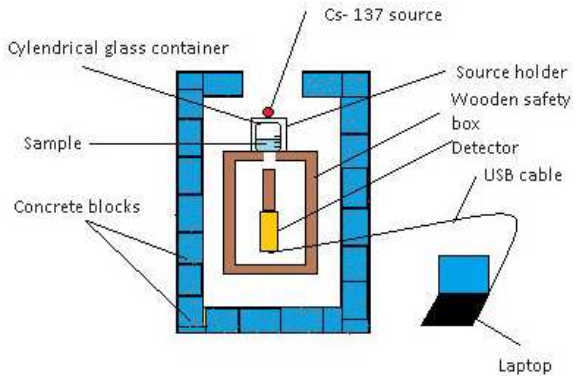


**Figure 1: Schematic of Experimental Arrangement**

## RESULTS AND DISCUSSIONS

The γ-ray spectroscopy system used is simple and readily portable. The weight ratios of melamine and milk powder (9.09, 16.67, 23.08, 38.57 wt% of melamine in the mixture of milk were used for the measurements and the details are given in Table 1. The values of linear attenuation coefficient of the milk powder, melamine and the mixture of melamine and milk powder at different weight ratios were determined by following the above procedure. Table 2 shows the variation of ln ($I_0$/I) with respect to height of milk powder. The table 2 also shows the value of linear attenuation coefficient obtained by experiment and value of linear attenuation coefficient obtained by linear fit to the plot shown in the figure 2. Table 3 shows the variation of ln ($I_0$/I) with respect to height of melamine powder, experimental value of linear attenuation coefficient of melamine powder and the value of linear attenuation coefficient obtained by linear fit to the plot shown in Figure 3. To find variation of linear attenuation coefficient with %w/w of melamine in the mixture of milk powder and melamine, the experiment was carried out with fixed height of the mixture. The values of linear attenuation coefficient of the mixture for varying %w/w of melamine at 662 ke Vare tabulated in the Table 4. The Figure 4 shows the variation of linear attenuation coefficient with %w/w of melamine in the mixture with ±5% error bars.

**Table 1: Weight Ratios of Melamine and Milk Powder**

| % w/w of Melamine in Mixture | % w/w of Milk Powder in Mixture |
|---|---|
| 0 | 100 |
| 9.09 | 90.91 |
| 16.67 | 83.33 |
| 23.08 | 76.92 |
| 28.57 | 71.43 |

**Table 2: Height of Milk Powder and ln ($I_0$/I)**

| Height of Milk Powder (cm) | Counts under ROI for 101 Seconds | Count Rate (Counts/Sec) | ln($I_0$/I) | Linear Attenuation Coefficient | Mean Linear Attenuation Coefficient μ (Experimental) | Linear Attenuation Coefficient μ (Linear Fit) |
|---|---|---|---|---|---|---|
| 0 | 45606 | 451.5446 | ---- | ---- | $0.0368cm^{-1}$ | $0.0360cm^{-1}$ |
| 0.5 | 44768 | 443.2475 | 0.0186 | 0.0371 | | |
| 1 | 43941 | 435.0594 | 0.0372 | 0.0372 | | |
| 1.5 | 43157 | 427.2970 | 0.0552 | 0.0368 | | |
| 2 | 42336 | 419.1683 | 0.0744 | 0.0372 | | |
| 2.5 | 41577 | 411.6502 | 0.0925 | 0.0370 | | |
| 3 | 40839 | 404.3465 | 0.1104 | 0.0368 | | |
| 3.5 | 40165 | 397.6733 | 0.1270 | 0.0363 | | |
| 4 | 39474 | 390.8317 | 0.1444 | 0.0361 | | |

**Table 3: The Height of Melamine Powder on the Linear Attenuation Coefficient and ln ($I_0$/I) Values**

| Height of Melamine Powder (cm) | Counts under ROI | Count Rate (Counts/Sec) | ln($I_0$/I) | Linear Attenuation Coefficient | Mean Linear Attenuation Coefficient-μ (Experimental) | Linear Attenuation Coefficient-μ (Linear Fit) |
|---|---|---|---|---|---|---|
| 0 | 45316 | 448.6733 | ---- | ---- | 0.0622cm$^{-1}$ | 0.0612 cm$^{-1}$ |
| 0.5 | 43909 | 434.7426 | 0.0315 | 0.0631 | | |
| 1 | 42570 | 421.4895 | 0.0625 | 0.0625 | | |
| 1.5 | 41254 | 408.4604 | 0.0942 | 0.0626 | | |
| 2 | 39991 | 395.9505 | 0.1250 | 0.0625 | | |
| 2.5 | 38800 | 384.1584 | 0.1552 | 0.0621 | | |
| 3 | 37659 | 372.8614 | 0.1851 | 0.0617 | | |
| 3.5 | 36553 | 361.9109 | 0.2149 | 0.0614 | | |
| 4 | 35419 | 350.6873 | 0.2464 | 0.0616 | | |

