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Selection of the Optimal Concentration of Nitro Blue Tetrazolium Dye in Polyvinyl Alcohol Film for Diagnostic Radiology

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Abstract

Ensuring the limited exposure of both patients and medical professionals to radiation during examinations is paramount. One effective approach is the utilization of a film dosimeter that is not only efficient but also easy to carry. In this research, we employed polyvinyl alcohol/nitro blue tetrazolium (PVA/NBT) with varying concentrations of NBT dye (ranging from 0.015 to 0.3 g) to identify the most optimal concentration. These films were subjected to low doses of gamma rays (8, 25, 55 and 96 mGy) emitted from 3 Cs-137 sources. XRF analysis confirmed the effectiveness of the preparation method used, and the NBT salt exhibited excellent solubility ($R^2 = 0.93$). The films exhibited an increasing yellow hue as the concentration of NBT dye rose. Following irradiation, they took on a yellowish-brown tint, intensifying with higher gamma doses. PVA/NBT films, when stored in the dark, demonstrated good stability for 30 days post-irradiation. Analysis using ImageJ software unveiled that the relative density of the red color heightened proportionally with the concentration of NBT dye. XRD patterns showcased the clarity of structural rearrangements occurring after irradiation with high NBT concentrations. Two distinct peaks were registered at $2\theta = 20.12$ and 40.94 °, which shifted to $2\theta = 19.74$ and 41.30 ° post-gamma irradiation. The CIELab system study and K/S color strength indicated that increasing the NBT dye concentration led to a linear rise in color strength with escalating low gamma doses. Through a comprehensive assessment, it is concluded that PVA/NBT film can be effectively employed as a dosimeter for low gamma doses in diagnostic radiology.

Keywords: Nitro blue tetrazolium, X ray diffraction, CIELab, ImageJ, Color strength K/S, Film dosimeter

Introduction

Tetrazolium salts, classified as heterocyclic organic compounds and quaternary ammonium compounds, possess a notable property of forming water-insoluble, highly colored formazans through reduction [1]. Two categories of tetrazolium salts exist: mono-tetrazolium salts and di-tetrazolium salts [2]. Nitro blue tetrazolium (NBT)is an example of di-tetrazolium salt. When exposed to radiation, it undergoes a color change as NBT^{+2} is reduced to mono-formazan (MF^{+}), subsequently transforming into di-formazan (DF) with increasing doses. This unique characteristic renders it valuable for dosimetry applications [3,4].

Kovács *et al.* [5] were among the 1st researchers to investigate the efficacy of NBT dye on PVA film for dosimetry. They utilized different concentrations (1 and 2 mM) of NBT dye to prepare PVA/NBT films, which were exposed to a ^{60}Co γ-source at doses ranging from 1 - 50 kGy. The film exhibited a color change

post-irradiation, turning lilac at lower doses due to the formation of mono-formazan (MF^+) radiolysis product. As doses increased, the color shifted to blue with the appearance of di-formazan (DF). The study concluded that PVA/NBT film is suitable for dose determination within the 1 - 40 kGy range. Absorbance at 540 nm increased with higher NBT concentrations, widening the range of covered doses, affirming the reliability and effectiveness of PVA/NBT film in measuring doses in the suggested range.

Moussa *et al.* [6] demonstrated the radiation sensitivity of NBT^{2+}-PVA film. They tested various NBT^{2+} concentrations (0.5, 1.0 and 2.0 mmol/dm^3). Exposure to ^{60}Co γ-source and electron radiation (1 - 50 kGy) showed absorbance increased with higher NBT^{2+} concentrations, favoring 2 mmol/dm^3 for dosimetry. This suggests NBT^{2+}-PVA film could serve as a reliable dosimeter for process control. Another study by Basfar *et al.* [7] explored the impact of NBT dye concentrations (1 - 5 mM in ethanol) on PVB film. Gamma irradiation (5 - 55 kGy) showed increased dose sensitivity with higher NBT concentrations. Thus, researchers recommended these films for routine dosimetry in industrial radiation processing.

