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# Effect of Gamma radiation, bloom strength and annealing on pig skin gelatin with high bloom (PGH)

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**ABSTRACT**
*Radiation processing of gelatins is considered to be important tool to bring out changes in chemical structure making it suitable for different applications. The authors presents results on gamma radiation effects on porcine gelatin with high bloom (PGH) and compare them with low bloomprocine(PGL). ESR spectra of gamma irradiated PGH at RT possess four hyperfine line spectrum / quartet. The spectrum is not due to one free radical: instead it is caused by atthree different free radicals generated by cleavage of chains on irradiation of gelatin.Though ESR spectra of PGL and PGH appeared to have similar shapes spectral differences are observed, which are explained in terms of variations in bloom strength. Temperature dependent ESR spectra suggest that spectral signal vanished around $90^0$C. Activation energy associated with free radical decay is calculated using Bloch analysis. FTIR spectra of non-irradiated and irradiated PGH are recorded and resultant changes are explained in terms of chemical changes induced by gamma irradiation. Due to changes in chemical structure, thermal properties are also varied as per DSC data. Effect of radiation on morphology is also evidenced from SEM studies.*

**Keywords: ESR:** Electron Spin Resonance, **FTIR:** Fourier Transform Infrared Spectroscopy, **PGL**: Porcine Gelatin Low Bloom, **PGH**: Porcine Gelatin High Bloom,**DSC:** Differential Scanning Calorimetry,**SEM:** Scanning Electron Microscopy.

## INTRODUCTION

Gelatins are important class of polymers having applications in food package [1], bio-medical articular cartilege[2, 3] and microelectronics [4]. They alsohave antimicrobialapplications [5] and or used in breast cancer treatment [6]. Due to the chemical functionalities the Gelatins are considered to be more advantageous then conventional polymers. During the course of applications there isa risk of exposure to high energy radiations which alter their structure associated by compositional variations in amino acids. Further, authentication of gelatins has become a major issue due to religious concerns as well as health issues[7]. Gelatins are biodegradable biopolymers composed of various types of amino acids in different compositions. The amino acid sequence and composition depends on their source i.e. bovine, porcine, fish etc. [8] Bloom value of gelatin is an important parameter, which depends on processing conditions of base material i.e. collagen. Gelometer is used to measure the bloom of the gelatin. Gelatins with a bloom of 50 -125 are called low bloom, soft molecular structure and available creamy form; while gelatin with bloom value of 125 – 225 are called medium bloom and are used in frosting cream stabilizers. Gelatin with a bloom value of more than 225 is called high bloom gelatin made from cow or pig collagen, which are

used in food deserts and jelly filling. High bloom Gelatins generally have high values of melting (Tm) and gel temperature (T gel) but shorter gelation times .This is due to longer peptide chains of high bloom Gelatins when compared to shorter peptide chains of low bloom gelatins [9].The peptide chain length determine the $T_m$ and $T_{gel}$of Gelatins.

Though degradation of gelatins by different radiations i.e.thermal [10, 11], photo degradation [12,13],electron beam irradiation [14]are available, gamma irradiation effects on high bloom porcine gelatin is not attempted previously.Since gamma irradiation is a free radical process, ESR technique in conjunction FTIR is used to characterize the irradiated gelatins. ESR spectra are interpreted by computer simulation methods. Effect of bloom strength on ESR spectra is also attempted in the present studies.

Islam et al [15] have reported on effect of gamma irradiation on bloom value of gelatin and reported that the bloom value decrease with increase of radiation dose except the intermediate dose of 1 K.Gy. This is attributed to the crosslinking of gelatin chains at initial radiation doses. However with the increase of radiation dose beyond this value (1 K Gy) degradation occur, which resulted in the decrease of bloom value.

Yasmin et al [16] have reported that the gelatin with high bloom will have high glass transition (Tg), denaturation temperature (Td). The increase of Tg is due to triple helices present in Gelatin, which act as physical cross links restating the chain movements [17]. But denaturation temperature is not influenced by bloom value, subjecting that the dimension of triple helix is not effected by bloom value. Gelatin with high bloom contains more crystalline ordered domains in the form of triple helices. Decrease of bloom value with decrease of triple helix content is correlated to the decrease ofintensity of XRD peak centered on $2\theta=8^0$. Consequently gelatin with high bloom value had high denaturation enthalpy associated with triple helix content.

Gelatins extraction temperature affects its bloom value.An increase in bloom index lead to improvement in mechanical properties and reduction in water absorption capacity[18]. Gelatin extracted at low temperature is harder and has high bloom value and possess high melting point [19]. Bloom strength is reported to depend on the base material (from which it is extracted).For example the bovine gelatin had a bloom value of 225; while for porcine the value is 300[20]. Chuyanika et al [21] have observed a bloom value of 252 for bovine gelatin and 205 for fish gelatin. Though gamma irradiation effects on porcine gelatin with low bloom are available [22, 23], such studies are not available for high bloom geletins(PGH). In this study the authors focus investigations in this aspect with Electron Spin Resonance (ESR),Fourier Transform Infrared Spectroscopy(FTIR), Differential Scanning Calorimetry (DSC),Scanning Electron Microscopy(SEM)techniques.

