JSR Publications

Available Online

J. Sci. Res. **14** (2), 395-404 (2022)

JOURNAL OF SCIENTIFIC RESEARCH

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Temperature-Dependent Changes in Gamma Irradiated Porcine Gelatin with Low Bloom (PGL) Value: an ESR Investigation

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Received 9 August 2021, accepted in final revised form 9 January 2022

Abstract

Gamma irradiation of Gelatin brings important changes in its chemical structure, making it suitable for different applications. The irradiated Gelatin may be subjected to different thermal treatments during these applications. Therefore, temperature-dependent changes in irradiated gelatins are a point of interest. Electron spin resonance (ESR) is vital for detecting free radical processes in irradiated polymers. As such, ESR spectra of temperature-dependent porcine Gelatin with low bloom are recorded, and they are analyzed by computer simulation techniques. Component spectra under different conditions are evaluated by the magnetic parameters employed to simulate the ESR spectra. The results indicate that the spectral shape at 300 K (RT) is stable up to 350 K, and signal intensity begins to decay beyond 350 K. The signal vanished around 355 K, designated as radical decay temperature. Bloch analysis is applied to evaluate activation energy associated with free radical decay, calculated around 35 KJ/mole.

Keywords: Porcine Gelatin; Bloom; Gamma irradiation; ESR; Bloch analysis.

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doi: http://dx.doi.org/10.3329/jsr.v14i2.55078 J. Sci. Res. **14** (2), 395-404 (2022)

1. Introduction

Gelatins are an important class of biodegradable biopolymers with specific properties like gelation and emulsification, making them use food, pharmaceutical, and cosmetic industries [1,2]. Recently Alipal *et al.* have reviewed and highlighted the sources, processing, and applications of gelatins [3].

Radiation-induced processes in gelatins are an essential part of their application. Due to the crosslinking of gelatins by different types of radiations (gamma, electron beam), changes in chemical structure occur, followed by a change in mechanical properties [4]. Therefore attempts have been made to investigate radiation-induced changes in different gelatins by various authors [5-9].

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Abraham *et al*. [5] have studied gamma-induced changes in gelatins by the ESR technique and predicted the cleavage of peptide bonds, generating two types of radicals that contribute to the observed quartet ESR spectrum. Sridhar Rao *et al*. [6] have reported on the ESR studies of different types of gelatins (porcine and bovine) with different bloom strengths (low and high). They have observed an ESR quartet spectrum attributed to the free radicals formed by the cleavage of the peptide bond [6]. Though the ESR spectra irradiated gelatins appear to be identical, types of free radicals and their concentration depend on the type and nature of Gelatin. Dian *et al*. [7] and Vaidya *et al*. [8] have reported gamma radiation effects in fish gelatins by spectroscopic and thermal methods. These authors [7,8] have also reported the cleavage of peptide bonds resulting in the formation of free radicals, which crosslink to improve the mechanical properties of gelatins. Sivri *et al*. [9] have reported on gamma irradiation changes in Gelatin by the ESR technique. The observed quartet spectrum is analyzed by computer simulations. Mubarak *et al*. [10] have studied gamma irradiation changes in native gelatins and crosslinked gelatins and proposed various types of free radicals. Though free radical formation is predicted, their structures are not determined. Identification of free radicals in irradiated gelatins is essential because they are responsible for the mechanical properties of the ultimate product. Hossana *et al*. [11] have reported on the composition effects of Gelatin by FTIR technique.

Capsules made up of Gelatin are used in biomedical applications. They suffer from the problem of microbial degradation during their usage. It can be prevented by the sterilization of capsules with gamma radiation. In this context, Rodrigues *et al*. [12] have measured chemical and physical changes in Gelatin induced by gamma radiation and shown that sterilization by radiation is an efficient method to address this problem. Further, Proton irradiation of polyethylene terephthalate (PET) [13] and gamma, UV irradiation of gelatins considerably improved mechanical and thermal properties [14].