An investigation was recently conducted by Alashrah *et al.* [8] to study the effects of low doses of X-rays on PVA/NBT films. The films were exposed to X-rays ranging from 220 mGy. Their analysis of UV-Vis absorption revealed a proportional increase in absorbance with increasing low X-ray doses. FTIR and XRD characterizations confirmed this finding. Hence, they found that the PVA/NBT film can serve as a reliable dosimeter for routine diagnostic medical procedures.

In the present study, a PVA/NBT film was prepared with varying concentrations of nitro blue tetrazolium (NBT) to be presented as a gamma ray film dosimeter in the mGy scale, applicable in diagnostic radiology. The study aimed to assess the effectiveness of films with different concentrations through a color study using the CIELab system and color strength K/S, as well as the ImageJ program, both before and after exposure to gamma rays at doses of 8, 25, 55 and 96 mGy. Additionally, XRD analysis was employed to study the crystalline structure of the films, and XRF analysis determined the change in elemental composition for different concentrations, investigating the radiation effect on thin films. The study also sought to identify the optimal concentration of NBT dye in PVA/NBT films best suited for the conditions of this work.

Materials and methods

Materials used

The polymer matrix was made of Polyvinyl alcohol (PVA) (85 - 124 kDa & hydrolysis degree of 85 - 90 %) and was acquired from Sigma Aldrich (St. Louis, MO, USA). The dye indicator used in the film was Nitro blue tetrazolium chloride (NBT) ($C_{40}H_{30}Cl_2N_{10}O_6$; M_W = 817.65; +98 %) from the Alfa Aesar brand. It was purchased from Thermo Fisher Scientific in the USA. During the film preparation process, solubilization was carried out using ultrapure water (Milli-Q® Direct, Darmstadt, Germany).

Preparation of thin films

Nine samples of PVA/NBT films were meticulously prepared through a standardized procedure. Initially, 2 g of Polyvinyl alcohol was dissolved in 20 mL of distilled water per sample, ensuring thorough mixing. The solutions underwent agitation for 2 h at a constant temperature of 80 °C to attain homogeneity before being allowed to cool to room temperature. To achieve a diverse range of concentrations, Nitro blue tetrazolium (NBT) was incorporated. Varying amounts of NBT (0.015, 0.02, 0.025, 0.05, 0.1, 0.15, 0.2, 0.25 and 0.3 g) were dissolved in distilled water for each concentration level. Subsequently, the NBT mixture was blended with the PVA solution and stirred at room temperature for an hour across all samples. Each resulting solution was then evenly spread onto glass Petri plates and left to dry in darkness at room temperature for a standardized period of 2 days. Upon complete drying, the films were meticulously cut

into small 2 cm squares and securely stored in airtight envelopes to prevent light exposure until the irradiation phase.

The films were then subjected to gamma ray exposure emanating from 3 distinct Cs-137 continuous irradiation sources. These sources were calibrated to emit gamma rays at a uniform dose rate of approximately 50 μSv/h, accurately measured using the calibrated RaySafe 452 radiation survey meter. Various exposure durations were employed to achieve a spectrum of integrated absorbed doses, ranging from 8 to 96 mGy, ensuring comprehensive irradiation coverage. Post-irradiation, the samples were carefully stored in envelopes, maintaining their integrity, and prepared for subsequent measurement and analysis to evaluate their response to radiation.

Characterization and measurement of films

To conduct the stability test of the films and measure the relative density of the red channel, ImageJ software was employed. The PVA/NBT films were scanned using a Canon scanner (CanoScan LiDE 110), and the digital images were saved in TIFF format with a resolution of 300 dpi. All films were measured by an area of 0.028 in^2, which is adequate for small films. Relative density was calculated by subtracting the pixel value of the unirradiated film from the irradiated one, and the results were normalized. Finally, the percentage relative density was plotted against different concentrations of NBT.

