



Poly(vinyl alcohol) and liposomes as a synthetic biodegradable model membrane for probing radiation interactions

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Abstract

This work aims to investigate the effect of X-rays on poly(vinyl alcohol) (PVA) films and studying the ability to be a model of a synthetic cell membrane when compared to liposomes as biological models. The influence of X-ray irradiation on thin films of PVA doped with safranin (SAF) dye and liposomes encapsulated by SAF dye is studied using UV–visible spectrophotometry. These films are exposed to X-ray radiation with accumulated doses range of 10–50 Gy of 140 kVp at a constant dose rate of 2002 $\mu\text{Gy/s}$. The absorption spectra of the PVA/SAF and the liposomes/SAF are measured. The absorption spectra of the PVA/SAF showed a decrease in the absorbance band peak with the increase of the dose with values are similar to those observed with the liposomes/SAF. The dose response function indicates the same radiation sensitivity of both samples. The results indicated that full width at half maximum of SAF/PVA and SAF/liposomes show linear decrease with an increase of dose. The variation in dose response is relatively small and found to be within 3.5% and the variations in a decrease of the absorbance peak are found to be with maximum 5%. These values can indicate that both of SAF/PVA and SAF/liposomes have the same behavior under the applied X-ray dose range. The results viewed that the PVA doped with SAF dye behave similarly as the liposomes doped with SAF dye. PVA may be used as a synthetic model for a cell membrane for probing X-ray-membrane interactions.

Keywords PVA film · Safranin · Liposomes · Synthetic membrane · X-rays

1 Introduction

The use of nuclear irradiation for industrial applications has risen over the past 20 years. The irradiation processing is used in various applications, such as food irradiation, sterilization, polymerization and cross-linking of polymers. It is important to measure accurately the radiation dose delivered during processing. In particular, accurate measurement of low doses has been a problem due to non-availability of appropriate dosimeters in this range. The progress in the area of dosimetry right from early days while using the photographic films, Geiger–Muller and proportional counter has progressed rapidly. The use of solutions as standards, the change in the oxidation states and colors have been amply demonstrated. However, the solutions require careful handling and the changes need

to be detected by some auxiliaries such as UV–Vis spectrophotometer, ESR, NMR, IR and thermo-luminance.

Polymers are materials with many advantages as they are lightweight, flexible materials and can be manufactured at low cost. They have many advantages due to the ease of processing and ability to be molded to form thin films [1–3]. The structural properties of poly(vinyl alcohol) (PVA) continue to be a subject of active research. PVA has fascinating properties and a wide variety of applications. Also, it has good film forming by solution casting. It has a carbon chain backbone with hydroxyl groups which can act as a source of hydrogen bonding and it is a biocompatible polymer which may be potentially used as a synthetic membrane or an interchange resin. Several unique features of PVA, it is water soluble, crystallizable and capable of hydrogen bonding. PVA can be used in the

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medical field because they possess biosuitability [4–6] as PVA has great stability after doping by chemical and physical doper [7–9].

Liposomes are artificial membranous vesicles formed by phospholipids bilayers. Liposomes have long been receiving much attention because of their biocompatibility and appealing ability to carry both hydrophobic and hydrophilic compounds. Due to their similarity with the bilayer structure of natural membranes, liposomes are used as a membrane model to investigate the significance of X-ray-membrane interactions.

In the present work, PVA was chosen firstly, due to the ease of availability, water solubility and possibility to incorporate a range of dyes. Secondly, its films could be casted at moderate temperatures. The structural and morphological changes occurring in the PVA films subjected to gamma irradiation have already been reported [10, 11]. This work aims to investigate the effect of X-rays on PVA films and studying the ability to be a model of a synthetic cell membrane when compared to liposomes as biological models.

