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Chemiluminescence study of commercial type-B gelatines

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Abstract

The chemiluminescence emission of type-B commercial gelatines films was studied under different conditions. The influence of the gel strength resistance or bloom value of the gelatines on the characteristics of the emission has been investigated. Gelatine material of better quality, bloom 225, gives lower emission than the bloom 75 gelatine material under all the conditions employed in this work. The level of hydroperoxide concentration, determined in the gelatine films, correlates with the emission intensity. A mechanism of hydroperoxide and peroxide radical formation has been proposed in a similar way to that of the autooxidation reactions that take place on *N*-alkyl amide products. The emission is enhanced drastically in the presence of oxygen and at temperatures above the T_g of the gelatine materials. This fact confirms the important role of oxygen mobility in the material. Also, at temperatures higher than T_g the disproportionation reaction between peroxide radicals, responsible of the emission, is favoured.

Keywords: Chemiluminescence; Fluorescence; Type-B gelatine; Photographic gelatine

1. Introduction

Gelatines are a class of proteinaceous materials that are derived from the parent protein structure of collagen [1,2], by a procedure that involves the destruction of the collagen secondary structure and, in some aspects of the primary and tertiary structures. The obtained degraded protein from the collagen, which is the principal component of the white fibrous connective tissues, has a predominantly amorphous macromolecular structure. Gelatine is composed of long chains of amino acids joined through a peptide linkage and the acidic and basic nature of the functionalities present on the side chain confer polyelectrolyte characteristics that govern to some extent the interactions between gelatine macromolecules themselves and with the solvent. These interactions affect the viscosity and other solution properties.

The gelatine manufacture is carried out by acid pre-treatment (type-A gelatine) or alkali pre-treatment (type-B gelatine) from bone and skin collagen fibrils in a reproducible way, but always, gelatine is a degraded protein. Gelatine from successive extractions has different physical and chemical properties due to the hydrolytic degradation that takes place during the manufacturing process. Its variety of properties have focused much scientific interest due to the large number of commercial applications such as edible, pharmaceutical and photographic uses. After the pre-treatment, the first extract (with hot water) is obtained at the lowest temperature and always exhibits a higher bloom value or gel strength. This is the common parameter to measure the gelatine quality. Colour and turbidity increases in later extracts, details of the manufacture process and the operating parameters are described in the bibliography [3].

The application of gelatine revolutionized photography, and nowadays, it is still the principal polymer employed as component in silver halide photographic films. The most photographic gelatine is type-B, obtained from cattle bone by the liming process and, high bloom values are required due to the important properties related with this high quality gelatine grade. Gelatine is important in the entire photographic process [4,5], covering the formation of the silver halide emulsions, through the manufacturing steps to the developing and printing operations.

The purpose of this research is to explore the possibilities of chemiluminescence emission to study type-B gelatines since this technique can be employed in the solid state gelatine which is an application advantage. The measurements of properties in the solid state offer the possibility to study

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different processes for these materials from a comparative point of view. In this work we have compared two gelatine raw materials differing in molecular weight (bloom value) and, also, gelatine hydrolytically degraded in water solution. The characteristics of the studied gelatines were determined by measurement of their viscosity in aqueous solution.

The technique of chemiluminescence [6,7] is useful for the study of polymer degradation, oxidation mechanisms, kinetics and stabilisation efficiency. The chemiluminescence is a week emission of light produced as a direct result of a chemical reaction [8,9], generally due to oxidation species. This emission is, in general, enhanced by the degradation of the polymer material, that in its term can be induced by different factors such us heat, oxygen, humidity among others. In hydrocarbon polymers the chemiluminescence emission ($h\nu_{\mathbf{CL}}$) is due to the decomposition of peroxides ($POO^{\bullet}$) which are related to the hydroperoxide (POOH) originated by the reaction of oxygen with radicals ($P^{\bullet}$) formed in the macromolecular chain. A simplified general mechanism can be as follows:

$$PH \xrightarrow{\Delta} P^{\bullet} \xrightarrow{O_2} POO^{\bullet} \quad (1)$$

$$POO^{\bullet} \xrightarrow{PH} POOH \quad (2)$$

$$POO^{\bullet} + POO^{\bullet} \rightarrow POH + {}^1O_2 + P^3(C{=}O)^* \rightarrow PCO + h\nu_{CL} \quad (3)$$

As mentioned above, gelatine is not stable in aqueous solvent systems and undergoes a progressive hydrolytic degradation lowering its molecular weight and consequently decreasing its useful physical properties. As will be shown in this work chemiluminescence emission is sensitive to the characteristics of the gelatine raw material. Hence, gelatine films were prepared to measure Chemiluminescence and were previously characterised by DSC and absorption and fluorescence spectroscopy.

2. Materials and methods

2.1. Materials

Gelatines used in this work were supplied by Aldrich Chemicals. Two powder commercial products of type-B gelatine with different gel strength value were used, bloom values 75 and 225. Also, films of 40 μm were prepared using aqueous solution of gelatine (6.67 wt.%) and solvent evaporation at 37 °C.