Unlike many polymers (which have the same repetitive structure), Gelatin is a heterogeneous structure built by different types of amino acids via peptide bonds. The composition of the amino acids in gelatins depends on their type (mammalian (porcine, bovine) and aquatic (fish)) and source (skin, horn) [15]. Therefore, identifying free radicals in this large polymer has become a complicated task. In this context, the authors report on the gamma irradiation changes in porcine Gelatin with low bloom value by the ESR technique. The observed ESR spectra are analyzed by computer simulation (easy spin and total curve fitting methods). Irradiated gelatin films are used in high-temperature applications. Therefore irradiated gelatins are heated to high temperatures in the range of RT (300 K) to its glass transition temperature (400 K). Usually, the ESR signals decay at high temperatures due to the recombination of free radicals near transition temperature [16]. The present studies attempt to study free radical decay and evaluate the activation energy using Bloch analysis.

2. Materials and Methods

Gelatins derived from pig skin (porcine Gelatin) designated as Gelatin A in the form of powder are used in the present studies. All gelatin samples are exposed to a Cobalt 60 gamma source with a dose rate of 15 K Gy/h in the air at room temperature. The radiation dose given to the sample is calibrated to the time of exposure. ESR spectra are recorded on a Varian E-line spectrometer, operating at X – band frequencies and 100 K Hz modulation. The spectrometer is fitted with suitable accessories to record variable temperature ESR spectra.

3. Results and Discussion

ESR spectrum of gamma-irradiated PGL at room temperature is as shown in Fig. 1. Curves 1, 2, 3, and 4 represent the ESR spectra recorded at 310, 330, 350, and 370 K.

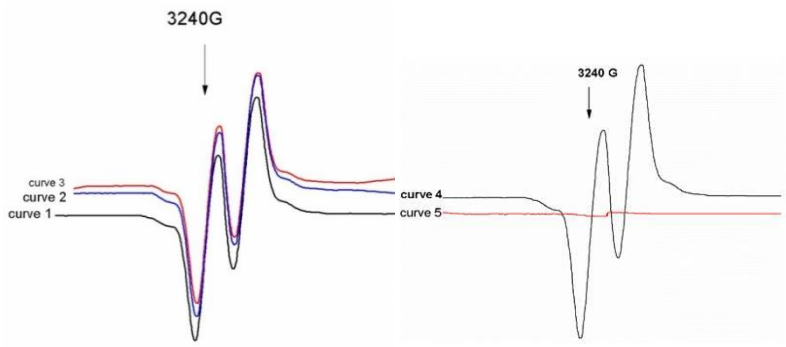

Fig. 1. Temperature-dependent ESR spectra of irradiated porcine Gelatin.

The spectra are quartets with the hyperfine splitting of 20-15 G and a spread of 80-70 G. Hyperfine pattern of the spectrum is almost stable up to a temperature of 350 K, but this pattern is suddenly lost at 370K. Since the ESR quartet spectrum observed for irradiated PGL is a quartet, the free radical responsible for the quartet might have had three interacting protons. In this case, an ESR spectrum with an intensity distribution of 1:3:3:1 and a hyperfine splitting of 20G might have resulted. However, the intensity distribution and the hyperfine splitting observed in the present studies appreciably deviate from the expected values. Further chemical structure of PGL is very complicated, and it does not allow such types of free radical configurations. Therefore exclusive protonic interaction is ruled out. In view of this computer, simulations are employed.

3.1. *Computer simulation: easy spin method*

The simulation of ESR spectra (Fig. 2) has been made using the easy spin program [17], which included parameters like several interacting alpha and beta protons, peak to peak

width W_{pp}, hyperfine splitting (A and B), and g-value. The parameters employed to simulate at RT spectrum are as listed in Table 1.

Table 1. Parameters used to simulate ESR spectra by an easy spin method.

Temperature (K)	g -value	Interaction protons	Peak to peak width Tow (τ)	Hyperfine splitting (G)
300	2.006	3	0,0.3	25
315	2.006	3	0,0.35	25
335	2.004	3	0,0.40	25
355	2.005	3	0,0.38	25

Fig. 2. Component ESR spectra as per the easy spin method.

The parameters employed in the easy spin method suggest that the free radical giving the ESR spectrum at RT should have three interacting protons, i.e., $\dot{C}H_3$. However, in view of the fitness of the spectrum and free radical proposed by this method, this method is discarded. Further, Abraham *et al*. [5] and Sridhar *et al*. [6] have reported the existence of NHCO (I) and CH (O)-N-(II) free radicals in irradiated PGL. Radical II gives an ESR doublet spectrum in protonic interaction. In contrast, radical (I) has interacting nitrogen with nuclear spin 1, which gives a quartet spectrum. However, hyperfine splitting observed in present studies is different from Abraham *et al*., [5]. The differences are attributed to the magnetic environment around free radicals' magnetic nuclei [18].