**EXPERIMENTAL**

Porcine Gelatin with bloom strength of 220 (PGH) in powder form is supplied by Sigma Aldrich. The sample is used as supplied, without any further treatment. PGH is irradiated with gamma radiation facility Cobalt – 60 ($Co^{60}$) radiation source with a radiation dose rate of 0.2 M Rad/hour in air at room temperature [RT]. The samples were immediately transferred into quartz tubes for spectral recording. ESR spectra of irradiated PGH are recorded using Varian E-line spectrometer operating at X-band frequencies and 100 K Hz modulation. FTIR spectra are recorded on Perkin Elmer spectrometer for pellets of sample. DSC thermograms are recorded on Meitler calorimeter under nitrogen conditions with a heating rate of $10^0$ C/ minute and empty aluminum pan as reference. SEM micrographs are recorded on Carl-Zeiss instrument.

**RESULTS AND DISCUSSION**

**ESR studies:**

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ESR spectra of irradiated PGH(10.M. rad radiation dose) in the temperature range of 300 – 360 K are as shown in Figure 1.The spectra appear to have quartet shape but intensity distribution is not that expected from the quartet ( 1:3:3:1). Thespectra are analyzed by computer simulations [24], whichenable evaluation of component spectra. The analysis indicate that the RT (300 K) spectrum is a superposition ofquartet(– NH – ĊH– C (= O)) triplet(ĊH2 – C ( =O )), (doublet)( (– ĊH– ) and asymmetric triplet(ĊH2 – NH –). When compared to the PGL an asymmetric triplet was additionally present in PGH.Magnetic parameters were almost similar to that of PGL [23]. Gelatin is a macromolecule consisting of different amino acids likeproline, hydroxyl proline, alanine connected by peptide bond. Radiation attack mainly occurs at peptide bond (CONH)[23]producing free radicals. In particular,chain cleavages also occur on glycine and proline. The difference in presence of different free radicals is attributed to compositional variations in amino acids. The results are in agreement with the earlier reports. When compared to PGL, the PGH contain one more tripletwith asymmetric shape. The presence two different triplets are also possible as cleavages occurred on glycine and proline and both of them give triplet spectra with different parameters [25].

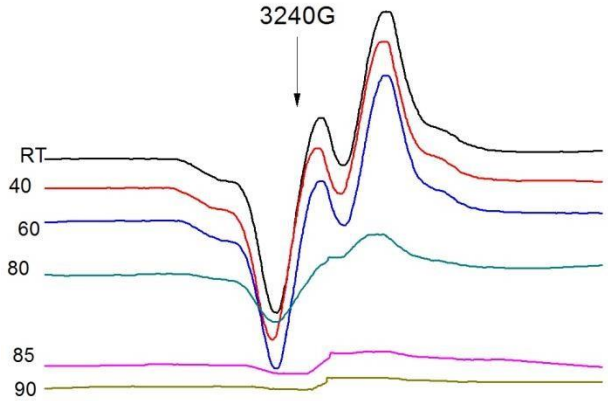


**Figure 1: Temperature dependent ESR spectra of PGH irradiated to 10 M. Rad dose**

**EFFECT OF BLOOM STRENGTH ON SPECTRSCOPIC PROPERTIES**.

Bloom strength has an influence on spectroscopic properties of gelatins as evidenced from the comparison of ESR spectra of PGL and PGH at different temperatures as shown in Figure2 ( Fig 2A to Fig 2D)and the results are summarized in the following table1.

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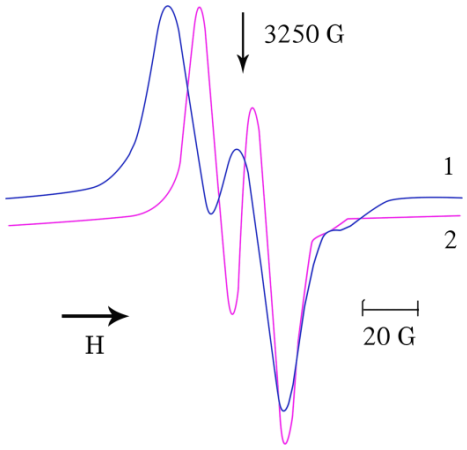


Figure2A:Comparison of ESR at 300K Curve 1 PGH Curve 2 PGL .

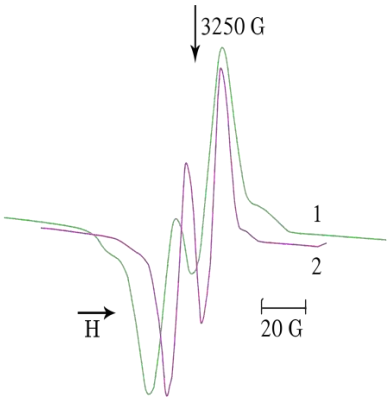


Figure2B:Comparison of ESR at 310K Curve 1 PGH Curve 2 PGL.