The study included the analysis of the color of both irradiated and non-irradiated films using the CIE L^*, a^*, b^* system, initially recommended in 1976, has since become a widely adopted method for specifying colors and color differences. The 3 parameters L^*, a^* and b^* are utilized to represent various colors. L^* signifies lightness, with a value of 0 denoting perfect black and a value of 100 indicating perfect white. Positive values of a^* suggest a more red color, while negative values indicate a green color. Similarly, positive values of b^* suggest a yellow color, while negative values indicate a blue color [9]. This was conducted using a spectrodensitometer, specifically the 3NH YD5010. Measurements were taken under specific conditions: A standard observer of 10°, the daylight standard illuminant D_{65}, and M0 for measurement condition. The color difference (ΔE^*_{ab}) was calculated using Eq. (1), while all other parameters were directly obtained by the spectrodensitometer using the SDQC program, recommended by the manufacturer, through a USB connection. All variables were then plotted as a function of different NBT concentrations using Microsoft Excel.

$$\Delta E^*_{ab} = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

$\Delta L^* = L^*_1 - L^*_0$, where L^*_1 is the value for irradiated film and L^*_0 is the value for non-irradiated film, similarly for Δa^* and Δb^*.

The color strength K/S was investigated using the Kubelka-Munk equation (1931) which is the most commonly used equation to relate reflectance to dye concentration [10]. Several studies have utilized it for dosimetry [8,11] and assessing fabric dye effects [12,13]. The K-M equation is expressed in terms of absorption (K) and scattering (S) and can be calculated using Eq. (2):

$$K/S = \frac{(1-R)^2}{2R} \quad (2)$$

where R represents the reflectance at wavelength λ, which was measured using the same 3NH YD5010 spectrodensitometer with Senior Mode (densitometer) at 480 - 700 wavelengths. The K/S values were then plotted with respect to the NBT concentration using Microsoft Excel.

For determining the concentration levels and types of elements present in PVA/NBT films, an ARL Quant'X EDXRF spectrometer, acquired from Thermo Scientific Inc. in the USA, was employed. The spectrometer's X-ray tube uses Rhodium (^{45}Rh) as its anode material and operates at voltages ranging from 4 - 50 kV, with a current of 0.02 - 1.98 mA. A Si(Li) semiconductor detector with a crystal area of 15 mm² and a crystal depth of 3.5 mm is used to detect the released X-rays. **Table 1** displays the filters that were utilized in the process.

Table 1 List filters of ARL Quant'X EDXRF spectrometer [14].

Filter	Voltage (kV)	Live time (s)	Elements
Cu thick	50	240	Sn, Sb
Cu thin	50	800	Mo, Cd
Pd thick	30	1,600	As, Br, Sr, Pb
Pd medium	20	960	Cu, Zn
Pd thin	16	600	Fe, Co, Ni, Mn
Aluminum	12	1,000	Ti, V, Cr
Cellulose	8	200	S, Cl, K, Ca
No filter	4	100	Mg, Al, Si

X-ray diffraction patterns were collected with the Rigaku Ultima IV X-ray Diffractometer. The measurements were taken with a step size of 0.02 ° at the 2θ angle, covering a range from 10 to 90 °. The instrument was operated at a voltage of 40 kV and a current intensity of 40 mA. The resulting 2θ values and their respective intensities were plotted for different gamma doses using the Matlab software.

Results and discussion

Stability of PVA/NBT film dosimeter after irradiation

The stability of a PVA/NBT film containing 0.1g of NBT dye was assessed. The test involved utilizing ImageJ software to compare the difference in values between the film irradiated with 55 mGy and a control film for the red color channel region. After irradiation, the films were stored in the dark under standard laboratory conditions and measured for 30 days. The results presented in **Figure 1** indicated that the film remained stable under γ-ray irradiation at various time intervals, with no significant changes observed during the test period. This finding aligns with the outcomes of previous research conducted by Moussa *et al.* [6].

The changing color after irradiation

The nanocomposite PVA/NBT films exhibited a yellowish-brown hue after exposure to low doses of γ-rays ranging from 0 - 96 mGy. In **Figure 2**, 2 film samples with different concentrations of NBT dye −0.02 and 0.2 g, respectively- were displayed. As the concentration of NBT dye increased, the films took on a more pronounced yellowish color. Additionally, with an increase in γ-ray doses, the films' color

deepened to brown across all concentrations, each showing varying intensity. The color change was more noticeable in higher concentrations of NBT, particularly at 0.2 g. This effect resulted from the elevated amount of NBT dye, possibly caused by the reduction of NBT^{2+} after exposure to radiation. This process led to the formation of mono-formazan, followed by di-formazan [5]. These visible changes, discernible to the naked eye, highlight the films' sensitivity to γ-rays, even at low doses. Consequently, the films proved well-suited for use in diagnostic radiology.