3.2. *Computer simulation total curve fitting method*

The total curve fitting method also simulates ESR spectra of irradiated PGL at different temperatures [18,19]. Based on the chain cleavages expected on gamma irradiation of Gelatin, different types of free radicals are expected. Component spectra corresponding to these free radicals are simulated and superposed to match the experimental spectrum. The goodness of the fit is determined by verifying intensifies at different line positions. The mean square deviation is calculated between the observed and calculated values of intensities for each set of simulations. The set with a minimum value of mean square deviation is treated as the best fit. The chemical composition of free radicals is identified using magnetic parameters used in computer simulations.

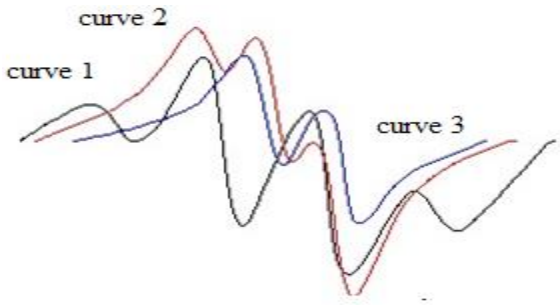

Fig. 3a. Component stectra of PGL.

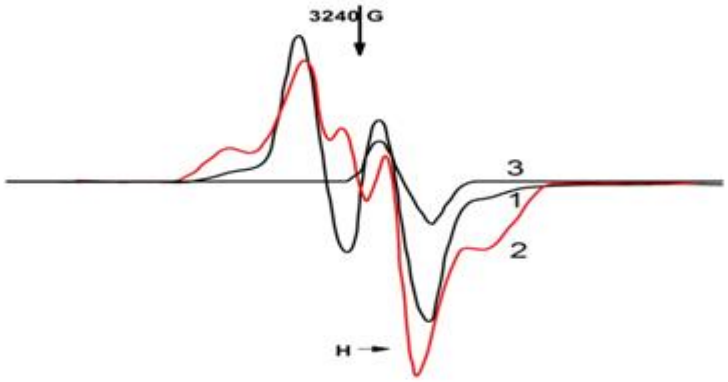

Fig. 3b. Computer simulation by total curve fitting method.

The RT spectrum is simulated with the component spectrum shown in Fig. 3a, with the magnetic parameters listed in Table 2. The component spectra are (Fig. 3a) superposed to give the superposed spectrum shown as curve 2 in Fig. 3b. The first component is the component quartet (Curve 1 in Fig. 3a) simulated with n_i = 3, m_i = 2, and hyperfine splitting values of 7.5 G, 6.0G. The values of coupling constants suggest that the splitting constants do not correspond to protons as these values usually are in the range 20–23 G [20]. Therefore, the presence of magnetic nuclei other than the proton is proposed. Considering degradation studies of amino acids like alanine, glycine, and lysine, the formation of nitrogen-centered free radicals is reported [17]. In gelatins, amino acids are bound by peptide bonds (CONH). Therefore formation of nitrogen-centered free radicals is also expected in irradiated Gelatin. Such free radicals have also been reported previously [21,22]. The nuclear spin of nitrogen is 1 (I = 1); hence three hf lines are expected (n_i = 3), and the presence of even one proton in beta position causes multiple hyperfine structures. The simulated component quartet in the present studies indicates the presence of magnetic nitrogen in the alpha position (n_i = 3) and at least one proton in the beta position (m_i = 2). Therefore, values of n_i = 3 and m_i = 2 are used for simulation of the

component quartet in the present studies, which is appropriate in the case of PGL. Free radical having such a structure is of the type - N H –, and it is expected to have been formed due to the cleavage peptide bond (- CONH -).

Table. 2. Magnetic parameters of irradiated PGL at RT.