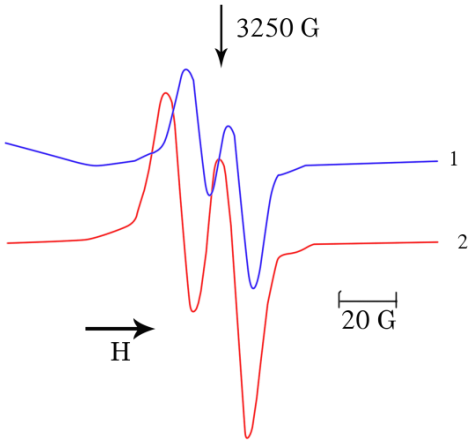


Figure2C:Comparison of ESR at 330K Curve 1 PGH Curve 2 PGL.

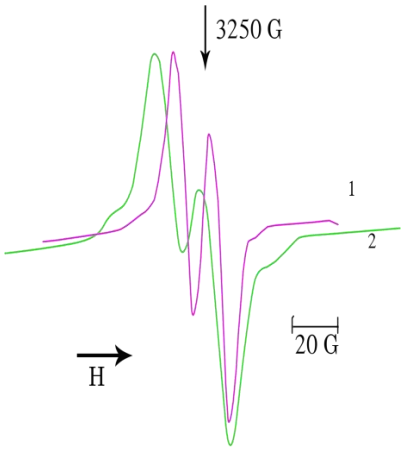


Figure2D: Comparison of ESR at 350K Curve 1 PGH Curve 2 PGL.

Figure 2: Comparison of ESR spectra of PGH and PGL at different temperatures

**Table 1: SPECTRAL DIFFERENCES OF PGL AND PGH**

| S No | Spectral Change observed | PGL | PGH |
|---|---|---|---|
| 1 | Stability of spectrum | The quartet shape is stable up to $80^0$ C and signal vanished around $100^0$ c | The quartet decayed at $60^0$ c and ESR signal vanished around $95^0$ C |
| 2 | Radical decay temperature | $85^0$ C and $100^0$ C | $60^0$ C and $95^0$ C |
| 3 | Spread of the spectrum | Varied stably up to $80^0$ C and then abruptly decreased | Varied stably up to $60^0$ C and then abruptly decreased |
| 4 | Activation Energy | 35 KJ/ mole | 30 K J / mole |

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**VARIATION OF ESR LINEWIDTH AGAINST TEMPERATURE**

The free radicals are considered as molecular chains of polymer produced by cleavage of chain. Since polymer which also undergoes different types molecular relaxations / transitions with the change of temperature, the free radical molecule will also experience relaxations when temperature of observation is nearer to it. The molecular relaxations are associated with changes in physical change properties. As such line width variations are monitored to detect such transitions in polymer. Variation in ESRline width against temperature is plotted as shown in Figure 3. As expected the ESR intensity suddenly became minimum around 380 K. It is understandable that the free radicals increase with the increase of temperature and it is maximum measure to detect and for this purpose variation of inverse of temperature against inverse of log 1 / $r$is as shown in Figure 4. Similarly line width (Bloch) analysis is used to evaluate activation energy associated with free radical decay. In the present studies the activation energy is calculated around 30 K J/ mol. Parameters used in Bloch analysis are as listed in Table 2.

