Visual Indicator Based on Leuco Crystal Violet for Radiation Processing Technology

S. H. Shinde, S. Mondal, and V. Sathian

Abstract—**One of the important technologies for food preservation and processing is radiation processing, which is growing at ever increasing rate in India. Efforts are being done to make it more cost-effective, so there is always a need for cost-effective, indigenously developed visual indicators for providing an easy identification and segregation of irradiated products. Thus development of cost-effective visual indicator based on leuco crystal violet for doses ≥ 10 kGy was under taken. Current research works deals with fabrication and characterization of various parameters such as optimum composition, light stability, temperature effect and effect of relative humidity on the new indicator.**

Keywords—**Visual indicator, Radiation processing technology, Leuco crystal violet, Reflectance.**

I. INTRODUCTION

Radiation processing technology is a field that makes use of large doses of radiation such as gamma rays, accelerated electrons and X-rays in-order to achieve specific biological, chemical or physical effects in a specified product [1]. In all radiation processing, accurate dosimetry ensures that the radiation treatment required for the process is correctly applied. But in-order to determine whether a product has been irradiated or not, the techniques used are generally based on physical, chemical, biological, and microbiological changes in irradiated products. However, none of these methods have the potential to effectively characterize all products in accordance to their irradiation history and usually multiple methods are need to be applied. However, a simpler method is to use self-adhesive visual indicators on product boxes. These indicators change color on irradiation to a specified dose; thus indicates visually whether or not a product has been irradiated and help in distinguishing irradiated process loads from the unirradiated process loads [2].

Availability of high intensity cobalt-60 gamma ray sources and high power electron beam accelerators has led to a continuous growth of radiation processing industry in India. There is a huge demand for indigenously developed cost-effective visual indicator. Hence an attempt was made to develop a visual indicator for high dose food radiation processing applications i.e. for doses ≥10 kGy.The mechanism of the radiation-induced color change of the LCV from colorless to deep purple can be attributed to formation of the highly coloured quinoid chromophore as a part of resonant carbonium cation (CV^+). Use of thin films containing leuco crystal violet (LCV) in poly vinyl butyral for high dose dosimetry in the range of 1 – 100 kGy has been reported [3]. These PVB-LCV films were meant to be used as dosimeters and not as visual indicators hence light stability for short period of 3 days, was considered insufficient for visual indicator. Also, when such a system is to be proposed as a visual indicator, it should be least affected by varied environment factors such as light, temperature and humidity. This became the hypothesis for the development of visual indicator; as LCV is easily available commercially and also very cost-effective as compared to diacetylenes used in some of the visual indicators [4][5][6][7], and [8].

II. METHOD OF RESEARCH

All reagents and solvents were obtained from Merck, Germany and used without further purification. All glasswares were cleaned as per the recommended procedure [9]. The optimum LCV concentration in the indicator required for obtaining the significantly visible color change;was determined by preparing 1, 2, 3 and 4 % solution of LCV in solvent mixture of trichloroethanol and toluene (1/4 v/v) containing 20% polystyrene,1% tinuvin-327 i.e. 2-tert-Butyl-6-(5-chloro-2H-benzotriazol-2-yl)-4-methylphenol and 1% Irganox-1076 i.e. Octadecyl 3,5-di-tert-butyl-4-hydroxyhyrocinnamate. All the reagents were mixed thoroughly.

20 number of paper strips each having dimensions of 30 mm width and 60 mm length, were cut from a single spotless whiteA4 size paper. Length of 40 mm of these strips were dipped into these solutions and kept hanging for drying in dark placefor 24 hrs and further stored at room temperature under normal laboratory conditions. This coating method produced uniformly thick indicator, each having an average thickness of 0.052 ± 0.0035 mm, measured by thickness gauge (Mitutoyo, Japan). Gamma Chamber-1200 was used for irradiation. It was calibrated at the centre position of its irradiation volume as per the recommended procedure [10]; using Fricke dosimeter - a reference standard [11]. Specially designed perspex jig was used for providing reproducible geometry and electronic equilibrium during irradiation of the indicators. Gamma Chamber-1200 was found to have a dose rate of 25.35 Gymin^{-1}. Irradiation temperatures encountered during irradiation were around30°C.