2.2. Viscosity measurements

Water solution viscosity, often determined at 6.67 wt.% is a widely characteristic property of the gelatine used in the industry as an estimation of the relative molecular weight [10]. Values measured at 40 °C or above range from 3 to 10 cP ($mm^2\,s^{-1}$) for commercial gelatines [11]. In this work, we have measured the viscosity of the gelatines at 37 °C in unbuffered water solution at 6.67 wt.%. The temperature was maintained constant using a glass-panelled thermostatised bath of ±0.02 °C.

To measure the viscosity an Ubbelohde micro-viscometer (filling capacity of about 2.5 ml) from Schott, having a 0.40 mm capillary diameter was used. The instrument constant of the employed viscometer was $K = 0.01\,mm^2\,s^{-2}$, being suitable for the measurements of viscosities ranging from 0.4 to 6 centipoises (cP). The viscosity of the gelatine solutions in cP can be calculated using the instrument constant by the equation: $\eta\,(cP) = Kt$, were K is the instrument constant and t the flow time in seconds which was corrected when necessary according to the manufacturer of the viscometer and following DIN 51 562. The measurement of flow time of the solution though the capillary was obtained using a digital chronometer of ±0.01 s of accuracy and using the timing mark printed above and below the measuring sphere of the micro-viscometer. In such conditions, at 37 °C water flow time (69 s) was in agreement with the viscosity of water [12] at 37 °C. The viscometer was conveniently cleaned with suitable solvents (hot water, ethanol and acetone) and dried in an oven after each run.

2.3. Chemiluminescence

Chemiluminescence spectra were obtained using a CL400 chemiluminescence analyser developed by Atlas Electric Devices Co. Samples are placed in temperature controlled cells using specimen holders consisting of disposable aluminium dishes. Such cells are closed by optical lenses which focus the corresponding emission light of each sample in a photon counting photomultiplier (Hamamatsu R1527P), which is water-cooled at 17 °C. The photomultiplier was previously calibrated using a radioactive standard provided by Atlas. The instrument has an internal calibration for the photomultiplier tube of photon counts versus milivolts. Depending on the expected chemiluminescence emission of the samples and in order to prevent saturation of the photomultiplier tube (PMT) different sensitivity level of the PMT (gain setting) were selected. Hence, in the measurements under oxygen and nitrogen, low gain and high gain of the PMT were chosen, respectively.

Two different types of tests were performed. (A) Isothermal: samples of material are preheated up to the test temperature under nitrogen, and the media is charged with oxygen and the temperature of the test maintained constant. (B) Dynamic: material samples are heated up with pre-test ramp (10 °C min^{-1}) under constant flow (50 ml/min) of gas, nitrogen or oxygen.

The acquisition and analysis of the data collected by the PMT were processed using the specific software supplied with the instrument. Samples for chemiluminescence

measurements were prepared by cutting circular specimens of 0.8 cm in diameter and the emission area was maintained constant in all the determinations. The gelatine films were obtained from gelatine solutions after water evaporation. To ensure the same thickness of the gelatine films the solvent evaporation was carried out in Petri-recipient cages of 10 cm of diameter filled with 10 ml of the gelatine solution 6.67 wt.%. In such conditions gelatine films of 100 μm thickness were invariably obtained.

2.4. Thermal analysis

Differential scanning calorimetry was undertaken using a Shimatdzu DSC-50 instrument. Gelatine films (5 mg) were analysed in standard cells for comparison at a constant heating rate of 5 °C min^{-1}. The instrument was calibrated with an indium standard (T_m = 429 K and H_m = 25.75 J g^{-1}).

In order to determine the moisture content of the gelatine films, thermogravimetric analysis (TGA) was performed with a Perkin–Elmer TG model TGA 7. The initial mass of the sample was about 5 mg. The moisture content for the gelatine films was 4% in all the tested materials. Such value was determined as the loss of weight in the sample between 50 and 105 °C. The thermograms were obtained under nitrogen atmosphere (flow rate of 20 ml min^{-1} and a purge time of 10 min) using a heating rate was 10 °C min^{-1} and the temperature was maintained at 105 °C for 15 min.

2.5. Absorption and fluorescence measurements

UV-Vis spectra were recorded by means of a Perkin–Elmer Lamda-35 spectrophotometer. Fluorescence emission spectra were recorded on a Perkin–Elmer LS-50B spectrofluorimeter. To improve accuracy in the determination of maximum wavelengths first derivative spectra were obtained in all the wide emission bands. All the spectra were corrected using the response of the photomultiplier.

2.6. Hydroperoxide determinations

The hydroperoxide concentration of polymer film samples were determined by a combination of the methods of Mair and co-workers [13,14]. Fifteen micrograms of film fragments were refluxed with 2.0 ml of a sodium iodide solution in isopropanol (200 g l^{-1}) after acidification with 7 ml of an acetic acid/isopropanol solution (1:10 ratio by volume). After 30 min of reflux, the solution was cooled, diluted with 10 ml of distilled water and the iodide, generated as I_3^- by the reaction, $ROOH + 3I^- + 2H^+ \rightarrow I_3^- + H_2O + ROH$, was determined spectrophotometrically at a wavelength of 360 nm using an absorption coefficient of 2.5×10^4 l mol^{-1} cm^{-1}. In all the cases, reagent blanks were carried out on duplicate refluxes to avoid hydroperoxide impurities.

