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On the action of ozone on gelatin

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Abstract

Gelatin, the lower molecular weight derivative of collagen, was treated with ozone and the structural changes were studied with polarimetry, electronic absorption and FT-IR spectroscopy. The resulting ozonized gelatins were studied by thermal analysis (DSC and TGA) in comparison to a reference gelatin. It has been found that at relatively low ozone dose, for instance at ozone/gelatin molar ratios between 0.1 and 0.35 the structural damage introduced by ozone oxidation of gelatin is minimal to negligible and this may facilitate the industrial utilization of ozone in the cold sterilization of gelatin as well as in its bleaching processes required for certain applications. At higher O_3/gelatin molar ratios the gelatine damage is evident both polarimetrically as suggested by the drop of its specific optical rotation and by spectrophotometrical analyses.

Keywords: Gelatin; Collagen; Protein; Optical activity; Optical rotation; Chiro-optical properties; Polarimetry; Oxidation; Ozonation; Ozonolysis; Ozone; Cold sterilization

1. Introduction

Gelatin is the hydrolyzed collagen industrially derived from the chemical and thermal processing of pig or cattle skin [1,2]. A minor fraction of the industrial gelatin is also derived from pig and cattle bones, fish and poultry [1,2].

Two types of industrial processes can be distinguished, the alkaline process and the acid process. The first process is used for the extraction of collagen from cattle skin and it requires several weeks while the acid process is dedicated to the collagen extraction from pig skin and requires just few hours [1,2].

Collagen is the raw material of the connective tissues and in mammals it represents about 30% of the total body protein [3]. In fact, its chemical structure is characterized by protein chains which are heavily interconnected with each other. The typical superstructure or tertiary–quaternary structure of collagen involves a triple stranded and left-handed helix structure [1–3]. However, the industrial extraction process of collagen involves its denaturation so that after the industrial processing it acquires solubility and a lower molecular weight but it looses its tertiary and quaternary structure i.e. the triple chain structure is lost. The product obtained from collagen extraction is called gelatin. The collagen denaturation in its passage to gelatin can be followed polarimetrically. The specific optical rotation $[\alpha]_D$ of collagen is comprised between -380 and -420 [4] but drops to -137 in common gelatine [3] (see Fig. 1).

Because of its origin, the industrial production of gelatin requires also a sterilization step. In a patent [5], the use of diluted hydrogen peroxide as sterilizing agent in gelatin production has been reported. Hydrogen peroxide can be used also as a bleaching agent in gelatine production from collagen [5] and as solubilizing agent of collagen with or without a hydrogen bond breaking agent such as a salicyclate or more common lyotropic agents like LiCl [6].

Depending on the final application, the industrial gelatin may also have special colour characteristics as disclosed in a patent [7]. Particularly important is the absorbance at 420 nm which is called “colour of the gelatin” and which should be as low as possible.

In the present paper, we are reporting about the use of ozone for the treatment of gelatine which could find application both for the gelatin cold sterilization but also for the bleaching of its colour.

2. Experimental

2.1. Materials and equipment

Gelatin from porcine skin was obtained from Fluka (Switzerland). Gelatin used in this work was a standard grade for

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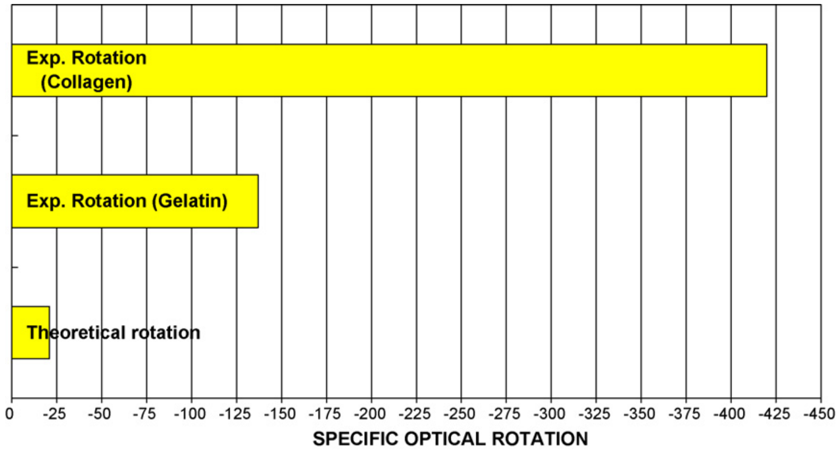