UV-Vis spectrophotometer (Shimadzu UV 3600 Plus, Japan) along with ISR-603 integrating sphere attachment was used to measure the reflectance spectra of unirradiated and irradiated in the wavelength range of 450-700 nm. Absorbance values were then calculated from these reflectance spectra assuming diffuse reflectance for these indicators irradiated to various doses. Fig. 1 represents the absorbance spectra for indicators having varied LCV concentrations irradiated to different doses. For each of the LCV concentration, indicators were irradiated in triplicates and then absorbance values were calculated. Errors bars in Fig. 1 represent the standard deviation in absorbance values.

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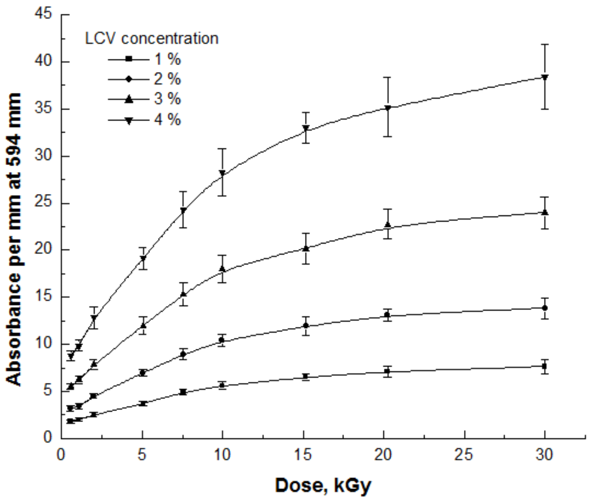


Figure 1. Change in response of irradiated labels having varied LCV concentration.

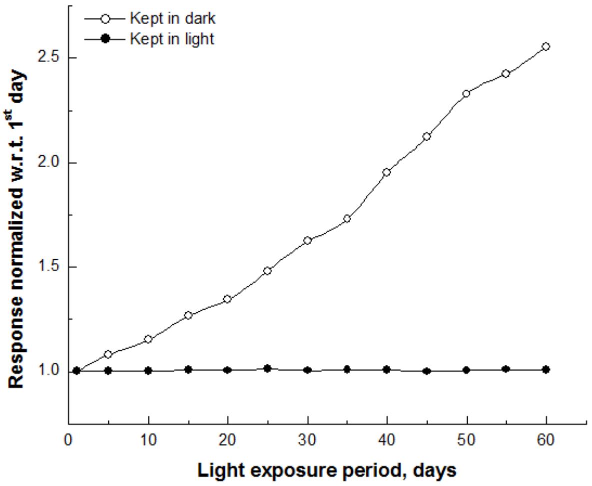


Figure 3. Light stability of unirradiated labels.

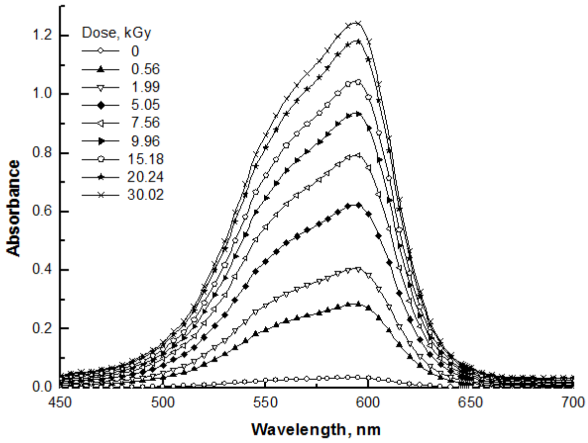


Figure 2. Absorbance spectra of unirradiated and irradiated labels.

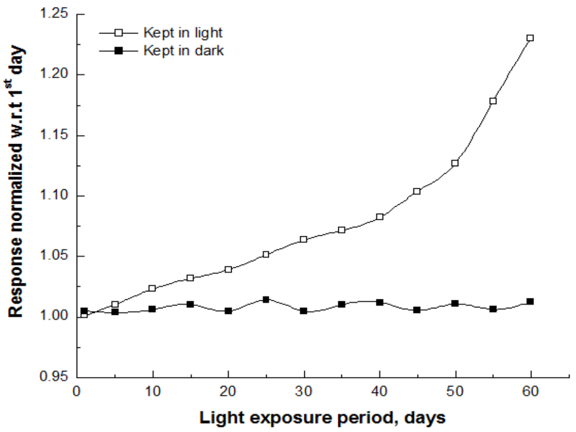


Figure 4. Light stability of irradiated labels.