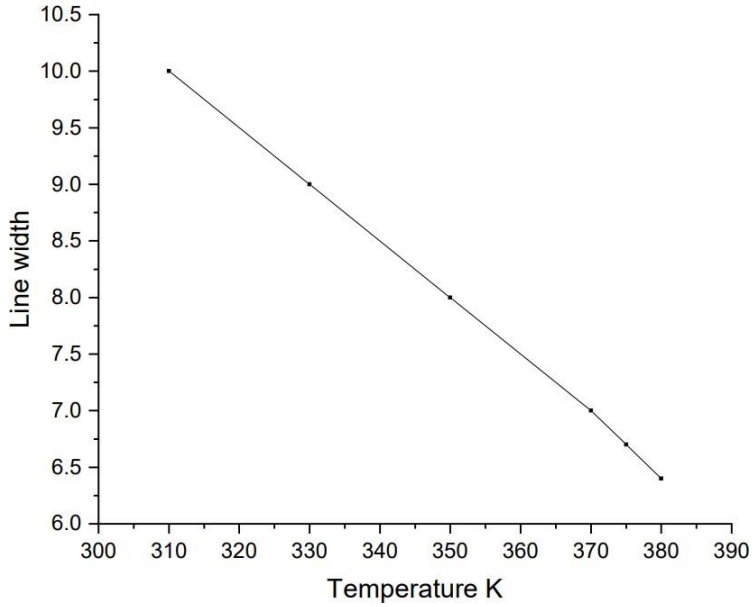


**Figure 3: Temperature- Line width variation.**

**Table 2: Parameters used in Bloch analysis**

| S.No | Temperature $^0$C | Temperature K | 1/T x $10^{-3}$ | Line width | $r$ | 1/τ | log(1/τ) |
|---|---|---|---|---|---|---|---|
| 1 | 30 | 300 | 3.22 | 10 | - | - | - |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 2 | 40 | 310 | 3.03 | 9 | 0.0069 | 144.09 | 2.184 |
| 3 | 60 | 330 | 2.85 | 8 | 0.0081 | 123.456 | 2.114 |
| 4 | 80 | 350 | 2.7 | 7 | 0.0093 | 107.526 | 2.051 |
| 5 | 85 | 355 | 2.66 | 6.7 | 0.0093 | 107.31 | 2.03 |
| 6 | 90 | 360 | 2.63 | 6.4 | 0.0094 | 101.01 | 2.002 |

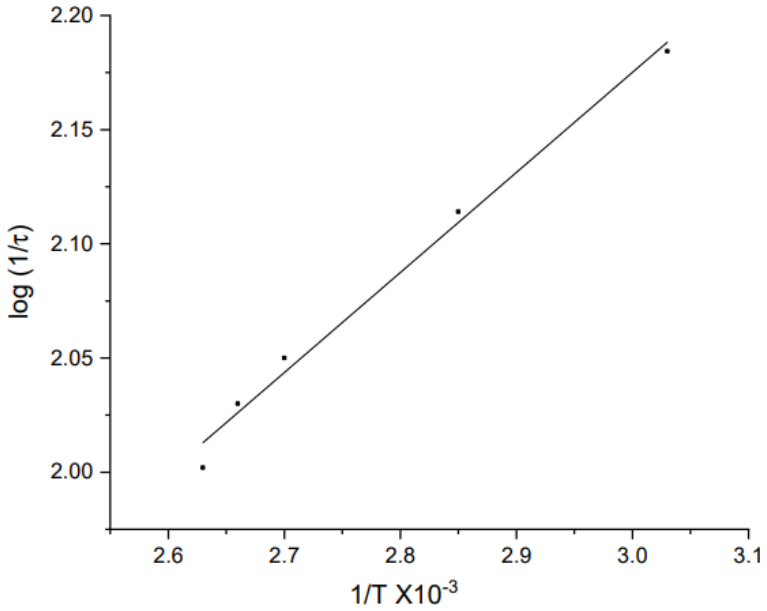


**Figure 4: Bloch analysis of (1/T X10$^{-3}$ – log (1/τ) ofirradiated PGH**

**FTIR studies:**

Gamma irradiation leaves changes in chemical structure of polymers either by removal/ generation of functional/chemical groups, which can bedetected by FTIR spectroscopy. As such FTIR spectra ofun-irradiated (Curve 1 figure 5)and irradiated, (lowdose) curve 2 and curve 3

(Highdose)are recorded as shown in Figure6. Variation in band positions / intensities are noted and the results are given in table 3. Comparison of Chemical changes induced by irradiation(curve2)and irradiation + annealing(curve3)are alsostudied with respect to un-irradiated (Curve 1) are made as shown in Figure6. The absorption band position and their assignments are given in table 3. Effect of annealing on chemical changes can be asserted by comparing the FTIR of un-irradiated, irradiated and annealed.

FTIR spectra of PGH are shown under two different conditions. They are i) radiation dose and ii) annealing. For comparison purpose base spectrum (Un-irradiated) [Curve1 Figure 6] is compared with the irradiated spectra [Cuev2 Figure 6(low dose) and Curve 3 Figure 7(high radiation dose]. Similarly to study the effect of annealing (at 100$^0$C) the base spectrum (curve1 Figure 6.