Relative density

The examination of the relative density of PVA/NBT films exposed to low doses of γ-rays was conducted using ImageJ software. The software was employed to measure the RGB colors of digital images, with the red color channel being the sole focus for this study, given its more significant variation in the values obtained. The results revealed that concentrations less than 0.025 g, as well as those exceeding 0.2 g, proved ineffective when exposed to a dose range of 8 - 96 mGy of γ-rays. However, PVA/NBT films containing varying amounts of NBT between 0.025 and 0.2 g exhibited an excellent response, as illustrated in **Figure 3**. The graph in **Figure 3** displayed a linear increase in redness with an escalating amount of NBT dye in the dose range of 8 - 96 mGy. The relative density demonstrated an increase with an escalating dose up to a concentration of 0.2 g, beyond which it gradually decreased with a rising concentration.

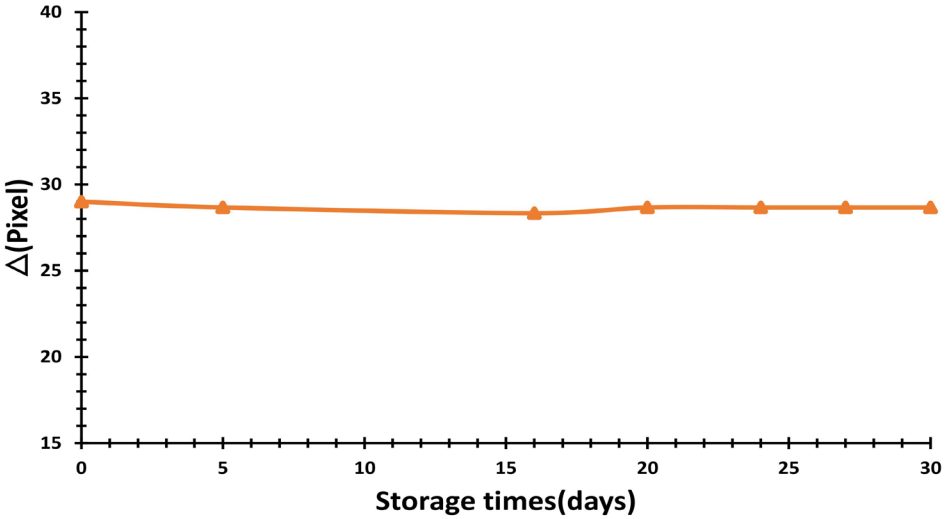

Figure 1 Stability of PVA/NBT film containing 0.1 g of NBT after exposure to 55 mGy γ-ray dose and stored in dark.

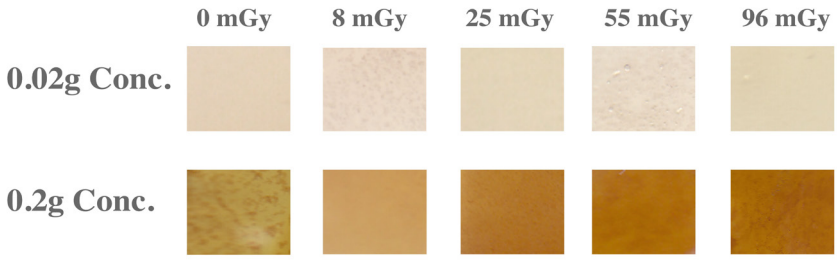

Figure 2 The color shifted in PVA/NBT films for 2 different concentrations of NBT dye after exposure to varying doses of γ-rays.