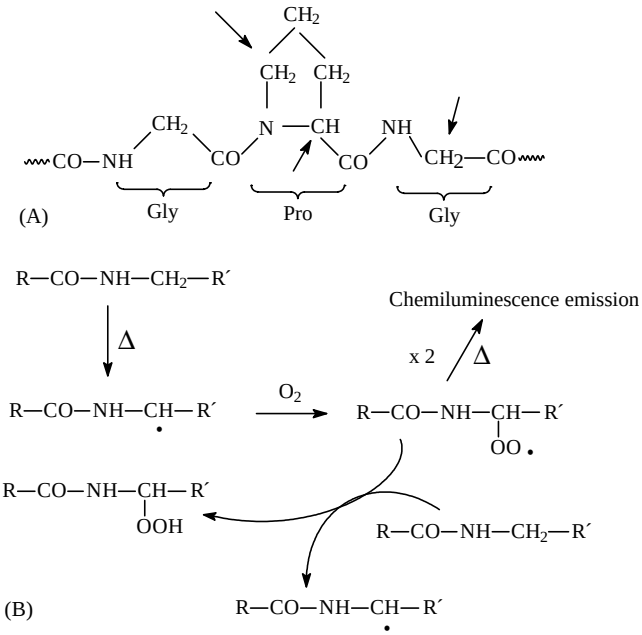
3. Results and discussion

3.1. Spectroscopic and DSC characterisation of type-B gelatines

In this work, we have studied commercial type-B gelatines having two different gel strength resistances, bloom values 75 and 225. The bloom value is a measure of the gelatine quality, since it is the value of the force in grams necessary to apply in a standard plunger to deform 4 mm the surface of the gelatine gel [15]. In general, technical uses of gelatines require strict specifications and, particularly, in photographic applications where gelatines of high bloom and type-B are preferred because of their better quality. At higher bloom value better mechanical properties due to the higher molecular weigh of the gelatine are achieved.

Absorption spectroscopy in the study of collagen provided an early means of detecting the presence of impurities in the preparation of this protein [16]. In contrast, little attention has been paid to the application of UV spectroscopy toward the study of collagen and gelatine in the solid state. The UV absorption spectra of the type-B gelatine films exhibit a strong absorption below 240 nm that can be attributed to the non-aromatic amino acids of the structure, (A) in Scheme 1. The observed band at the longest wavelength interval 250–320 nm is due to the presence of aromatic amino acids. In Fig. 1, the spectra of the two gelatine films in this region are shown.

The absorption band of the gelatines in the longest wavelength region is due to phenylalanine and tyrosine that are the aromatic amino acids presents in gelatines at proportions of 14 and 1.2 residues per/1000 total residues [17,18].



Scheme 1.

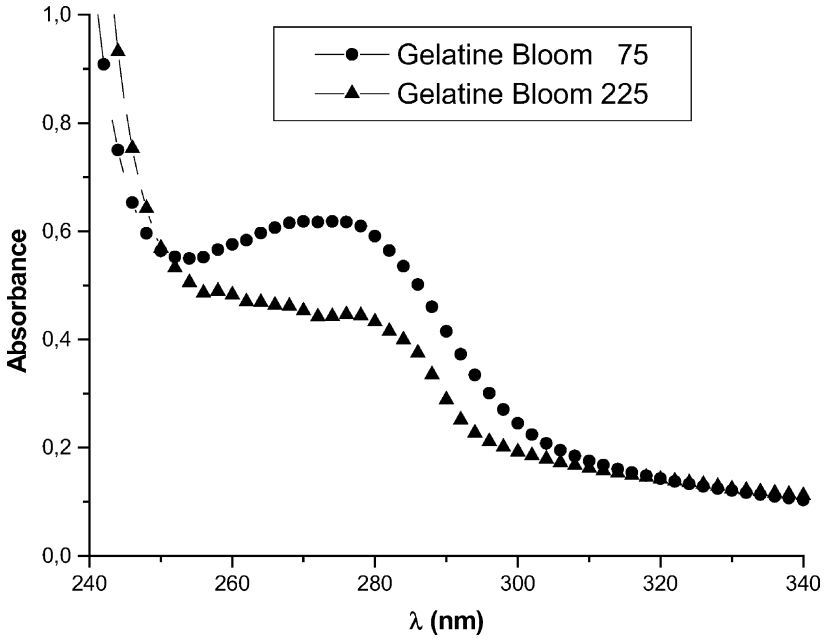


Fig. 1. UV-spectra of the gelatine materials in films (40 μm).

There are differences between both materials which, maybe due to the effect of the strong absorption of the structure under 240 nm and the thickness of the films (40 μm). To determine the presence of absorbing impurities in the gelatine films second derivative spectra were recorded, and these are shown in Fig. 2.

Due to the strong sensitivity of the second derivative spectra, different maxima (minimum in the second derivative) can be distinguished in the aromatic amino acid absorption band. Such maxima differ in relative intensity but not in wavelength position for the two bloom grades of gelatine. This fact confirms the presence of the same amino acids in both materials. Also, no signals are detected in the second derivative spectra on the spectral region until 400 nm indicating that no visible absorbing oxidising species are present in the films.