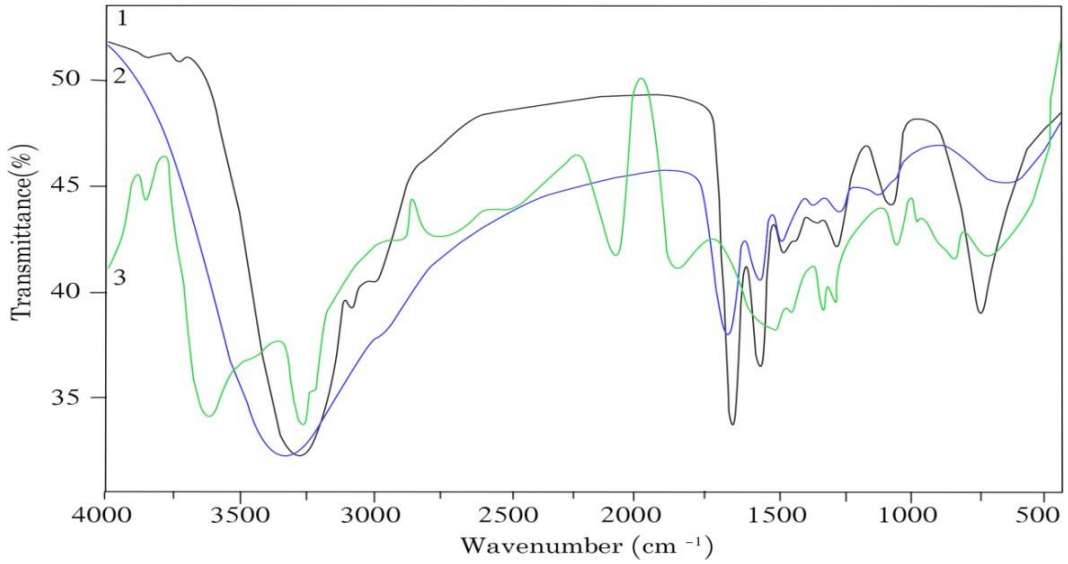


Figure 5: FTIR spectra of PGH
1.Un-irradiated 2.Low dose and 3.High dose.

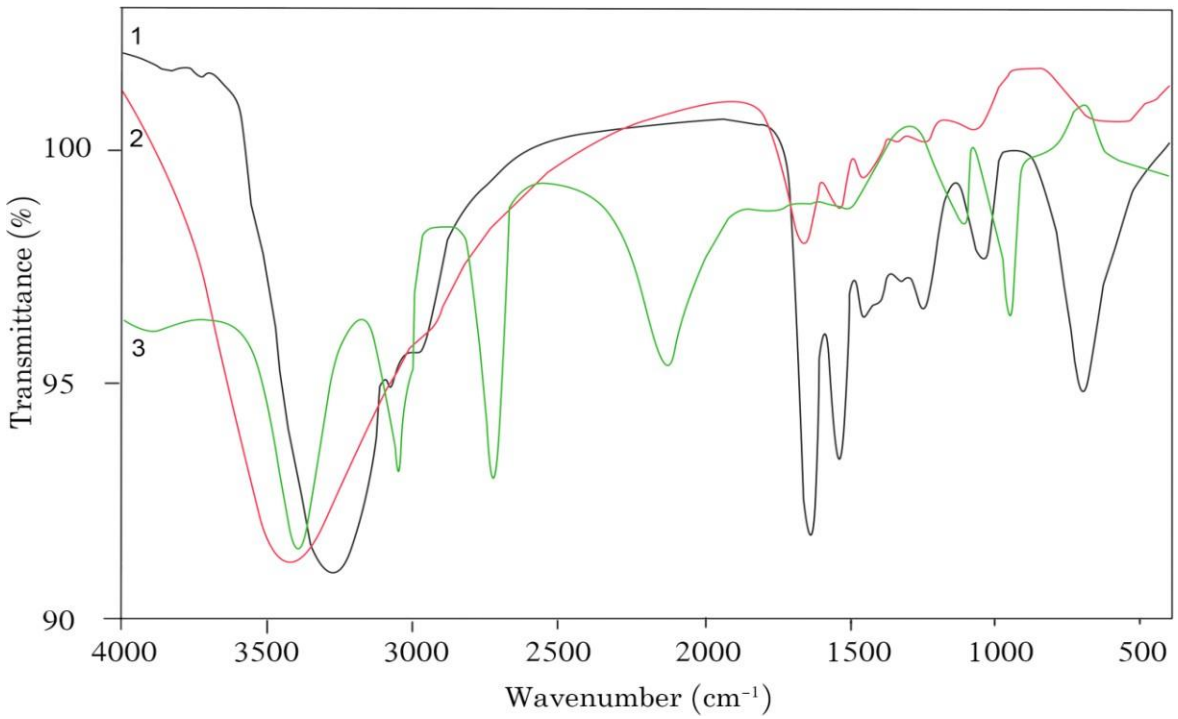


**Figure 6: Comparison of FTIR spectra of PGH under different conditions**
1. Un-irradiated 2.Irradiated 3.Irradiated and Annealed

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Collagen and gelatin based materials are constituted by different types of amino acids bond together by peptide bonds. Vibrational bandsof these materials are reported (26). The studies indicate the presence of 3300-3250 $Cm^{-1}$(amide A), 3100 – 3050 $cm^{-1}$(amide B), 1690 -1600 $cm^{-1}$ (amide – I), 1550 – 1480 $Cm^{-1}$(amide II), 1300 – 1230 $cm^{-1}$(amidIII), 767 – 625 $cm^{-1}$, (amide IV), 800 -640 $cm^{-1}$(amide V) and 606 - 573 $cm^{-1}$(amide VI).