Color strength K/S

The color strength K/S of PVA/NBT films was examined using a spectrodensitometer before and after irradiation with γ-rays. **Figure 4** illustrates the relationship between the concentration of NBT dye and K/S as a function of γ-ray doses applied to PVA/NBT films. Upon examining films without irradiation and within a concentration range of 0.05 - 0.3 g, an increase in the concentration of NBT dye resulted in a linear increase in K/S, indicating an augmented yellowing of the films. Furthermore, an increase in γ-ray doses led to a linear rise in K/S, attributed to the significant color change in PVA/NBT films after irradiation. Lower concentrations ranging from 0.015 to 0.025 g proved ineffective and did not yield satisfactory results compared to higher concentrations. The findings of this research suggest that the dose-response of PVA/NBT films significantly increases as the concentration of NBT dye rises. This outcome aligns well with the conclusions of a previous study [15]. The primary objective of this paper is to determine the optimal concentration of NBT dye for the best dose-dependent performance. The study determined that the range between 0.05 to 0.3 g exhibits the highest sensitivity to γ-rays, surpassing other concentrations in the range of 0 - 96 mGy.

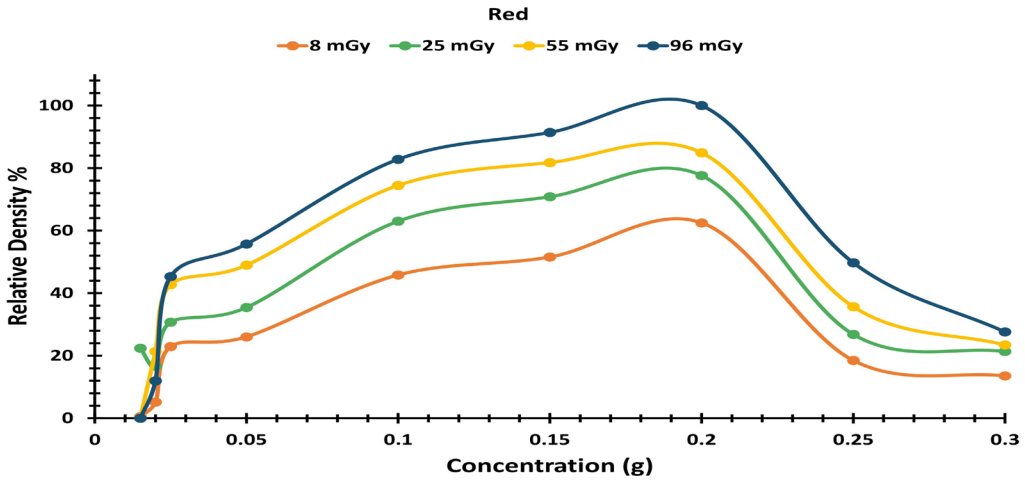

Figure 3 Relative density of PVA/NBT film dosimeters containing 0.015 - 0.3 g of NBT in dose range 8 - 96 mGy of γ-rays.

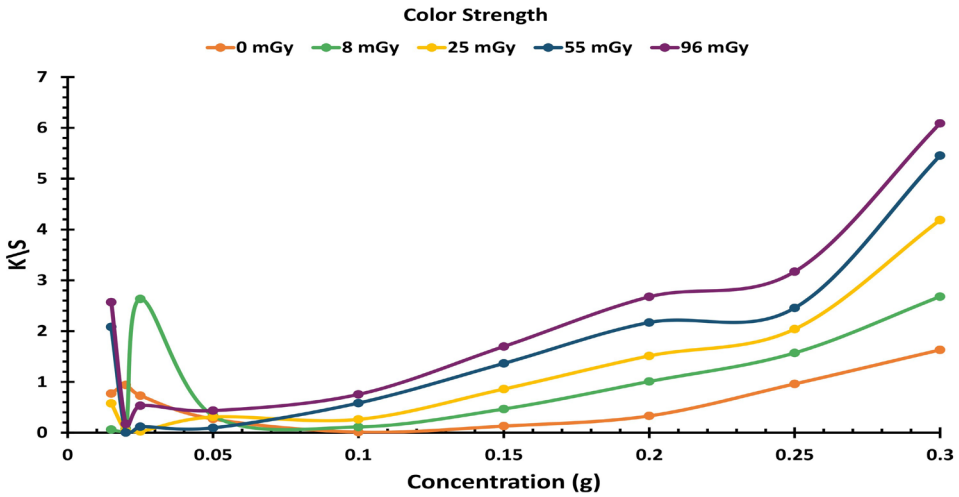

Figure 4 Color strength K/S of PVA/NBT films for different concentrations exposed to various γ-ray doses.