Fluorescence emission was not detected from the films using an excitation wavelength in the absorption region. In

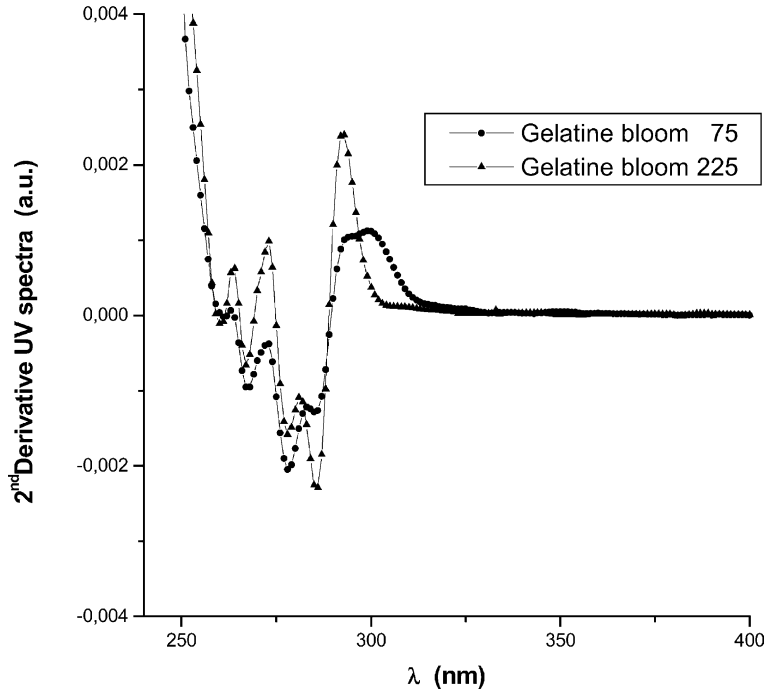


Fig. 2. Second derivative UV-spectra of the gelatine films.

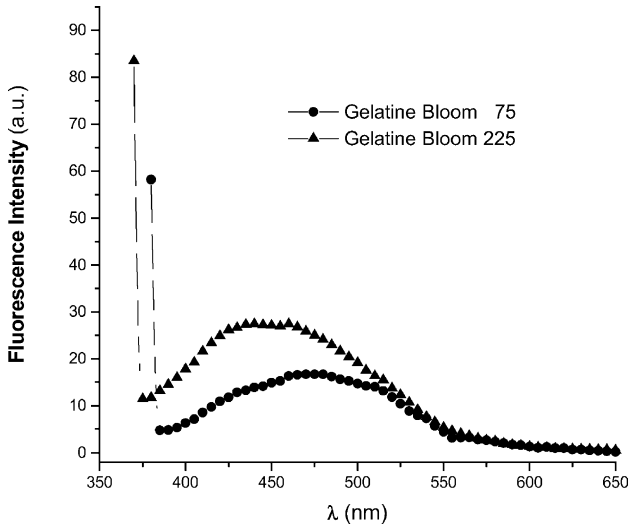


Fig. 3. Fluorescence spectra of gelatine films, $\lambda_{ex} = 355$ nm.

contrast, when 355 nm were used as an excitation wavelength fluorescence was detected. The emissions for both gelatine grades are shown in Fig. 3.

Gelatine of bloom 75 exhibits a lower emission intensity and its broad emission differs in shape to the corresponding emission of the higher bloom gelatine. These broad and low emissions of type-B gelatines have been assigned [19] to the presence of pentoxidine residues in the structure. The intensity of the fluorescence emission has been correlated with gelatine colour and, also, with the animal age from which the collagen, precursor of the gelatine, is obtained. The excitation spectra recorded with our gelatine films, fixing the emission wavelength at 470 nm gives a very low absorption band in the 335/385 nm wavelength region with a maximum at 355 nm. This absorption is in agreement with the presence of a pentoxidine residue.

Gelatine films cast from aqueous solution at temperatures below 35 °C ("cold cast") show evidence of collagen residues, whereas gelatine films cast above this temperature, as prepared in this work (37 °C, "hot cast"), appear to be completely amorphous [20,21]. Amorphous gelatine, solid and dry, undergoes a glass transition at a temperature [22] of 217 °C and a melt temperature [23] T_m of 230 °C determined by DSC. The glass transition temperature of the gelatine is dramatically reduced by moisture [24,25] and the T_g is less than 100 °C when the content of moisture is 10%. If gelatine is heat-dried below 1% moisture, it tends to crosslink becoming more glassy and brittle [26]. Another complication in the determination of the T_g for gelatine is the occurrence of the melting and the chemical decomposition in the region just above the glass transition.

The two gelatine grades used in this work were analysed by DSC in order to estimate the glass transition temperature of the prepared films and their comparison with the corresponding raw materials in powder form. From the thermograms, the gelatine of bloom 75 has a T_g of 183 °C lower than that of the gelatine of bloom 225 which is 187 °C. These values are in agreement with the glass transition temperatures of solid state gelatines having 4% of content of moisture (determined by TGA). In Table 1, some comparative values of viscosity, thermal properties, absorption and fluorescence emission are summarised for the two type-B gelatine grades.