**Table 3:FTIR absorption bands of PGH under different conditions**

| S. No | **Base Spectrum Un-irradiated** | **Condition of recording** | | | |
|---|---|---|---|---|---|
| | | **Low dose** ($cm^{-1}$) | **High dose** ($cm^{-1}$) | **Irradiated and Annealed** ($cm^{-1}$) | |
| 1 | 3732-3100 | 3424(40) | 3448 | 3455(41) | I, II |
| 2 | 2740 | 2930 | 2924 | 2925 | C-H Alkane |
| 3 | - | 2850 | 2850 | 2850 | C-H Alkane |
| 4 | 1630 | 1657 | 1654 | 1652 | III |
| 5 | 1540 | 1550 | 1550 | 1550 | IV |
| 6 | 1494 | 1454 | 1450 | 1458 | New |
| 7 | 1332<br>1320 | 1347 | weak | weak | V |
| 8 | 1242 | 1240 | weak | 1260 | V |
| 9 | - | 1163 | 1160 merged | 1158 | New |
| 10 | | 1087 | 1080 merged | 1087 | New |
| 11 | 1031 new | 1026 | 1020 merged | 1028 | New |
| 12 | 800,700,600 merged | 800,700,600 merged | 800,700,600 merged | | VI, VII, VIII |

From the FTIR data the following results are suggested:

i) The 3424 $Cm^{-1}$ absorption band shifted to higher wave lengths i.e. 3455 $Cm^{-1}$; together with a reduction in intensity. The shift is in the order of about 31 $cm^{-1}$

ii) The 1657 $cm^{-1}$ band shifted to 1650 $Cm^{-1}$sufring a shift of 7$cm^{-1}$

iii) The intensity of 1550 $Cm^{-1}$ absorption band is reduced.

iv) 1464 $Cm^{-1}$ absorption band shifted to 1459 $Cm^{-1}$ with a decrease of intensity

v) The 1260 absorption band shifted to 1240 $Cm^{-1}$ , with a decrease in intensity

The results further suggest cleavage of N-H and or hydrogen bonds (O-H) bonds on irradiation. Since gelatin's molecular chains inter-connected by peptide (CONH) and molecular hydrogen bonds[27] above interpretation is more appropriate. In addition cleavage of C - C and or C - H is also anticipated.

**DSC STUDIES:**

Un-irradiated PGH (shown in Figure7) consists of DSC peaks centered around $108^0$ C, $160^0$ C and $168^0$ C and $198^0$ C and $284^0$ C, (shown as curve1 & curve2 figure7). Thermal transitions in gelatins mainly occurs in two regions i.e. I) 100 – $200^0$C and II) 200 - $225^0$C (28). Among them, the first transition is minor and observed in the temperature region of 80 – $100^0$ C associated with formation of glass like structure involving alpha –amino acid blocks of peptide chains. The second transition is in the temperature range of 180 – $200^0$C and it corresponds to the blocks of amino acid proline, hydroxyl proline and glycine (29). Presence of water in the gelatin influence its Tg and depending on percentage of water, the Tgis shifted (30). The peak positions of presentstudies are nearer to the reported in literature value.

On irradiation the first peak shifted to $98^0$ C; while second set of peaks (at $160^0$ Cand $168^0$ C) merged in to a single peak at $162^0$ C.In case of third set of peaks shifting is not observed but decrease of intensity is decreased. The first peak corresponds to glass transition. The second set of peaks ($160^0$ C and $168^0$ C) is reported to be due glass transition temperature of gelatin [15]. Since gelatin is a heterogeneous molecular system, different types of structural relaxations are possible. Further the structural transition temperatures may decrease on irradiation due to extensive chain cleavages resulting in decrease of molecular weight. Thermal properties of PGH are listed in the following table 6. The third set of peaks are appear at $198^0$ C and $284^0$ C are due to either chemical or melting transitions. The appearance two melting peaks suggest that multiple transitions are possible in gelatins in view of its heterogeneous molecular structure.

When compared to PGL, the DSC peaks of PGH have appeared to be sharpened shape when compared to the broadened peaks of PGL. This is a consequence of multiple cycles of processing involved to obtain high bloomed gelatins; while low bloomed gelatins can be obtained by less number of processing cycles. Due to the variations in processing conditions, the water content and inter-molecular hydrogen bonds are more in PGL resulting broadened DSC peaks. In case of PGH, the processing cycles are more resulting in low water content and less degree of hydrogen bonding resulting in sharpened DSC peaks. On irradiation a shifting peaks ( Tg shifted to $100^0$C and two melting peaks merged to a single peak at $100^0$ C) together with decrease in intensity is observed(curve1 in Figure 7). For comparison purpose both the thermo grams are as shown curve 2in Figure 7. Thermal properties of PGH are as listed in table 4.

**Factors influencing glass transition temperature:**

**1** Increase in pressure of surroundings leads to decrease of free volume resulting in high glass transition temperature.

**2** Tg depends on the mobility of chains. If mobility of chains is more, the Tg will be more. This is due to the fact that if the chains are movable, the transition from glass to rubber state passes at low temperature. In contrast, if the chains are immovable, it requires high temperature to transform from rubber to elastic state.