Colorimetric study

The colorimetric properties of non-irradiated and irradiated PVA/NBT films were determined through spectrodensitometric analysis. According to **Figure 5(a**), an increase in the concentration of NBT dye resulted in a linear increase in the total color difference ΔE^*_{ab} within range 0.05 to 0.2 g. Furthermore, a linear correlation was identified between the total color difference (ΔE^*_{ab}) and the applied gamma dosage. These findings align with a previous study conducted by Oberoi *et al.* [16]. However, beyond a concentration of 0.2 g, the color change was not very noticeable as the film became too dark. Therefore, the impact of γ-rays on the film was not directly observable. A similar observation was made for concentrations below 0.05 g. The lightness L^* (**Figure 5(b)**) followed the same trend but decreased with γ-radiation exposure due to the darkness of the film and increased with higher NBT concentration, consistent with a previous study [17]. **Figure 5(c)** indicated a linear rise with increasing NBT concentration, leading to a more significant amount of red color ($+a^*$), irrespective of the order of dosing. Similarly, **Figure 5(d)** showed a linear increase in yellow ($+b^*$) color up to 0.2 g, followed by saturation. Based on the above results, a concentration of 0.2 g PVA/NBT film proved to be an effective dosimeter for detecting low γ-ray doses.

X-ray fluorescence study

X-ray fluorescence (XRF) analysis was conducted to assess the elemental composition of PVA/NBT films before and after exposure to gamma irradiation. The study focused primarily on chlorine (Cl), the element with the highest atomic number in the PVA/NBT nanocomposite films, making it particularly susceptible to radiation-induced changes. **Figure 6** presents the mass percent (m/m%) of Cl for average values of unexposed and irradiated films plotted against NBT concentration. A direct relationship between Cl concentration and NBT amounts was observed, aligning with expectations. Post-irradiation, a slight decrease in Cl concentration in PVA/NBT films was noted across all NBT concentrations. Notably, the dissolution method for NBT influenced the observed outcomes. The data indicate that the optimal concentration range lies between 0.05 and 0.25 g, with a coefficient of linear regression of $R^2 = 0.93$, suggesting a strong correlation. Conversely, concentrations below 0.05 g and above 0.25 g exhibited inconsistent behavior, likely attributable to NBT's limited solubility in the solution. These findings underscore the importance of considering NBT concentration and its dissolution method when evaluating the radiation response of PVA/NBT films, providing valuable insights for further optimization and application in dosimetry.