2 Materials and methods

2.1 Chemicals

Pure poly(vinyl alcohol), Fig. 1a, in the form of powder of molecular weight 115,000 of purity 98% was obtained from Oxford laboratory agent Co. India. Safranin (SAF) dye of molecular weight 350.85, Fig. 1b, obtained from

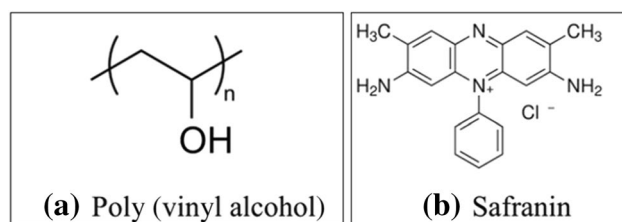
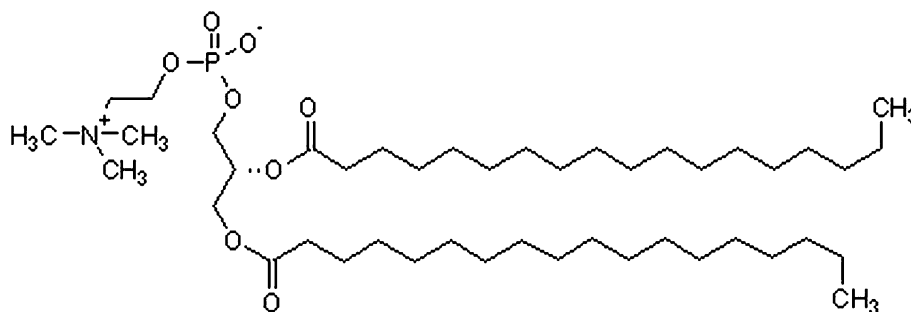


Fig. 1 The chemical structure of PVA and SAF

Fig. 2 Schematic chemical structure of DSPC



Lobochemie Fine Chemicals Pvt, Ltd., Mumbai, India. 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC) in powder form with molecular weight of 790.145 and purity 99% is presented in Fig. 2, was purchased from Lipoid KG, Ludwigshafen, Germany. Ethanol was of analytical grade 99% pure and obtained from Merck, Heliopolis, Cairo, Egypt. All other chemicals used for this work were of research grade. Solutions were prepared in de-ionized ultra-pure water.

2.2 Preparation of stock solutions

The stock solution of PVA was prepared by dissolving 5 g of PVA in 100 ml of double distilled water at 60 °C, with stirring for 24 h—to assure a complete solvation of all parts, then left to cool for 24 h at room temperature. The stock solution of the SAF was prepared by dissolving 0.08 g of SAF in 50 ml distilled water at room temperature for 24 h to assure a complete solvation of all parts.

2.3 Preparation of SAF/PVA films

The polymer films were prepared through a solvent casting method [12]. The films were prepared by dissolving 13 ml of PVA and 2 ml of SAF and kept stirred for 4 h at room temperature to obtain a uniformly dyed solution. The SAF/PVA solutions were casted on a 10×10 cm horizontal balanced glass plate to obtain a uniform film thickness and left to dry at room temperature for 120 h. The uniform and transparent films were obtained then were detached from the glass plates. Yellow–red colored films of SAF/PVA with the average thickness of 0.042 ± 0.005 mm were obtained and stored in the ambient controlled conditions at 50% relative humidity (RH) and at room temperature (23 ± 5 °C).

2.4 Preparation of liposomes

Liposomes were prepared by thin film hydration method [13]. Briefly, Aliquots of 10 mg of Distearoylphosphatidylcholine (DSPC), Fig. 2, were dissolved in 15 ml ethanol in around bottom flask. The liposome mixture was

shacked well for a few minutes then a vigorous vortexing took place to assure a complete solvation. The organic solvent was removed gradually using a digital rotary evaporator (WEV-1001L, Korea) under a steam of nitrogen gas in a warm water bath (45 °C) to produce a uniform thin film of lipids on the inner wall of the flask. The lipid film was hydrated with 4 ml of SAF stock solution in a water bath at 55 °C for 10 min to form multilamellar vesicles (MLV). The SAF/liposomes suspension was divided into six groups. These groups were left to dry at 60 °C for 2 h in their tubes.

2.5 Irradiation facilities

The X-ray irradiations were carried out by using YXLON MG 165145 (manufactured at YXLON-Germany). The dose rate was a constant beam of 2002 $\mu\text{Gy/s}$ (with uncertainty 1.28% obtained from calibration certificate) through 140 kVp–max 5 mA with focal point to film distance (FFD) = 100 cm. The groups of films were irradiated with different X-ray doses (10, 20, 30, 40, 50) Gy, the X-ray device was calibrated using National Institute of Standards (NIS) labs in Egypt, and it was calibrated against UNIDOS meter App. Nr. 10001-10522 (PTW, Freiburg). Because of some limitations related to long exposure time using the X-ray machine, the total exposure was fractionated on an equal basis of 10 Gy per day (i.e. 84 min daily for 5 days).