**3** Chain stiffness: Stiffness of chains reduces flexibility of chains and raise Tg value.

**4** Intermolecular Forces: Stronger inter molecular forces lead to high value of Tg. For example the dipolar bond between carbon (C) and chlorine (Cl)[C-Cl dipolar bond]

**5** Pedant Groups: Pendant groups restrict rotational freedom and increase Tg value. Flexible pendant groups decrease the Tg Value.

6. Crosslinking of chains restricts rotation motion and raises the Tg value

In the present studies some the above reasons are responsible for variations of transition temperature of gelatin.

**Table 4: Thermal Parameters of PGH( Enthalpy values are given in brackets)**

| Condition | Peak1 ( enthalpy ) | Peak 2 | Peak 3 |
|---|---|---|---|
| Non-irradiated | $108^0$ C (28) | $160^0$ C,$168^0$ C | $185^0$ C,$190^0$ C |
| Irradiated | $98^0$ C (24) | Two peaks merged and single peak at $162^0$C observed. | Intensity Decreased |

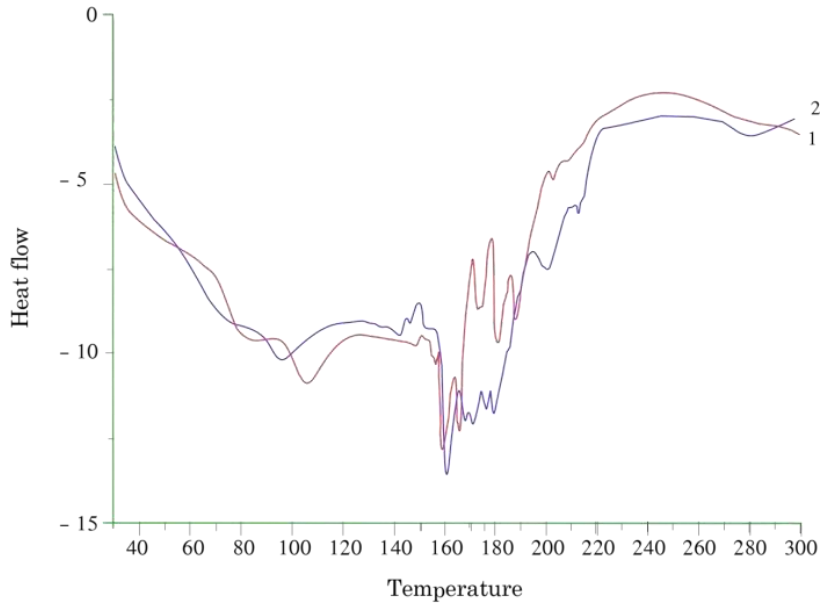


**Figure7: DSC Thermogramof PGH curve1unirradiated,curve 2 irradiated**

**STUDY OF MICRO STRUCTURE:**

Effect of gamma irradiation on surface morphology of non- irradiated and irradiated PGH has been examined by SEM pictures shown in Figure8. As expected non irradiated PGH possess smooth surfaces; when compared to rough surface of irradiated sample. Further cracks and fine holes are also visible on irradiated surface,