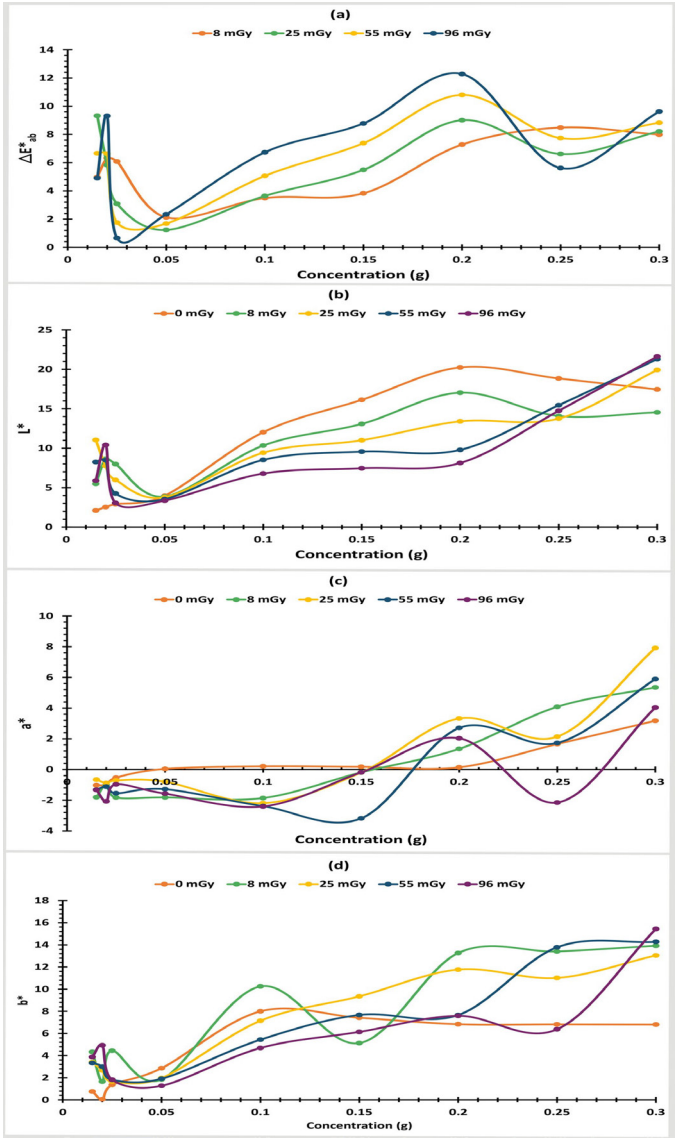

Figure 5 The CIEL*a*b* parameters and color difference ΔE^*_{ab} of PVA/NBT films as a function of NBT concentration at various gamma doses. Where (a) represents ΔE^*_{ab}, (b) L^*, (c) a^* and (d) b^*.

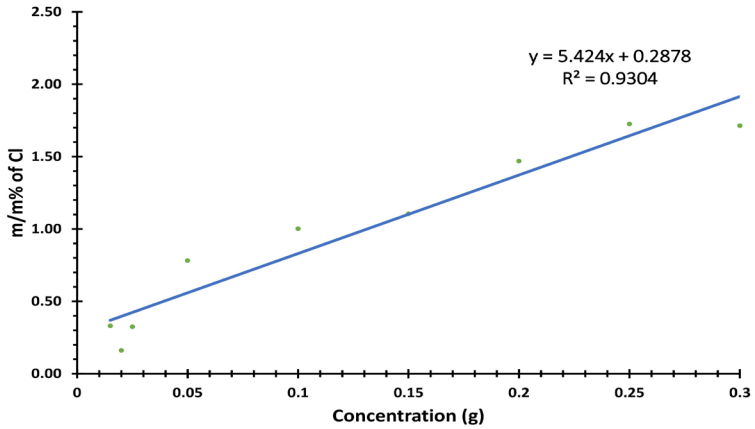

Figure 6 The Cl mass percent (m/m%) against NBT concentration amounts in PVA/NBT films for both unexposed and irradiated films.

X-ray diffraction study

The impact of X-radiation on the structural and crystalline characteristics of PVA/ NBT nanocomposite films was investigated via XRD patterns. Two samples, one with 0.02 g and the other with 0.2 g of NBT dye were examined before and after being subjected to different doses of gamma rays, as shown in **Figure 7**. The analysis of diffractograms revealed a characteristic peak observed at 2θ = 19.82 ° for the 0.02 g film and 2θ = 20.12 ° for the 0.2 g film; this peak corresponds to the reflection plane of (101) [18] and suggests the semi-crystalline nature of pure PVA [19]. Additionally, a minor peak was observed at 2θ = 40.36 ° for the 0.02 g film and 2θ = 40.94 ° for the 0.2 g film, confirming the crystalline phase of PVA [20], which corresponds to the (111) reflection plane [21]. The observations indicate that the peaks have shifted after exposure to gamma rays. At a concentration of 0.02 g, the intensity gradually decreased with an increase in gamma-ray doses, except at 96 mGy. However, at a higher concentration of 0.2 g, the intensity of the peaks increased with increasing gamma-ray doses. Additionally, the shift in the minimum peak increased gradually with increasing gamma doses, up to 55 mGy (from 2θ = 40.94 to 41.30 °), as indicated in **Table 2**. The findings confirm that the PVA/NBT films underwent structural rearrangement, as indicated by the shift in peak position and change in intensity after irradiation. These results align with previous studies on PVA films doped with other compounds and exposed to high doses of gamma radiation [22-24].