Fig. 2 represents absorbance spectra for indicators having 3% LCV irradiated to different doses.

Pre-irradiation and post-irradiation light stability of these indicators was studied by storing the unirradiated and irradiated indicators under normal fluorescent light along with scattered daylight in laboratory at room temperature for a period of 60 days. These indicators were not exposed to direct sunlight. Figure 3 & 4 shows the change in response of unirradiated and irradiated indicators respectively.

Effect of pre-irradiation storage temperature on response of the indicators was studied in the temperature range of 25 to 65ºC. Indicators were kept in a pre-cleaned petri dish and placed inside constant temperature hot air oven (Shital Industries, India) at the required preset temperature for a period of 5 hrs and then irradiated to a dose of 10 kGy. The reflectance values at 594 nm for each of these indicators were measured. Response of these indicators were normalized with respective to that at 25ºC and are as represented as Fig. 5.

Effect of post-irradiation storage temperature on irradiated indicators was studied in the temperature range of 25 to 65ºC. Indicators were initially irradiated to a dose of 10 kGy and kept in a pre-cleaned petri dish and placed inside constant temperature hot air oven (Shital Industries, India) at the required preset temperature for a period of 5 hrs and then the reflectance values at 594 nm were measured for each of these indicators. Response of these indicators were normalized with respective to that at 25ºC and are as represented as Fig. 6.

The effect of relative humidity (RH) on the dose response of these indicators was investigated in the RH range from 0 to 97%. Unirradiated indicators were kept in a pre-cleaned petri dish and placed inside desiccators containing the required saturated salt solution in order to establish various relative humidity ranging from 12 to 97% [12][13]. Table 1 shows the list of salts used for this purpose. RH was measured using a digital hygrometer with an in-built temperature sensor. Zero percent RH was obtained by using dried silica gel.

Figure 7 shows the set-up for conditioning indicators to various RH conditions ranging from 0 to 97%. For each RH, indicators were conditioned for a period of 10 days.

All these conditioning exercises were conducted at average room temperature of 27.2 °C. Each of these indicators was then sealed packed in polythene bags and irradiated to dose of 10 kGy. The reflectance values at 594 nm for each of these indicators were measured. Response of these indicators were normalized with respective to that at 0 % RH and are as represented as Figure 8.

III. RESULT AND DISCUSSION

The reflectance spectra of unirradiated indicators as well as irradiated indicators with doses in the range of 2.5 to 50 kGy were measured in the visible spectrum

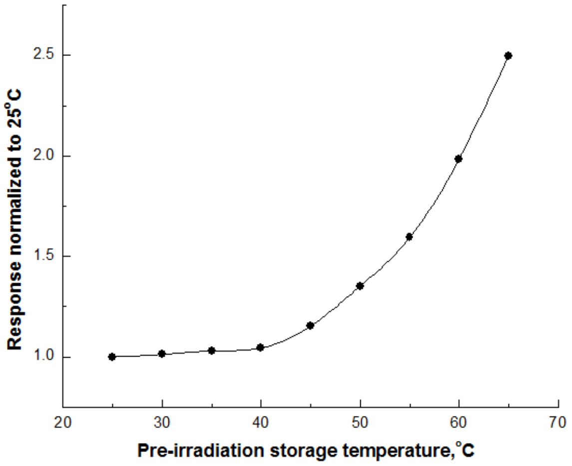
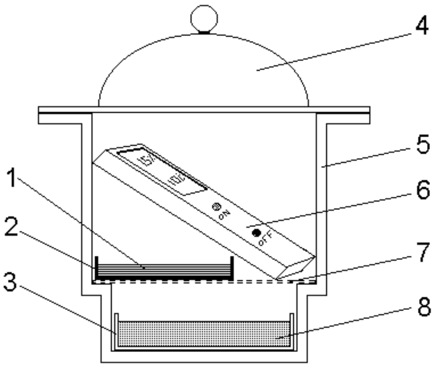


Figure 5. Change in response of labels with pre-irradiation storage temperature.