Component	$Y_{max\ i}$	a_i (G)	X_{oi} (G)	A_i (G)	B_i (G)	n_i	m_i
Quartet	66.5	28.3	3378	7.5	6.0	3	2
Triplet	86.5	38.3	3368	17.0	0	3	1
Doublet	86.5	38.3	3350	5.0	0	2	1

Apart from the quartet, two more components are contributing to the RT spectrum. They are component triplet (Curve 2 in Fig 3a), simulated with $n_i = 3$ and $m_i = 1$ (the presence of two protons) arising from - CH_2 –free radicals. The other component is doublet (Curve 3 in Fig. 3a) simulated with the values of $n_i = 2$ and $m_i = 1$, assigned to CH radicals. The superposition of all the components will result in the superposed spectrum shown as curve 2 in Fig. 3b, while curve 1 is the experimental spectrum. Subtraction of cure 2 from curve 1 results in curve 3 in Fig. 3b, the singlet spectrum, which is assigned to the free radicals of the type [17](IV). All the component spectra are as shown in Fig. 3c.

Besides this peptide bond, there are strong hydrogen bonds between adjacent (– C = O and - NH or CH groups) PGL molecules consisting of different amino acids, which form ladder networks. They are analogous to the hydrogen-bonded ladders of Polyamide 6 (PA6) [23]. The constituent amino acids of gelatins also have many reactive groups like hydroxyl (OH), carboxylic acid (COOH) groups, which react with adjacent carbonyl groups forming bridged networks. Such type of ladder networks or bridged networks makes the Gelatin almost radiation-resistant.

Glycine has the simplest structure because it has a methine group (CH) surrounded by peptide bound –NH group on one side and the C = O group on the other side considering the chemical structure of amino acids. Therefore, there is every possibility for the cleavage of a proton from the CH group (methine group) than the adjacent –C=O and NH – groups involved in peptide binding. Due to this reason, many authors report cleavage of glycine moieties on irradiation of Gelatin [10, 24]. This type of cleavage results in the formation of – NH – ĊH– C (= O) – (I) free radical, which is called macro radical. There is another possibility of chain cleavage in glycine, i.e., cleavage of either C- C (= O - or C – NH – bond. They will result in ŃH – (II) radical and $\dot{C}H_2$ – C (=O) – radical (III) [in case of C – N bond cleavage] and $\dot{C}H_2$ – NH – (IV) free radicals. Free radical I give component doublet or multiplet spectrum; while radical II gives both triplet or multiplet spectrum and free radicals III, IV gives component triplets.

As per the computer simulation studies, the ESR spectra of irradiated PGL are composed of the component quartet, triplet, and doublet spectra assigned to I, II, III, and IV. Further, if the irradiation is conducted in the oxygenated conditions, the free radicals abstract oxygen and convert into either alkoxy or peroxy radicals, which give either component singlet or asymmetric doublet spectra [25]. Therefore, the resultant ESR spectrum has a complex shape, as observed in the present studies.

Among the amino acids of gelatin, glycine is more prone to radiation attack as the large pendant groups of remaining amino acids are firmly bound by either the peptide/ hydrogen bonds. These bulky groups are buried under the ladder networks or bridge-type networks. They are considered to be prone to radiation attacks. Even in this case, if any chain cleavage occurs in any one of the C - C or C – N bonds of the AA it will result in the formation of III and IV radicals, which give identical component spectra with different g – values.

3.3. *Component spectra at RT*

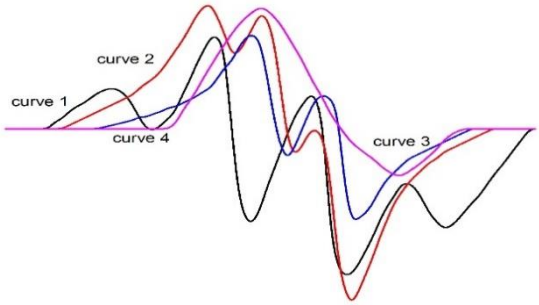

Fig. 3c. Component spectra of PGL.