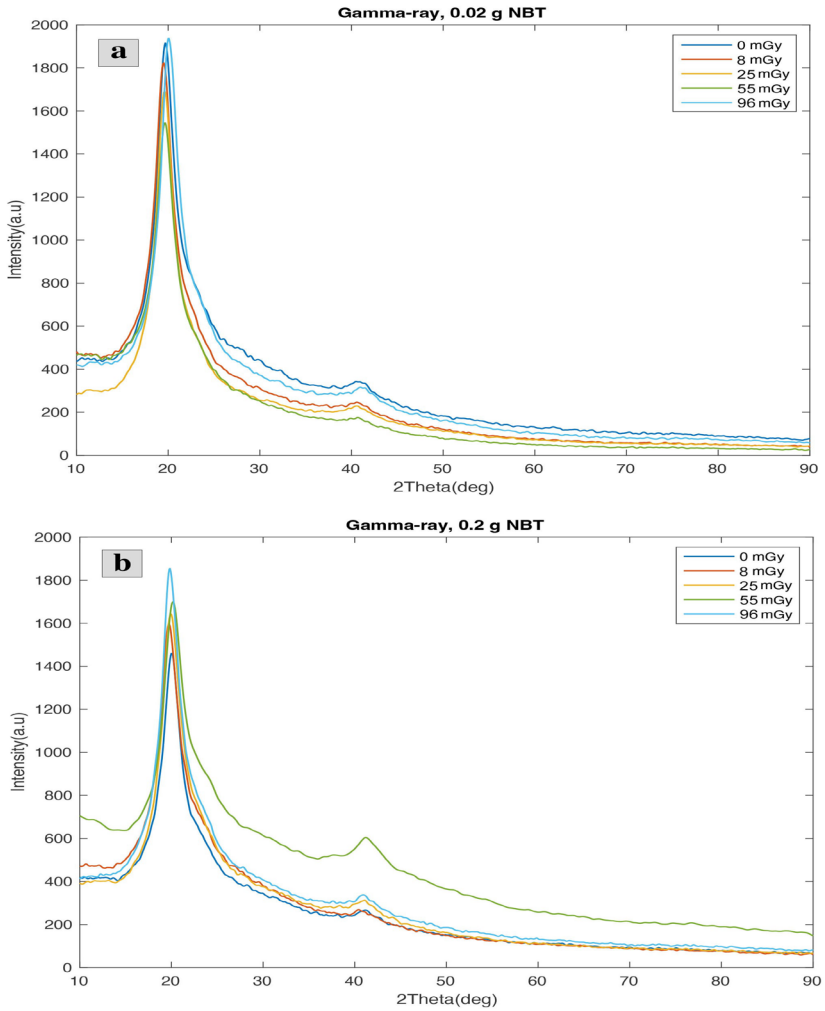

Figure 7 The XRD patterns of PVA/NBT films with (a) 0.02 g and (b) 0.2 g of NBT dye at various exposure doses.

Table 2 The list of peak values for PVA/NBT films containing 0.02 g and 0.2 g of NBT, exposed to varying γ-ray doses.

Dose(mGy)	0.02 g of NBT		0.2 g of NBT	
	2 Theta (degree)	Intensity (a.u.)	2 Theta (degree)	Intensity (a.u.)
0	19.82	1,999	20.12	1,548
	40.36	388	40.94	303
8	19.58	1,923	19.78	1,673
	40.86	274	41.06	302
25	19.54	1,760	19.98	1,738
	40.82	247	41.08	343
55	19.74	1,612	20.02	1,822
	40.28	202	41.30	674
96	19.94	1,994	19.74	1,921
	41.04	372	40.66	363

Conclusions

The PVA/NBT nanocomposite film, developed with varying concentrations of NBT dye, demonstrated a high sensitivity to low doses of γ-rays. This sensitivity was validated through chemical, physical, and colorimetric analyses. Increasing the NBT dye concentration enhanced the film's responsiveness to gamma rays, supported by the CIE L^*, a^*, b^* system analyses and K/S color strength tests. XRF analysis verified the effectiveness of NBT concentrations ranging from 0.05 to 0.25 g. The films remained stable after 30 days of irradiation in darkness, with XRD pattern analysis confirming structural changes post-irradiation, particularly noticeable at higher NBT concentrations. Utilizing NBT concentrations between 0.05 and 0.2 g is recommended, with 0.2 g identified as highly effective, especially for gamma rays in the low dose range.

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