The viscosity measurement in aqueous solution (6.67 wt.%) are in agreement with the order of molecular weight and the quality of the gelatine, hence, gelatine of higher bloom value exhibits the higher viscosity.

3.2. Chemiluminescence of gelatines under nitrogen

The measurement of chemiluminescence under an inert gas, such us nitrogen, gives information on the thermal story of polymers, for instance its manufacturing process. The intrinsic characteristics of the material play an important role in influencing the formation of minor amounts of anomalous structures or oxidation defects in the polymer. Hence, if some structural defects are formed in the gelatine materials during their extraction process, it would be possible to estimate relatively these defects by the highly sensitive chemiluminescence signal. As shown in Fig. 4, CL-temperature-ramping tests under nitrogen of the two bloom value gelatine grades are similar in shape but slightly different in relative intensity.

The obtained CL-profiles versus temperature confirm the week intensity of the emission under nitrogen. The low level of emitting species present in the materials and the absence

Table 1
General characteristics determined for type-B gelatines

Gelatine[a]	Viscosity[b] (cP)	Form	T_g	T_m	$\lambda_{F\text{-}max}$ (nm)[c]	λ_{ABS} (nm)
Bloom 75	3.6	Powder	183	215		
		Film	176	–	470, 430 (sh)	280[d]
Bloom 225	5.5	Powder	187	223		
		Film	181	–	470–430	280[d]

[a] Moisture content 4%, determined by TGA.
[b] Determined at 37 °C at the standard concentration of 6.67% in MiliQ Water.
[c] $\lambda_{EX} = 355$ nm.
[d] Second derivative spectrum shows maxima at: 286, 278, 267, 261 nm.

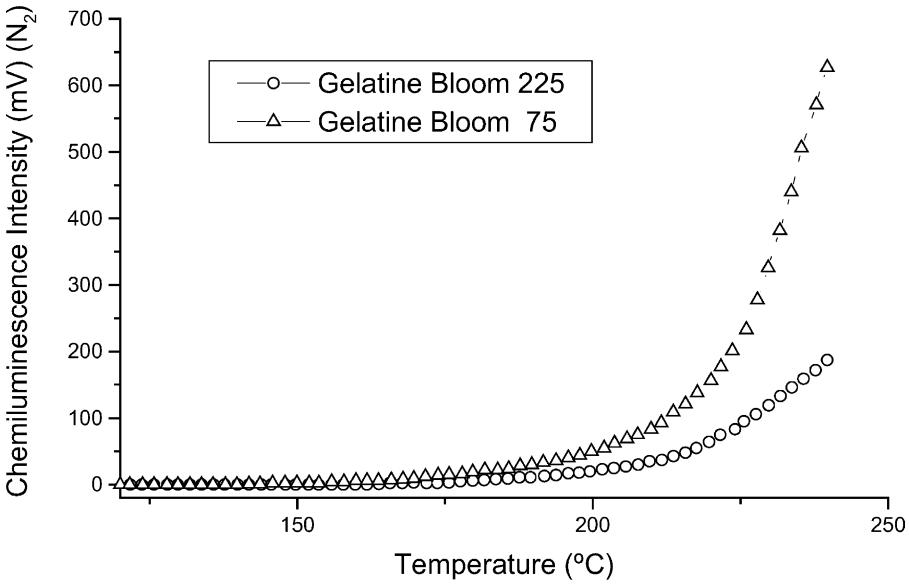


Fig. 4. CL temperature-ramping curves obtained for gelatine films under nitrogen.

of emission until high temperatures, >180 °C, confirms the thermal stability of dry gelatines. The gelatine of bloom 75 exhibits an earlier and more intense emission than the gelatine of bloom 225. This result is in accordance with the more degraded structure of gelatine bloom 75 because of its extraction at higher temperatures on the manufacturing process. The emission of chemiluminescence of both materials starts in the region of their glass transition temperatures confirming that the loss of rigidity favours the emission reaction.

The higher quality of the bloom 225 gelatine is evidenced by the behaviour of the films in isothermal chemiluminescence experiments under nitrogen. In Figs. 5 and 6 the isothermal emission at 160 and 225 °C are plotted.

At a temperature lower than the glass transition, 160 °C, the emission of gelatine materials (Fig. 5) is very weak but there is a significant difference between the two bloom types of gelatines. Higher bloom gelatine exhibited a negligible emission compared to the lower bloom material and both showed a unique CL-peek on the first 15 min. After this start of the emission the chemiluminescence is maintained (stationary CL-intensity, I_s) indicating that the gelatine materials are not degraded under nitrogen at this temperature and within this time scale. The CL-emission at 225 °C, temperature above the glass transition, is enhanced by the loss of rigidity of the film and shows the same behaviour, higher emission of the low bloom gelatine. The emission of both materials is nearly constant after the first 30 min indicating that at this temperature a progressive decomposition of the material takes place giving a constant emission. In such conditions, the intensity of the stationary emission is an indication of the resistance of the material to oxidation