2.6 Analysis of the samples

The transmission measurements of the unirradiated and irradiated samples were measured by using UV4 Spectrophotometer (Unicam Co. Ltd, England) in the wavelength range of 400–700 nm. From the transmission spectrum, the effect of X-ray irradiation on these films was investigated by estimating: (a) The optical properties of these films which were investigated from UV–Vis spectrophotometer, (b) Dose response and (c) the FWHM curve for SAF/PVA and SAF/liposomes was calculated.

3 Results and discussion

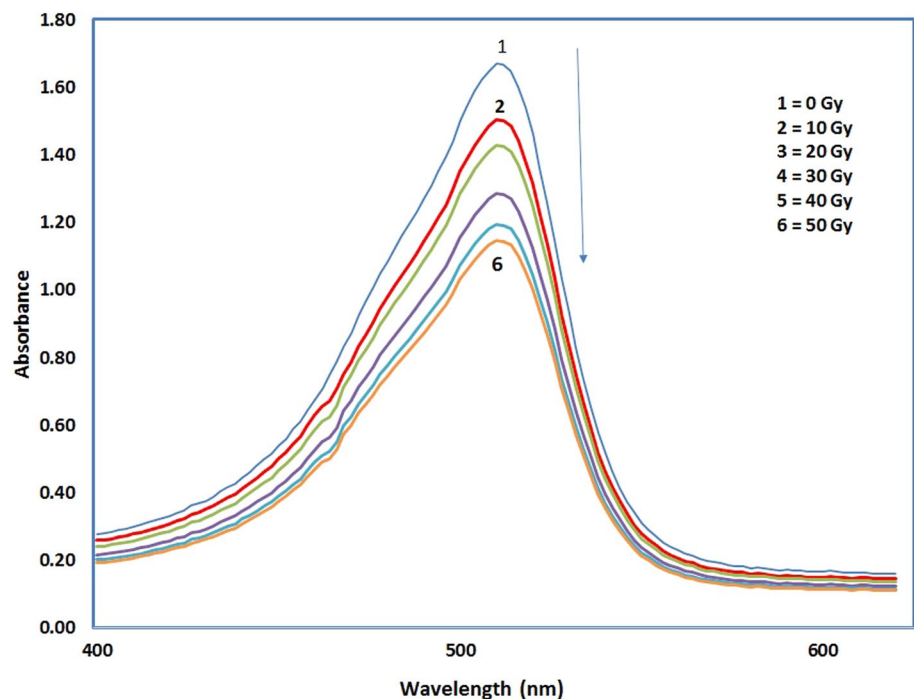
3.1 Effect of X-ray on SAF/liposomes

3.1.1 Absorbance spectra

The absorbance spectra of SAF/liposomes films (control and irradiated with different X-ray doses at 0, 10, 20, 30, 40, 50 Gy) were measured and are shown in Fig. 3.

The absorbance spectra of irradiated and unirradiated samples show a main absorption peak at 512 nm (the resonance absorption peak of SAF dye). The amplitude of this peak decreases in response to the increase in X-ray absorbed doses. Upon irradiation, these samples didn't show change in color from yellow–red due to the low irradiation energy dose (much greater doses in the range of

Fig. 3 The absorbance spectra SAF/liposomes films at different applied X-ray doses (0, 10, 20, 30, 40, 50 Gy)



hundreds of grays are needed to trigger bleaching in SAF/liposomes).

A graphical representation of the change in the absorption peak related to the exposure to different doses of X-ray is shown in Fig. 4. The decrease of the absorbance is found to be linear.

3.1.2 Response curves

Figure 5 shows the response curves of SAF/liposomes samples at 512 nm. It shows the response in terms of alteration

$\Delta A = A_o - A_i$, where A_i and A_o are values of absorbance for the irradiated and unirradiated samples, respectively. The curve showed a linear relation obtained up to 50 Gy.