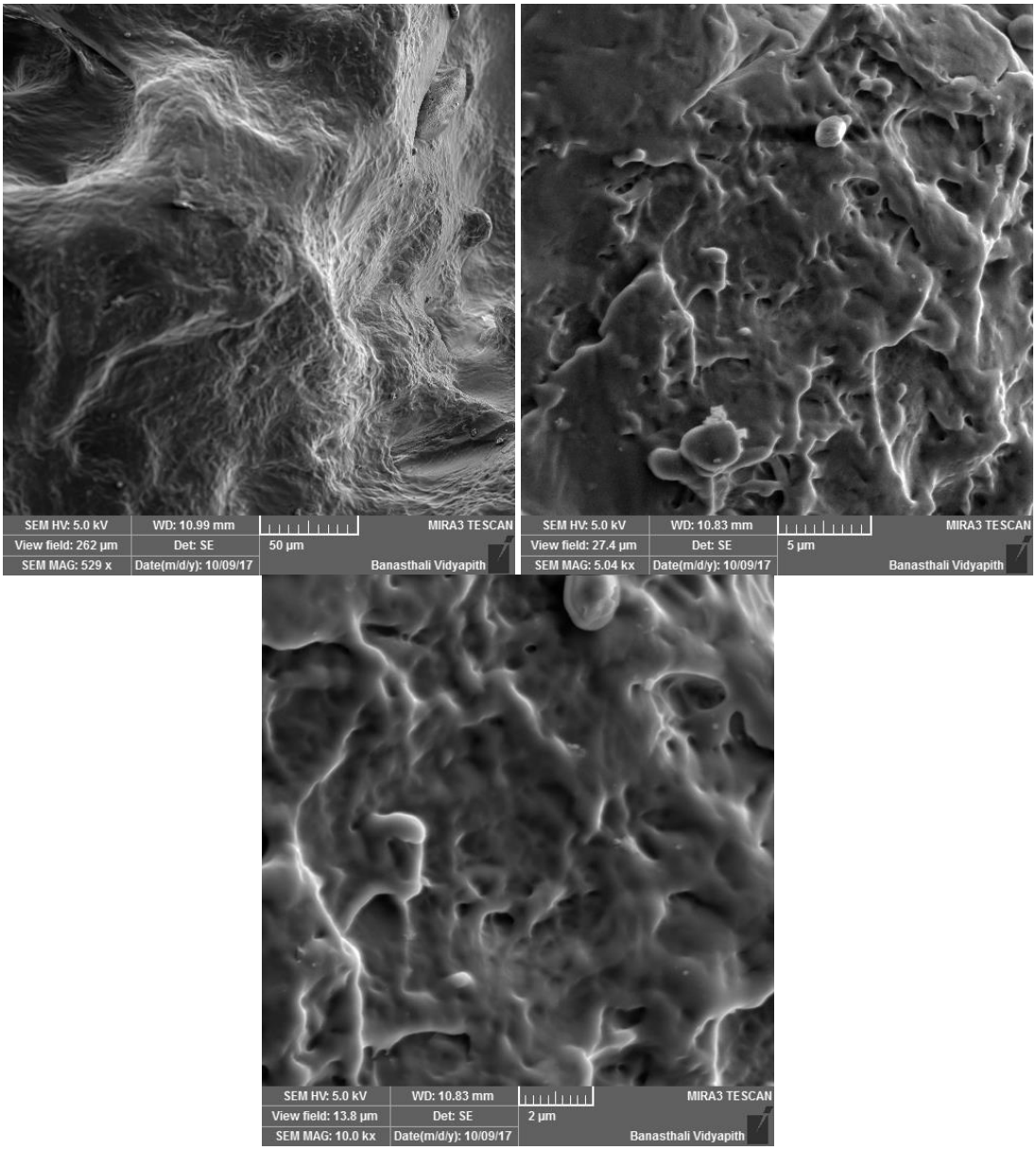


**Figure 8:Micro structure of PGH under different conditions.**

**Effect of radiation dose and Dosimetric applications:**

Dosimetric applications of PGH have been studied by recording the ESR spectra at differentradiation doses (shown Figure 10) . Spectral intensity of ESR spectra gradually increased though spectral features remain the same. The increase of intensity is due to the formation of more number of free radicals with the increase of radiation dose. To have a comparison concentration of free radicals against radiation dose is plotted as shown in Figure 11. The dosimetric applications of given polymer system is considered if ESR intensity varies linearly against radiation dose(24)as observed in the present studies.

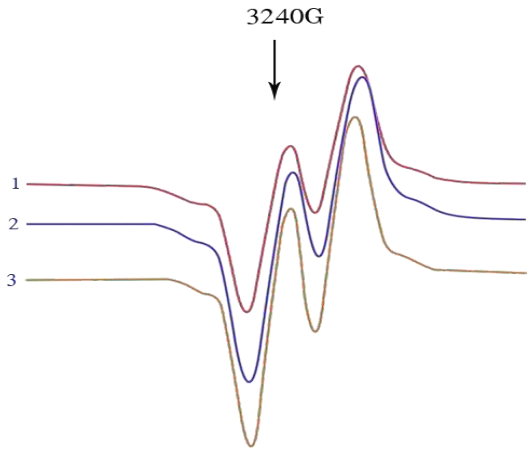


**Figure 10: ESR spectra of irradiated PGH at different radiation doses**
**Curve1: 3.5 M rad curve 2 : 7.0 M rad Curve 3 : 10.5 M rad**

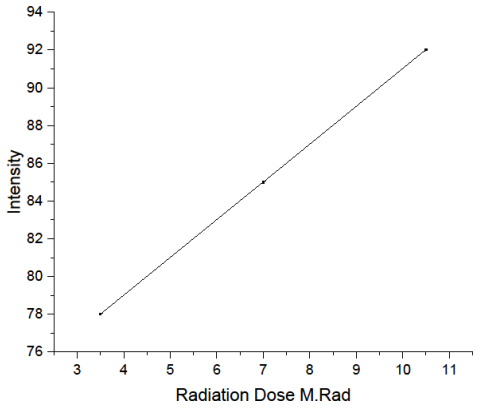


**Figure 11: Variation of free radical concentration with radiation dose.**

## CONCLUSION

Gamma irradiation effects of high bloom porcine gelatin(PGH) have been studied by spectroscopic (ESR, FTIR), thermal (DSC), and morphology (SEM) methods. Temperature dependency and annealing effects of free radicals is studied by recording ESR signals. Activation energy associated with free radical decay is estimated from Bloch analysis. Chemical groups effected by irradiation and annealed, irradiated samples is ascertained from FTIR data. Data obtained from ESR as well as FTIR are compared and analyzed. Thermal properties influenced by gamma irradiation are calculated from the DSC data. Influence of bloom strength on gamma irradiation of gelatin is also reported in this article.

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