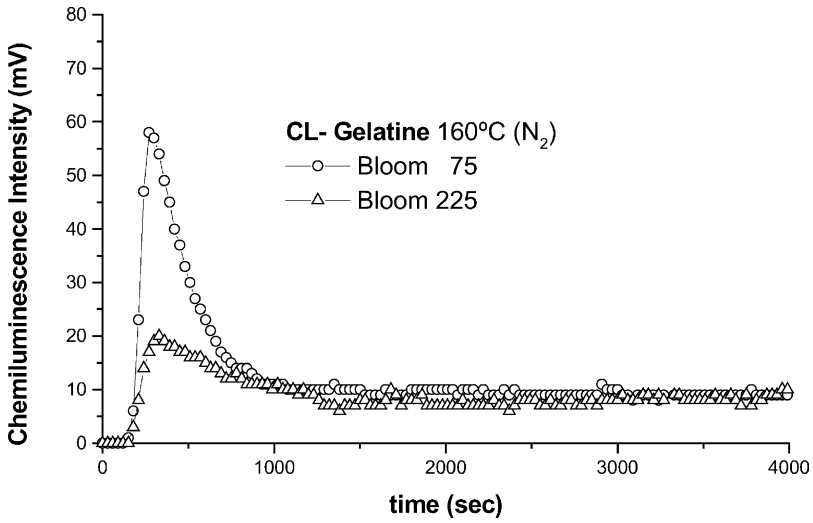


Fig. 5. CL-emission profiles for gelatines films obtained at 160 °C under nitrogen.

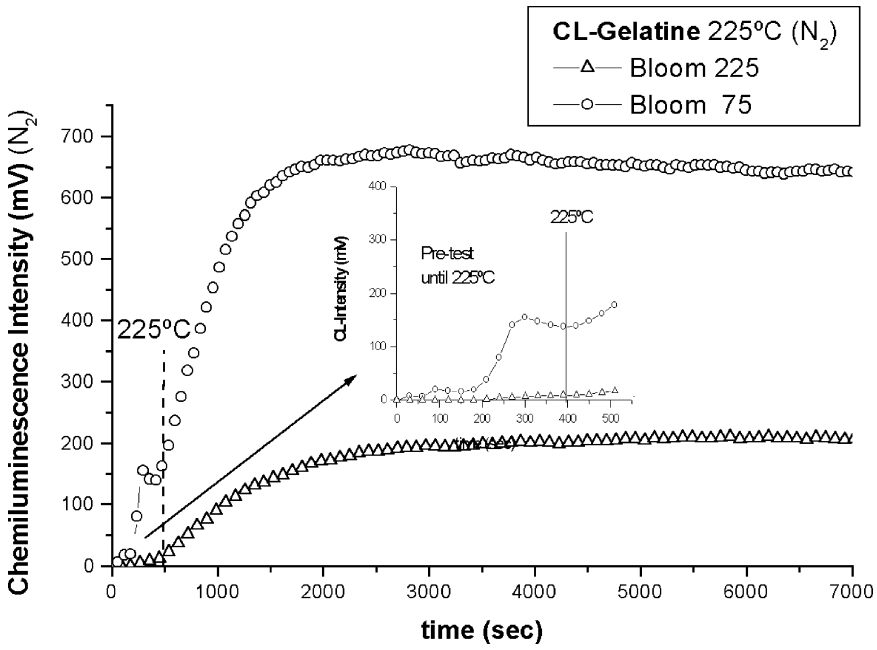


Fig. 6. CL curves for gelatine films obtained at 225 °C under nitrogen. The inset shows the emission during the pre-test, under nitrogen and heating rate of 10 °C/min.

and the higher bloom gelatine exhibits the higher stability. In the case of gelatine bloom 75, the emission starts during the pre-tests ($T < 225$ °C) in contrast with the bloom 225 gelatine. This behaviour confirms its lower rigidity and the higher oxygen mobility in the material. In Table 2, the chemiluminescence parameter of both gelatine materials are summarised.

The emission of the lower bloom gelatine is higher as it can be seen from the CL-parameters, intensity and area. The initial hydroperoxide content determined for the gelatine films are low but, are in agreement with the order of stability. Hence, higher hydroperoxide content corresponds with higher emission in the more degraded gelatine of bloom 75.

The structure of the commercial gelatines is heterogeneous with respect to the composition amino acids. Gelatine has a higher content of three amino acids, glycine (33 mol%), proline (10 mol%) and hydroxyproline (5 mol%). Proline and its hydroxy derivative are nitrogen heterocyclic organic compounds that can suffer, as well as glycine, an oxidation reaction in the α-carbon to the nitrogen of the *N*-alkyl amide of their structure in the gelatine. Such reactions have been observed in the autoxidation of *N*-alkyl amide [27–29] model compounds at temperatures lower than 100 °C and also photochemically at short (254 nm) and long ($\lambda > 300$ nm) wavelengths. This oxidation has also been observed in polymers such us nylon and polyamides [30,31]. In Scheme 1, a simplified fragment of the gelatine structure (A) containing glycine and proline—(Gly-Pro-Gly)—is shown together with the oxidation mechanism for peroxide formation that can take place in gelatine materials. This mechanism is analogous to the reactions proposed by other authors for the thermal autooxidation of *N*-alkyl amides at low temperatures.