3.2 Effect of X-ray on SAF dyed poly PVA films

3.2.1 Absorbance spectra

The absorbance spectra of X-rays irradiated and unirradiated SAF/PVA at concentration 49 mmol/l. Films were

Fig. 4 Plot of decrease in absorbance peak of SAF/liposomes samples at 512 nm versus the applied X-ray doses (0, 10, 20, 30, 40, 50 Gy)

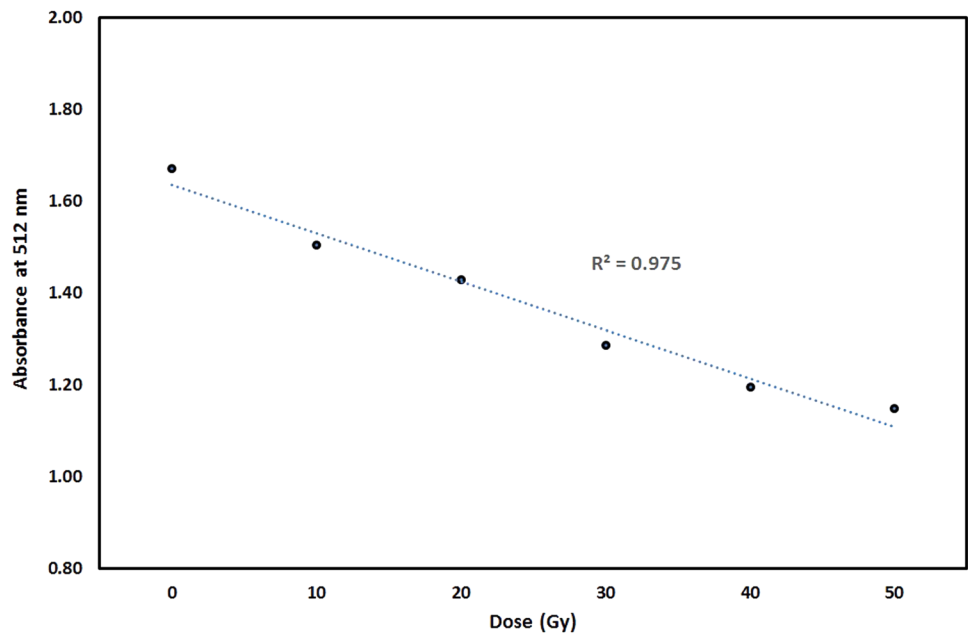
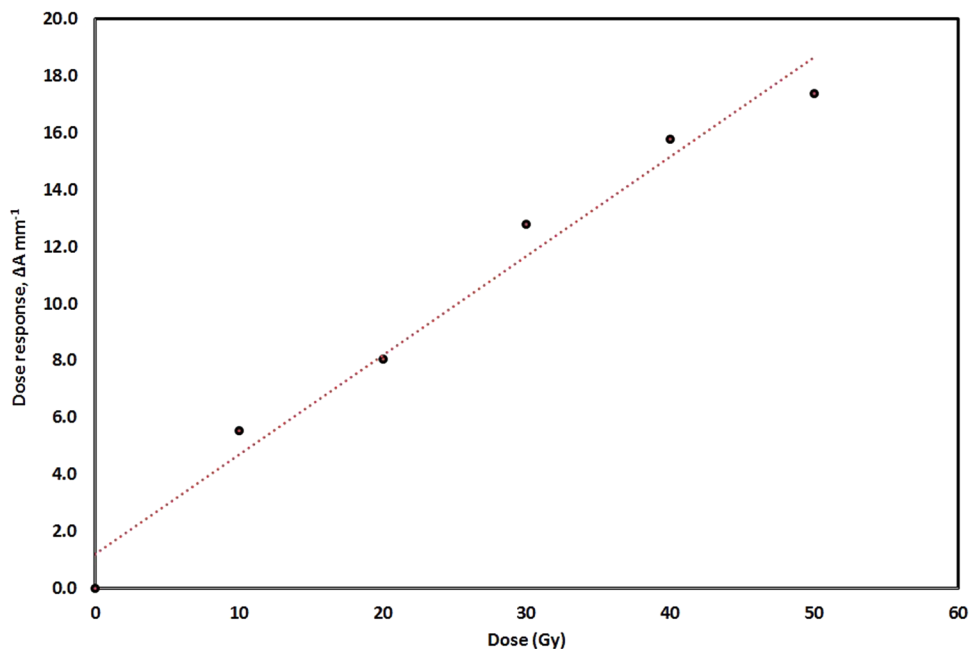


Fig. 5 Change of dose response $\Delta A \text{ mm}^{-1}$ of SAF/liposomes samples at 512 nm versus the applied X-ray doses (0, 10, 20, 30, 40, 50 Gy)



registered twice before and after irradiation to the applied doses as shown in Fig. 6.