Fig. 1. Experimental specific optical rotation [α] of collagen and gelatine in comparison with the theoretical value calculated on the basis of the specific optical rotation of the amino acid residues. The much higher [α] observed for gelatin and collagen are due to the chiral amplification of the optical activity of the amino acid residues. In fact, the secondary and tertiary superstructures assumed by the gelatin chains and also the quaternary structure of collagen are responsible for the considerable enhancement of the basic specific optical rotation due to the amino acid residues.

microbiology having a gel strength of 70 g. Ozone was prepared electrochemically as reported elsewhere [8]. Polarimetric measurements were performed on Pol-1 polarimeter from Optika using the conventional D sodium line as light source. All polarimetric measurements were made at room temperature. Electronic absorption spectra were recorded on Shimadzu UV160A spectrophotometer. FT-IR spectra were made on a Nicolet IR-300 spectrometer from Thermo-Electron Corp. on dried gelatin samples recovered after precipitation from aqueous solution by addition of acetone (see Section 2.1.1).

DSC (differential scanning calorimetry) was performed in static air at 5 °C/min in a Linseis apparatus model L63. TGA-DTA (thermogravimetric analysis with simultaneous differential thermal analysis) was made on a Linseis machine model L81 under nitrogen flow and at a heating rate of 10 °C/min.

2.1.1. *Polarimetric measurements during ozonation*

The changes in the specific optical rotation of gelatin under the action of ozone were made on an aqueous solution of 2.12 g of gelatin in 100 ml of distilled water. The gelatine solution was placed into a 2 l round bottomed flask. The flask was evacuated with the aid of an aspirator and then an ozone/oxygen mixture having an ozone content of about 200 mg was admitted into the flask. The flask was hand shaken violently for 2 min and then was left under magnetic stirring for about 3–4 h, a time judged sufficient for a complete reaction with ozone. Then the flask was opened and the optical activity of the gelatin solution checked polarimetrically. The same operation was repeated six times so that the curve of Fig. 2 was obtained.

It has been noticed that initially the gelatine showed an easy trend to produce foam but in the latest stages of ozonation this property was completely lost. After ozonation, the gelatin was isolated by precipitation with an excess of acetone and centrifugation followed again by acetone washing and then was left to dry in air for some days. These samples were used for the FT-IR measurements on a ZnSe plate in reflectance mode. A reference not ozonized gelatin sample was precipitated from aqueous solution with acetone and left to dry in air as in the case of the ozonated gelatin. After drying, it was analyzed by FT-IR in reflectance mode as for the ozonized samples.

Only for the highest molar ratio measurements of Fig. 2, a more diluted gelatin solution was used, i.e. a solution of 382 mg of gelatin in 200 ml of water in a 1 l flask with the same procedure described above for the more concentrated solution.

2.1.2. *Electronic absorption spectra of ozonated gelatin*

Gelatin (150 mg) was dissolved in 50 ml of distilled water. The electronic abosorption spectra were recorded in series during the addition of ozone.

In another run, use was made of a more concentrated gelatin solution: 4.28 g in 100 ml of distilled water. Under

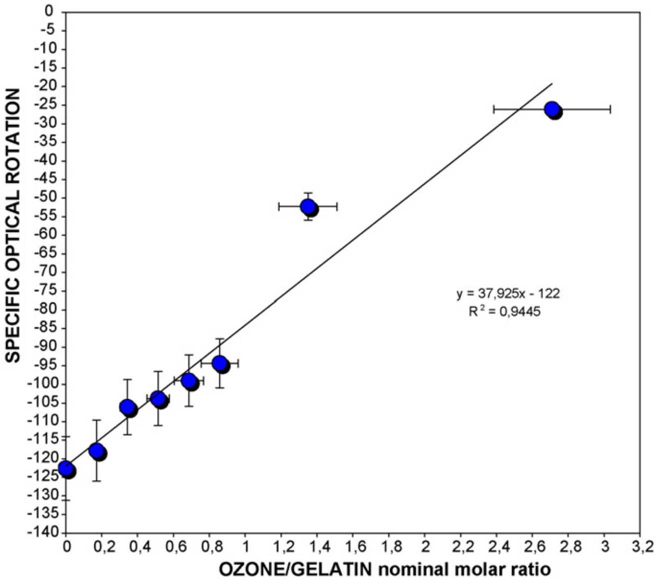

Fig. 2. Reduction of the specific optical rotation of gelatin as function of the amount of ozone added. The experimental data can be fitted by a linear relation with $[\alpha]_{\text{gelatin}} = 37.925\, m_r - 122$; m_r is the nominal molar ratio ozone/gelatin.