**Table 4: The % w/w of Melamine - Milk Powder Mixture and Linear Attenuation Coefficient**

| % w/w of Melamine in Mixture | % w/w of Milk Powder in Mixture | Counts under ROI | | | | Count Rate | ln(I/$I_0$) | Linear Attenuation Coefficient |
|---|---|---|---|---|---|---|---|---|
| | | Trail-1 | Trail-2 | Trail-3 | Mean | | | |
| 0 | 0 | 35626 | 35328 | 35226 | 35393 | 350.4290 | ---- | ---- |
| 0 | 100 | 31902 | 31616 | 31553 | 31690 | 313.7657 | 0.1105 | 0.0368 |
| 9.09 | 90.91 | 31302 | 30921 | 31240 | 31154 | 308.4587 | 0.1276 | 0.0425 |
| 16.67 | 83.33 | 30826 | 30737 | 30624 | 30729 | 304.2475 | 0.1413 | 0.0471 |
| 23.08 | 76.92 | 30315 | 30382 | 30531 | 30409 | 301.0825 | 0.1518 | 0.0506 |
| 28.57 | 71.43 | 30362 | 30017 | 29796 | 30058 | 297.6073 | 0.1634 | 0.0545 |

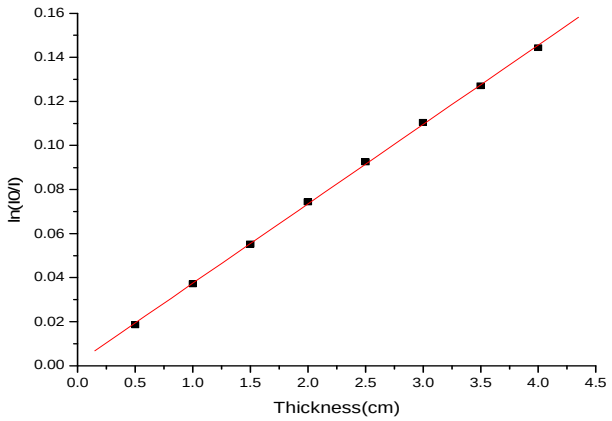


**Figure 2: ln $\left(\frac{I_0}{I}\right)$ v/s Height (h) of Milk Powder**

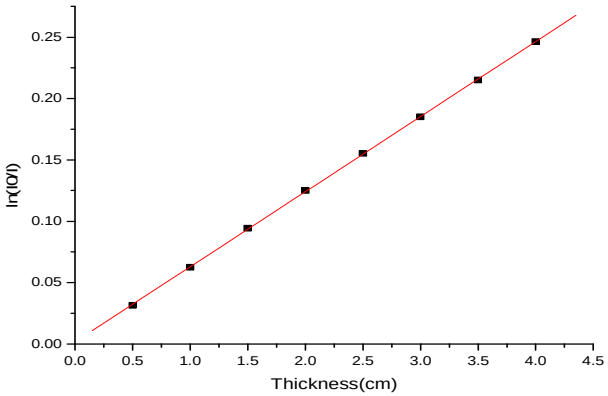


**Figure 3: ln ($I_0$/I) v/s Height of Melamine Powder**

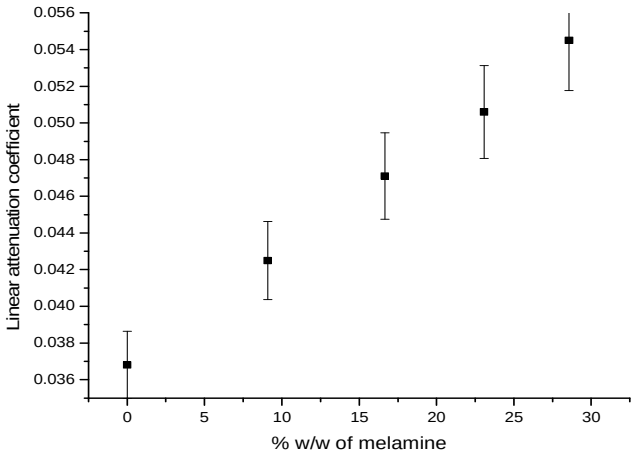


**Figure 4: The % w/w of Melamine in the Admixture of Melamine-Milk Powder and Linear Attenuation Coefficient**

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