The thermal fragmentation of labile bonds, such the C–H in the α-carbon to the nitrogen of the amide group, produces chain macroradicals that can be oxidized giving rise peroxide radicals. These peroxide radicals can form hydroperoxides through hydrogen atom abstraction as shown in Scheme 1(B). Also, two peroxide radicals can give chemiluminescence emission through the thermal disproportionation commented on before (Eq. (3)). In the gelatine there are few possibilities for radical formation, some of them are

Table 2
Chemiluminescence data of gelatines type-B obtained under nitrogen

Sample	T (°C)	Test atmosphere	$I_{CL\text{-}max}$[a] (mV)	$A_{CL\text{-}peak}$[b] $\times 10^3$ (mV)	I_s[c] (mV)	$[POOH]_{initial}$[c] (mol./g)
G-75	160	N_2	58	20.5	–	3.50×10^{-6}
G-225	160	N_2	20	6.4	–	2.75×10^{-6}
G-75	225	N_2	660	–	640	
G-225	225	N_2	170	–	200	

[a] $I_{CL\text{-}max}$: intensity of the chemiluminescence maximum.
[b] $A_{CL\text{-}peak}$: area of the chemiluminescence peak.
[c] Determined by iodometric titration.

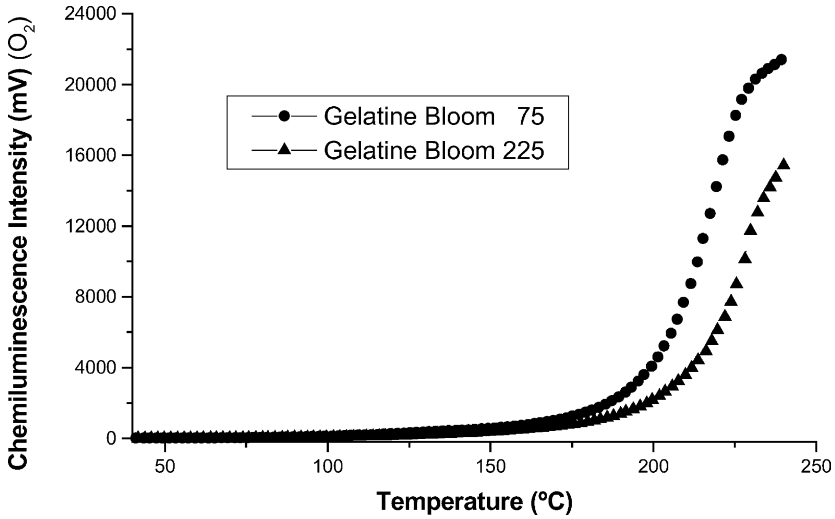


Fig. 7. CL temperature-ramping curves obtained for gelatine films under oxygen.

pointed out with arrows in the simplified gelatine structure shown in Scheme 1(A). During the manufacturing process of gelatines this mechanism can take place to a lower extent, as the low CL-emission under nitrogen and the low measured concentrations of hydroperoxides put in evidence.

3.3. Chemiluminescence of gelatines under oxygen

The non-isothermal chemiluminescence emission under oxygen for the two studied gelatines is plotted in Fig. 7.

The CL-emissions obtained in oxygen with the linear increase of temperature are much more intense than that obtained under nitrogen due to the oxidation conditions. Both materials behave with the same order of stability commented on before, again high bloom gelatine exhibits the lower emission. The oxidation rate and the subsequent chemiluminescence emission is a maximum in the interval of temperature above the T_g of the gelatines, where there is an increase of the oxygen mobility of the materials due to the loss of rigidity.

Table 3
Activation energies[a] of the CL-emission for commercial gelatines

Material	T_g region (175 < T <225 °C)	Low T region (T < 160 °C)
Gelatine bloom 75	89.8	42.3
Gelatine bloom 225	105.6	43.2

[a] E_a (kJ/mol).

Re-plotting the ramping temperature CL-emission profile in Arrhenius form, two different temperature intervals can be observed. The Arrhenius diagram is shown in Fig. 8 and the calculated activation energies are summarised in Table 3.

The process of low activation energy predominates at low temperatures, and that of high activation energy

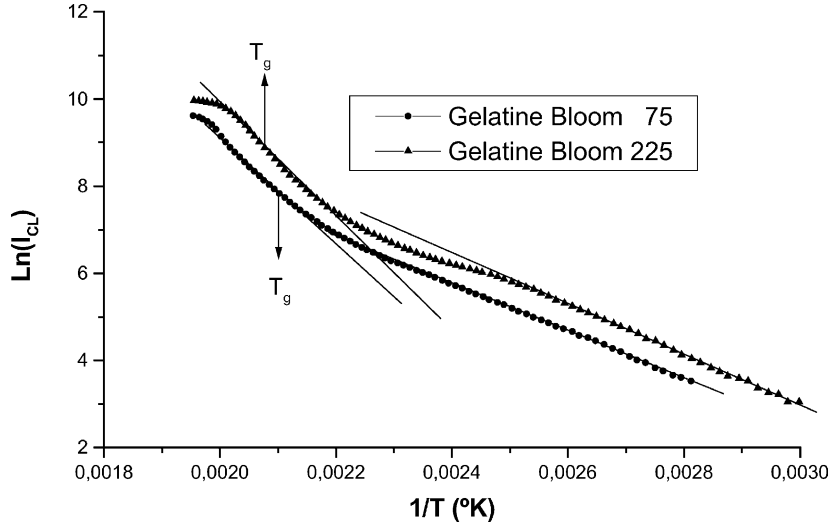


Fig. 8. Arrhenius plot for non-isothermal chemiluminescence profiles obtained with gelatine films under oxygen.