3.4. *Line width and line intensity - temperature behavior*

Annealing experiments were conducted to study the thermal stability of free radicals produced in irradiated PGL. It is observed that the ESR signal is stable up to 350 K, and the signal begins to vanish around 365 K. Melting temperature of PGL is reported to be around 335 and 480 K [16]. Therefore, the ESR signal observed for PGL is decayed below its melting point (T_m), suggesting that the free radicals are trapped in the amorphous phase of PGL. This phenomenon is observed for several semi-crystalline polymers like polyglycolic acid [17] and polylactic acid [26]. A plot of ESR intensity and ESR linewidth against temperature is as shown in Fig. 4. The graph also suggests a stepwise change of either line width or intensity at a particular temperature, suggesting that a transition is nearing. Such type of behavior has also been observed by Mosleh *et al*. [27], who observed sudden stepwise variation of storage modulus (E) at the glass transition temperature (T_g), and another stepwise variation is also observed near denaturation temperature (T_d).

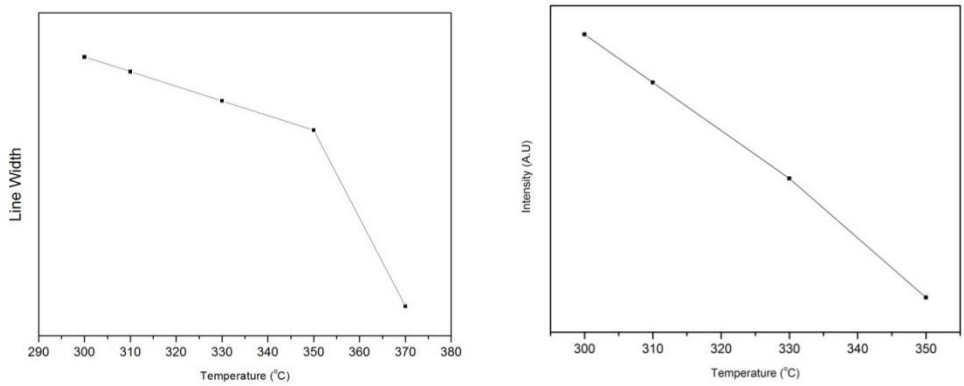

Fig. 4. Temperature-line width, temperature-intensity graph of PGL.

3.5. *Calculation of activation energy using Bloch analysis*

Bloch analysis is applied to evaluate activation energy associated with free radical decay [28]. The line width values at different temperatures are calculated, and the parameters are as listed in Table 4. The plot of inverse of temperature (1/T) against the inverse of 1/τ is drawn as shown in Fig. 5. From the slope of the straight-line value of activation energy is calculated. For irradiated PGL, the value is around 35 K J/mol. The low value of activation energy suggests that the free radicals formed in gelatins have a tendency to crosslink, which is responsible for the improvement in the observed mechanical properties of gelatins [12,14].

Table 4. Bloch analysis of PGL.

S. No	Temperature (K)	$\frac{1}{T}X10^{-3}$	Line width ΔH_{pp}	τ	$\frac{1}{\tau}$	$\log\left(\frac{1}{\tau}\right)$
1	300	3.33	8.5	-	-	-
2	310	3.22	8.0	0.008953	111.0944	2.04
3	330	3.03	7.5	0.00979	102.14	2.009
4	350	2.85	7.0	0.0107	93.45	1.9706
5	370	2.77	4.0	-	85.0	1.93

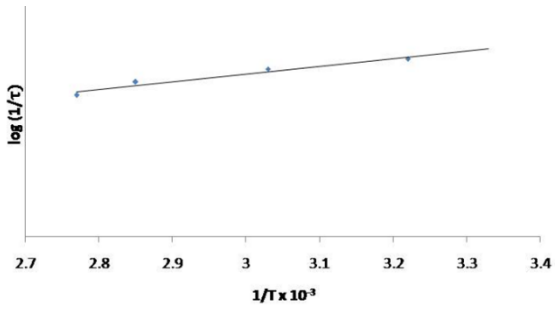

Fig. 5. Bloch analysis 1/T – log (1/τ) graph of PGL.

4. Conclusion

In conclusion, gamma irradiation PGL cause cleavage of molecular chains producing different types of free radical. Each has its component ESR spectrum, and the resultant spectrum of irradiated Gelatin has a complicated shape. The chemical structure and magnetic environment of free radical species are evaluated by simulation techniques. Annealing studies suggest that free radical decay is associated with the transition temperature of PGL. Activation energy associated with the free radical decay is evaluated by Bloch Analysis, and the value suggests that the free radicals have a tendency to crosslink.

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