The absorbance spectrum of irradiated and unirradiated films show absorbance band peak at 536 nm. The amplitude of this band decreased in response to the increase of the absorbed dose of X-rays. However, these films didn't show any change in their color from yellow–red due to the low irradiation energy dose. The change in the absorbance peak of the SAF/PVA films at 536 nm related to the exposure to X-ray different doses which is shown in Fig. 7.

The decrease of the absorbance is near to linear up to the 50 Gy dose. This shows that this dosimeter may be applied as a dosimeter in the dose range between 10 and 50 Gy.

Figure 8 represents the comparison between the decrease in absorbance of SAF/PVA and of SAF/liposomes versus X-rays. From this figure, it is worth noticed that the two trendlines of SAF/PVA and of SAF/liposomes are intersecting creating a small acute angle, which can give results that are rational to each other in the applied range.

Fig. 6 The absorbance spectra of SAF/PVA films at different applied X-ray doses (0, 10, 20, 30, 40, 50 Gy)

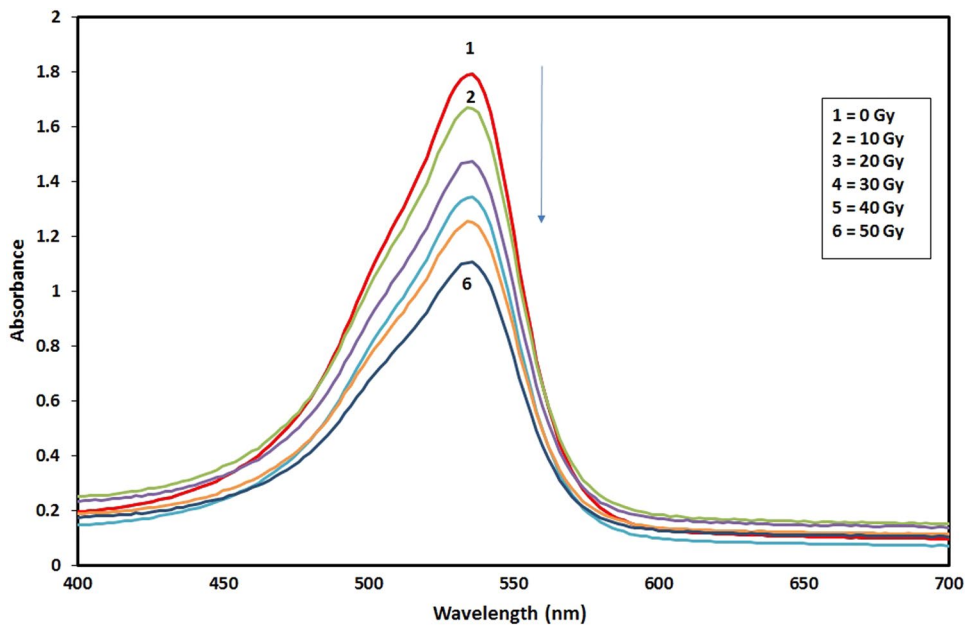


Fig. 7 Plot of decrease of absorbance peak of SAF/PVA films at 536 nm versus the applied X-ray doses (0, 10, 20, 30, 40, 50 Gy)