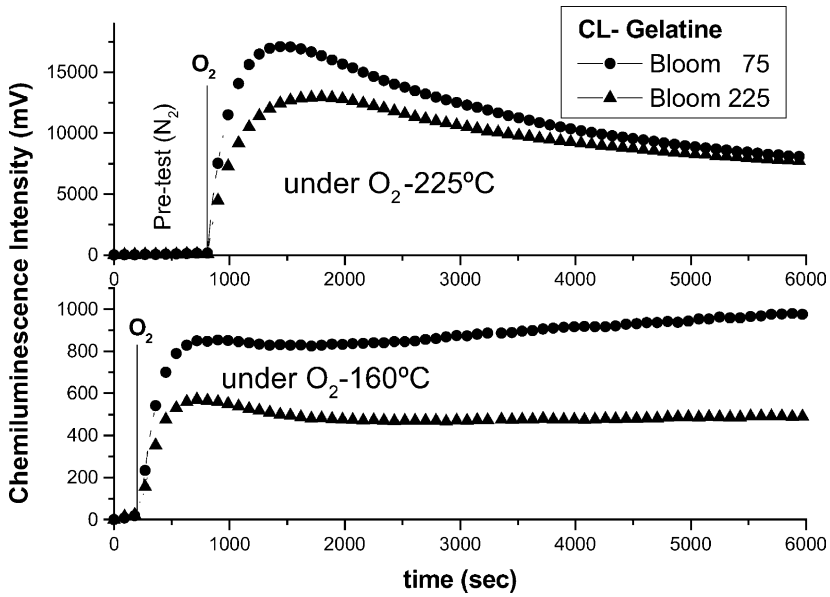


Fig. 9. CL-emission profiles for gelatines films obtained at 160 and 225 °C under oxygen. The pre-test (heating rate 10 °C/min) data under nitrogen are also included.

Table 4
Chemiluminescence data of gelatines type-B obtained under oxygen

Sample	T (°C)	Test atmosphere	$I_{CL\text{-}max}$[a] (mV)	$A_{CL\text{-}peak}$[b] $\times 10^3$ (mV)	I_s[c] (mV)	$[POOH]_{initial}$[c] (mol./g)
G-75	160	O_2	860	–	900	3.50×10^{-6}
G-225	160	O_2	550	–	500	2.75×10^{-6}
G-75	225	O_2	17000	219.9	–	
G-225	225	O_2	13000	207.1	–	

[a] $I_{CL\text{-}max}$: intensity of the chemiluminescence maximum.
[b] $A_{CL\text{-}peak}$: area of the chemiluminescence peak.
[c] Determined by iodometric titration.

predominates at higher temperatures in the glass transition region. This high activation energy value is an indication that bond cleavage and the subsequent formation and reaction of peroxyl radicals shown before in eq. [3] can contribute to the emission. For this mechanism, high temperatures (T_g region) and mobility in the material are required. The higher bloom gelatine exhibits higher activation energy of the CL-emission in the T_g region, confirming again its higher rigidity. The obtained values of the temperature coefficients from Arrhenius plot are in the order of those obtained for other polymers [32]. For example, with cellulose in sheet form, Matisová-Rychlá et al. founded 48 and 72 kJ mol $^{-1}$ for the intervals below and above the T_g of the cellulose which, as in gelatine, strongly depends on the content of moisture.

In the presence of oxygen, the CL of the gelatine materials is increased since oxidation takes place efficiently. Again, significant differences can be observed with temperature. At higher temperatures than the T_g the emission is clearly enhanced due to the higher oxygen mobility in the materials. The emission profiles at 225 and 160 °C are shown in Fig. 9.

From the emission under oxygen the CL-parameter before commented can be determined, the data are summarised in Table 4.

At 160 °C, a stationary emission (I_s) is observed indicating the lower oxidation rate, in contrast to that at 225 °C, where the emission gives a peak with much higher intensity. The gelatine grade of lower bloom shows the higher degradation rate in agreement with its lower quality.

4. Conclusions

The obtained results on chemiluminescence emission from gelatine materials confirm that this technique can be a useful tool to characterise the solid state of gelatines. It can be applied to different commercial grades and applications for oxidation control during the manufacture and use of gelatines. The differences observed in the chemiluminescence under different conditions are sensitive to the molecular weight and gel resistance value. The higher bloom gelatine exhibits lower emission, under nitrogen and under oxygen, confirming its better quality and its higher

resistance to oxidation, in comparison to the lower bloom gelatine.

The emission can be explained through the peroxide radical formation by the same mechanism that other authors have proposed for the oxidation at low temperatures related to *N*-alkyl amides. The mobility of the oxygen or the mobility of the peroxide radical to give the CL-reaction can be used at different temperatures for obtaining information on the gelatine materials. Hence, CL-emission could be used to study different processes that modify the gelatine structure.

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