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The Effect of Irradiation on Rheological and Electrical Properties of Collagen

Aleš Landfeld[1], Milan Houška*[1], Jan Skočilas[2], Rudolf Žitny[2], Pavla Novotná[1], Jaromír Štangl[2], Martin Dostál[2], David Chvátil[3]

[1]Food Research Institute Prague, Radiová 7, 102 00 Prague 10, Czech Republic
[2]Czech Technical University in Prague, Faculty of Mechanical Engineering, Department of Process Engineering, Technická 4, 166 07 Prague 6, Czech Republic
[3]Nuclear Physics Institute of the ASCR, v.v.i., Department of Accelerators – Microtron Laboratory, 250 68 Řež, Czech Republic

* Corresponding author: milan.houska@vupp.cz

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Abstract:
This paper describes the effects of irradiation on the rheological and electrical properties of a 7.7 % mass fraction of native bovine collagen in water. The radiation dose was in the range of 0 – 500 Gy. Rheological oscillation measurements were done at temperatures of 10, 20, and 30 °C. There was a statistically significant dependency of storage and loss moduli on irradiation dose and oscillation frequency. There was no significant change in the electrical conductivity of collagen during oscillation movements or any dependence on irradiation dose.

Key words:
Bovine collagen, electrical conductivity, storage modulus, loss modulus, electron irradiation, crosslinking

1 INTRODUCTION

Collagen is a fibrous protein that is abundantly found in animal connective tissue, constituting approximately 30 % of total protein [1]. Collagenous compounds exist in many forms, e.g. in combination with glutaraldehyde or polyvinyl alcohol. Such compounds are used for extruding vascular grafts in biomedicine [2] or for sausage casings in the food industry [3]. As mentioned in [4] the authors showed that physical methods like UV irradiation or dehydrothermal treatment can be used as safe and effective alternatives to chemical cross-linking for production of collagen casings.

Collagen (type I) is the most common type of collagen and is the main component of the extracellular matrix of animal connective tissue [5, 6]. Despite its relative abundance in animal connective tissues, there is surprisingly little published information regarding its rheological properties after irradiation with an electron beam. This lack of information is largely due to problems with the application of rotational rheometers (e.g. slip wall effects, and discharge of tested samples from gaps between the cone-plate geometry or the plate-plate geometry) [7]. The main problem with rheological properties measurements is caused by very high concentrations of dry matter in the collagen solution. Additionally, few articles exist with rheological measurements relative to collagen solutions. Publication [8] presents rheological and structural properties of collagen from the skin of large fin long barbel catfish. The rheological properties of collagen can be changed through the use of different crosslinking agents, e.g. basic chromium sulfate (BCS), tannic acid, catechin, and formaldehyde [9].

There are several papers dealing with the effects of gamma or UV irradiation on collagen. UV irradiation of porcine valves caused the formation of crosslinking matrices, see [10]. The influence of gamma irradiation on collagen fibril structure was described by Leontiou et al. [11]: The paper studied mouse skin collagen from the point of view of structure. Collagen fibrils of irradiated collagen samples were found to be much more substantial than fibrils in untreated samples. An excellent overview article by Parenteau-Bareil et al. [12] dealt with collagen as building material for tissue engineering applications. Grant et al. [13] provides an overview of the existing literature, at that time, describing methods to influence the mechanical properties of collagen through electron irradiation. The authors used rat tail tendon collagen and studied changes in structure and

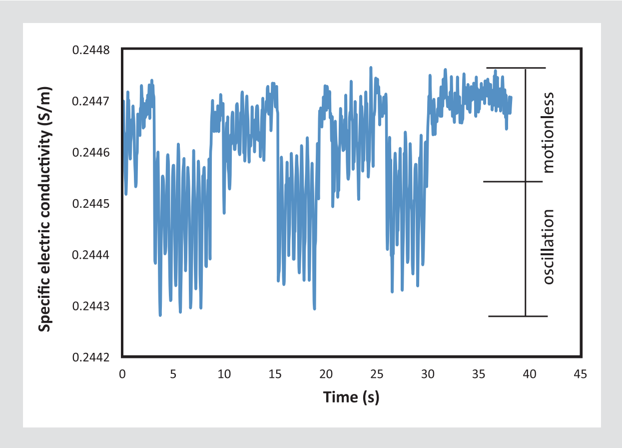

Figure 10: Changes in specific electric conductivity during oscillating deformations (1 Hz, 20 % relative deformation, 500 Gy).