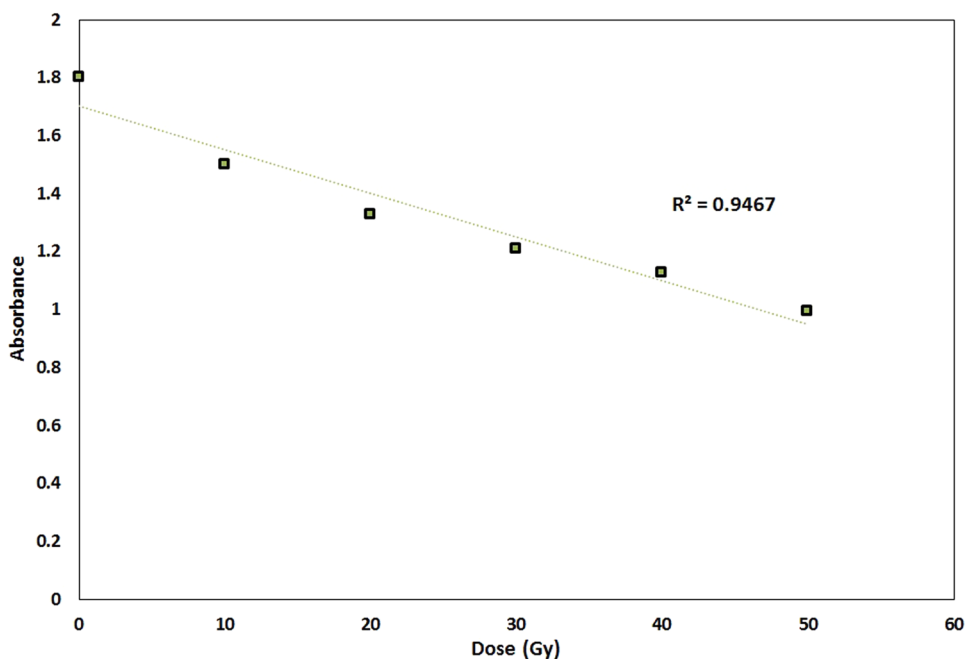
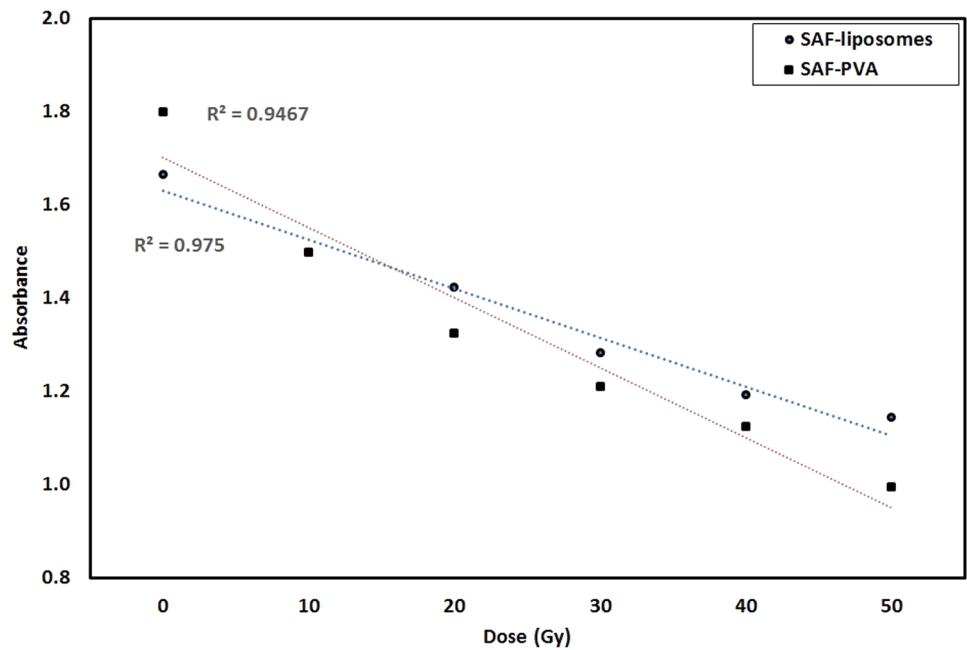


Fig. 8 Plot of the comparison between the decrease of absorbance peak of SAF/PVA films at 536 nm and SAF/liposomes at 512 nm versus the applied X-ray doses (0, 10, 20, 30, 40, 50 Gy)



3.2.2 Response curves

Figure 9 shows the SAF/PVA films' response curves. It shows the response in terms of alteration $\Delta A = A_o - A_i$, where A_i and A_o are values of absorbance for the irradiated and unirradiated samples, respectively. This figure shows the linearity of the obtained dose response with the increase in the applied dose.

Figure 10 represents the comparison between the dose responses of SAF/PVA and of SAF/liposomes versus X-rays. This comparison shows the small Acute Angle starting from the starting point of the two trendlines which

indicates the close results of both SAF/PVA and of SAF/liposomes.