1→ Labels, 2→ Petri dish (small), 3→ Petri dish (large), 4→ Lid, 5→ Desiccator
6→ Digital hygrometer, 7→ Wire mesh, 8→ Saturated salts

Figure 7. Set-up for conditioning labels to different RH.

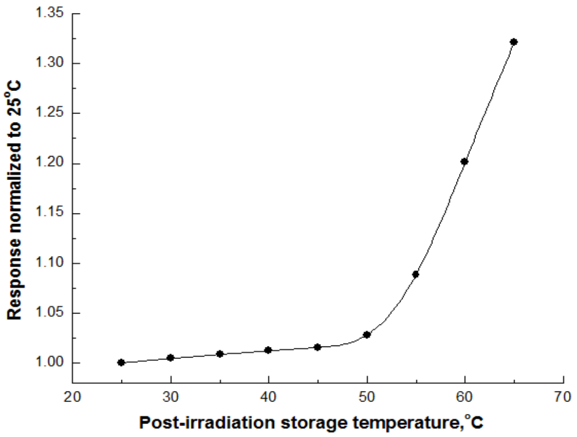


Figure 6. Change in response of labels with post-irradiation storage temperature.

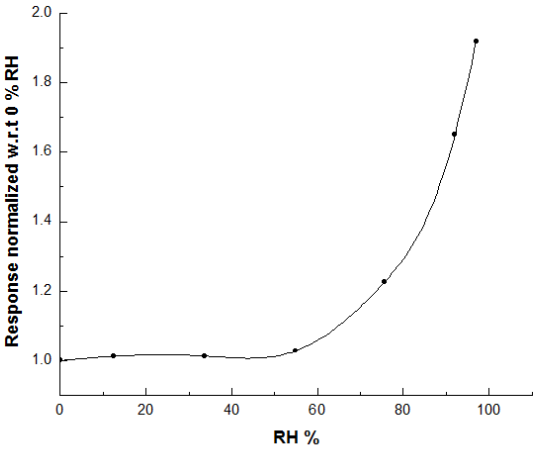


Figure 8. Change in response of labels with varied humid during pre-irradiation storage.

range of 450-700 nm. Absorbance values were then calculated from these reflectance spectra assuming diffuse reflectance for these indicators irradiated to various doses. It is evident from the Fig. 1, that with increasing LCV concentration; the response also increases. The response for 1 & 2 % LCV indicators is significantly low and for 4% LCV indicators, the response increases significantly even after 10 kGy. As it was attempted to develop indicator for doses in the range of 10 kGy, optimum concentration of 3% was selected, as the response is significantly high for doses ≥ 10 kGy and it increases steadily till 30 kGy as compared to that for 4% LCV indicators.

Figure 2 represents absorbance spectra for these indicators having 3% LCV. It is clear from Fig. 2 that wavelength of maximum absorbance is 594 nm. In-order to normalize the response for variations in indicator thickness, absorbance values at 594 nm were divided by the average indicator thickness of 0.052 mm, response thus obtained was then plotted for respective dose values.

Effect of light during pre-irradiation and post-irradiation storage of these indicators was studied by storing these indicators under normal fluorescent light along with scattered daylight in laboratory at average room temperature of 26.2 ºC for a period of 60 days. At regular intervals of time, reflectance values were measured at 594 nm and normalized with respect to that at 1st day. From Fig. 3 & 4, it can be inferred that irradiated and unirradiated indicators stored in dark place are unaffected in terms of response during 60 days of storage period as variation in response does not exceed 1.5% and 2% over the storage period for irradiated and unirradiated indicators, respectively. But, unirradiated indicators stored under laboratory fluorescent light show significant increase in response after 40 days of storage period, however the increase of response of irradiated indicators is much slower. As the amount of leuco-form of LCV dye in unirradiated indicator is much higher than that in irradiated indicator, the unirradiated indicator is more affected by light than the irradiated indicator. During the storage period of 60 days, it was found that the increase in response for the unirradiated indicators was drastically high to about 250% and was significantly low to 22% for irradiated indicators.