3.3 FWHM of (SAF/PVA) and (SAF/liposomes)

FWHM is a technique for distinguishing between doses absorbed by the variation of FWHM as an inference group in reaction to distinct doses in the dose spectrum of (0–50 Gy). The films are exposed to different doses and the FWHM was calculated of each along with alone can determine which films exposed to high or low doses. Figure 11 shows FWHM variation of SAF/PVA and SAF/liposomes

Fig. 9 Change of dose response $\Delta A \text{ mm}^{-1}$ of SAF/PVA films at 536 nm as a function of the applied X-ray doses (0, 10, 20, 30, 40, 50 Gy)

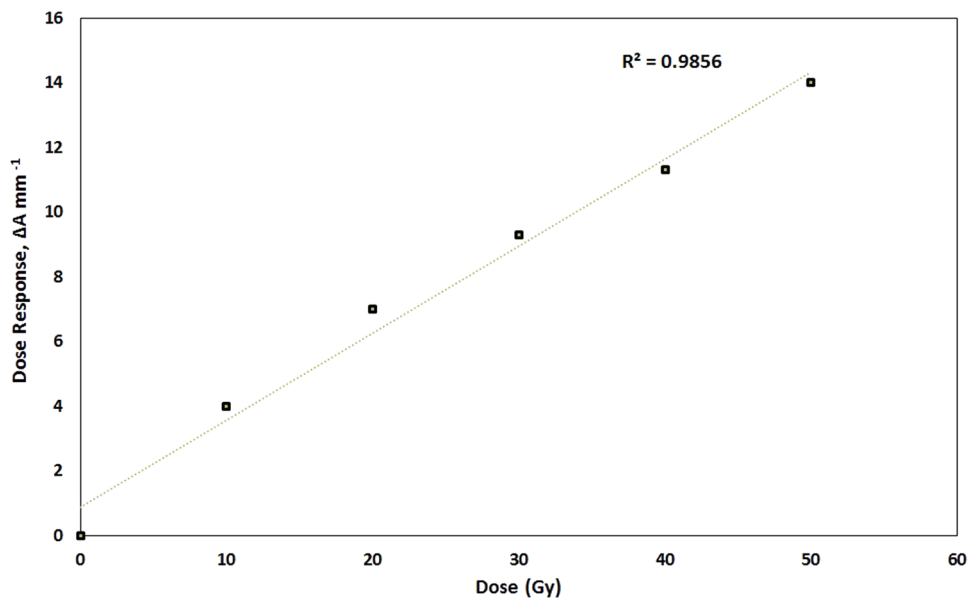


Fig. 10 Plot of the comparison between the dose response of SAF/PVA films at 536 nm and SAF/liposomes at 512 nm versus the applied X-ray doses (0, 10, 20, 30, 40, 50 Gy)

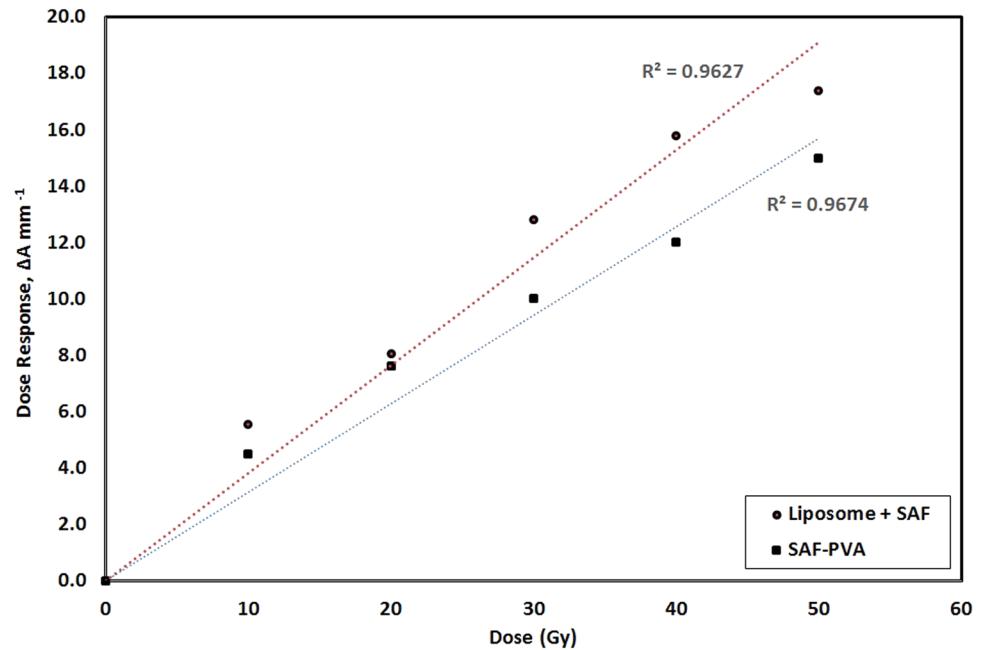
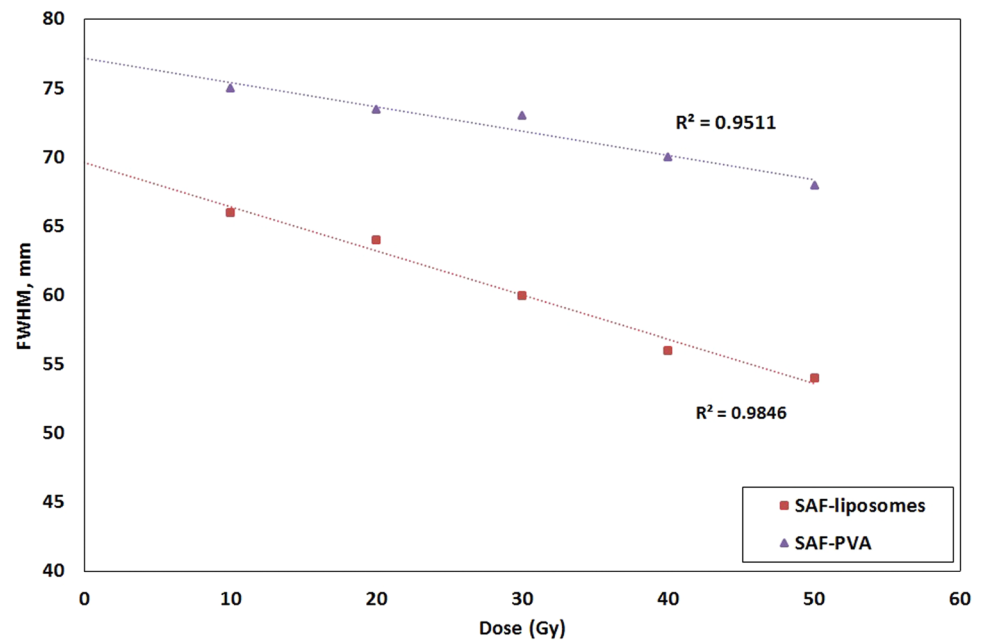


Fig. 11 The variation of FWHM of PVA/SAF and SAF/liposomes with the applied X-ray doses (0, 10, 20, 30, 40, 50 Gy)



with different doses of X-ray. The data are obtained from UV/VIS spectra and estimated by using origin pro 9 program. The response of the band at 536 nm and at 512 nm at different doses for the films is studied. The variation in FWHM (appeared from the Figs. 3, 6) as a reference to different absorbed doses in range (10–50 Gy). FWHM in SAF/PVA and SAF/liposomes decreases with increasing the dose.

Based on the above results, both SAF/PVA and SAF/liposomes showed linear response to different X-ray

doses (10, 20, 30, 40 and 50 Gy). The variation in dose response is relatively small and found to be within 3.5% and the variations in a decrease of the absorbance peak are found to be with maximum 5%. These values can indicate that both of SAF/PVA and SAF/liposomes have the same behavior under the applied X-ray dose range, this also can be concluded from the small variation in FWHM in SAF/PVA and SAF/liposomes which didn't exceed 5%.

4 Conclusion

The effect of accumulated doses of X-ray irradiation (10–50 Gy of 140 kVp 10 mAs at a constant dose rate) on thin films of poly(vinyl alcohol) doped with SAF dye, is compared with the effect of the same dose on liposomes doped with SAF dye. This study indicated that radiation of dose range of 10–50 Gy caused changes as follows:

- The spectrum of the prepared SAF/liposome samples released a decrease of the absorbance peak linearly with the increase of the irradiated dose which was very close to that of the SAF/PVA films.
- The dose response of the SAF/liposome samples was found to be linear with a ratio that is very close to the linear response of SAF/PVA.
- FWHM in SAF/PVA and SAF/liposomes show linearly decreasing values with increasing the dose.

The results indicated that the poly(vinyl alcohol) behaves similarly as the liposome which indicates that poly(vinyl alcohol) can be used as a synthetic model for a cell membrane for probing X-ray-membrane interactions.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

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