Pre-irradiation and post-irradiation storage temperatures may have profound effect on the response of unirradiated and irradiated indicators respectively; hence these indicators were exposed to temperatures in the range of 25 to 65ºC using constant temperature hot air oven. Each of the irradiated indicators was exposed to a dose of 10 kGy. Response of these indicators was normalized with respect to that at 25ºC and plotted against respective temperatures as shown in Figs. 5 & 6. The response of unirradiated indicators as represented in Fig. 5; increases gradually i.e. <5 % in the temperature

TABLE 9.
SATURATED SALT SOLUTIONS REQUIRED FOR OBTAINING THE DESIRED RH IN A CLOSED CONTAINER

Saturated salt solution	Relative humidity %
Lithium Chloride monohydrate	12.4
Magnesium Chloride Hexahydrate	33.6
Magnesium Nitrate Hexahydrate	54.9
Sodium Chloride	75.5
Potassium Nitrate	92.0
Potassium Sulphate	97.0

range of 25 to 40^oC however after 40^oC it increases drastically to more than 250% upto65^oC. As seen from Fig.6, the response of irradiated indicators increases <2.5 % in the temperature range of 25 to 50^oC however after 50^oC it increases significantly to more than 30% in the temperature from 55 to 65^oC

The effect of relative humidity during pre-irradiation storage conditions on the response of unirradiated was investigated by storing these indicators at different relative humidity ranging from 0 to 97% as shown in Fig.7. These indicators were then irradiated to dose of 10kGy. Fig. 8 shows the change in response normalized with respect to that at 0% RH, as a function of RH%. The variation in response of these indicators is <3% upto 55% RH but then it increases significantly with the increase of RH%.

IV. CONCLUSIONS

Newly developed indicator was prepared using polystyrene instead of polyvinyl butyral (PVB) as it is cost effective than PVB and commercially easily available. Optimum concentration for LCV was determined to be 3 % in solvent mixture of trichloroethanol and toluene (1/4 v/v) containing 20% polystyrene, 1% tinuvin-327 and 1% Irganox-1076. Trichloroethanol was added to toluene solvent to sensitize the oxidation of LCV on irradiation into crystal violet carbonium cation (CV+), as is reported elsewhere [14]. Tinuvin-327 was used instead of Tinuvin-P i.e. 2-(2H-benzotriazol-2-yl)-p-cresol; as it is cost effective. This was added as an UV absorber to protect from fluorescent lights in the lab and sunlight in outdoor conditions. There was no protection from thermal degradation in PVB-LCV films; hence Irganox-1076 was used as an anti-oxidant to protect the indicators from thermo-oxidative degradation in outdoor conditions.

The response of these indicators was studied in the dose range of 2.5 to 50 kGy were measured in the visible spectrum range of 450-700 nm. Wavelength of maximum absorbance was found to be 594 nm. Effect of light during pre-irradiation and post-irradiation storage of these indicators was studied under normal laboratory fluorescent light along with scattered daylight for a period of 60 days. Irradiated and unirradiated indicators stored in dark place are unaffected in terms of response during 60 days of storage period as variation in response does not exceed 2%. But, unirradiated indicators stored under laboratory fluorescent light show significant increase in response after 40 days of storage period, however the increase of response of irradiated indicators is much slower. It therefore is recommended to store unirradiated indicators in dark condition.

Effect of pre-irradiation and post-irradiation storage temperatures on the response of unirradiated and irradiated indicators was studied for temperatures in the range of 25 to 65^oC. The response variation of unirradiated indicators is <5% upto 40^oC however then it increases drastically upto 65^oC. Therefore, it is recommended to store unirradiated indicators under 40^oC. However, variation in response of irradiated indicators is <2.5% upto 50^oC and then it increases significantly upto 65^oC. Hence it is recommended to use these indicators in the temperatures <50^oC.

As the variation in response is significantly high, when stored in relative humidity more than 55%, but the variation was insignificant (<3%) when stored in 0-55% humidity range, it is recommended to pack and seal unirradiated indicators under RH ranging from 0-55%.

Thus, the newly developed indicator is having better light stability, thermal stability and more resistance to relative humidity as compared to the PVB-LCV film. Since indicators are used as qualitative rather than a quantitative tool, hence estimation of uncertainty involved in dose estimation is not relevant. Overall, this indicator has good potential to be used as indigenously developed cost-effective visual indicator for various food irradiation facilities.

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