irradiation (Gy)	Relative deformation (%)	Specific conductivity (S/m) motionless region	I value (S/m)	Specific conductivity (S/m) oscillation region	value I (S/m)	Decrease of specific conductivity (%)
0	4	0.247086	0.004296	0.247086	0.004296	0.00
0	10	0.245417	0.002453	0.245417	0.002453	0.00
0	20	0.244726	0.000243	0.244515	0.000197	0.09
0	190	0.241588	0.000655	0.240616	0.000603	0.40
500	4	0.256847	0.000770	0.256847	0.000770	0.00
500	10	0.254578	0.000478	0.254578	0.000478	0.00
500	20	0.244538	0.000426	0.244483	0.000046	0.02
500	190	0.242878	0.001341	0.241888	0.001006	0.41

*Table 4: Specific conductivity as a function of irradiation dose, relative deformation, and deformation regime (motionless, oscillation). I value is the variability interval defined as $I = t_{crit}s/n^{0.5}$ (n = number of points, t_{crit} = Student coefficient critical value). Decrease of specific conductivity is defined as D = 100 – specific conductivity at oscillation/specific conductivity at no-motion*100.*

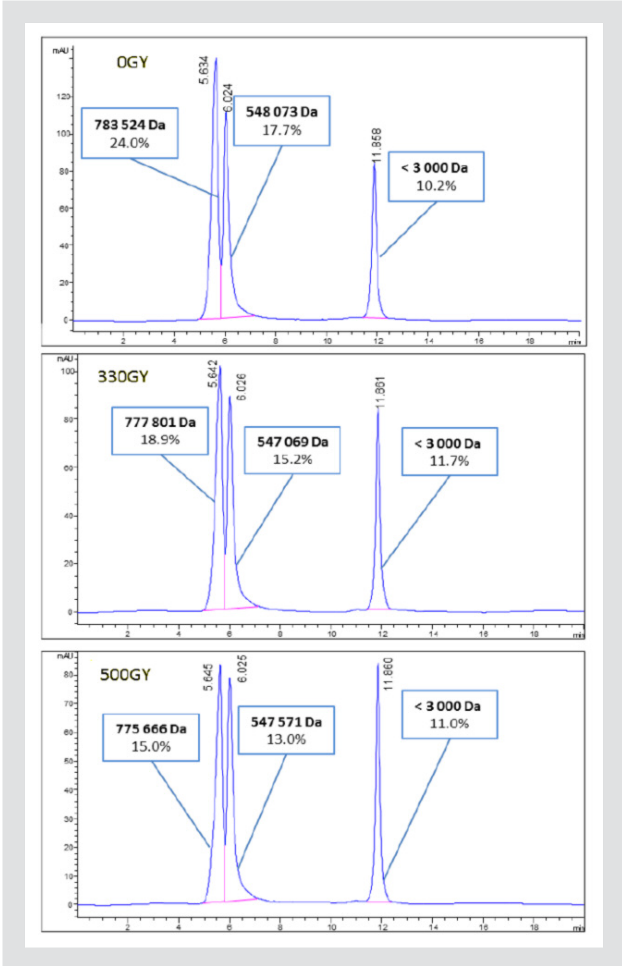

Figure 11: Percentage of fractions of collagen molecular size for differently irradiated samples. Graphs represent the shapes of chromatography peaks (horizontal axis represents time in minutes).

dependence of specific electric conductivity during oscillating deformation at a frequency of 1 Hz, shear relative deformation of 20 %, and an irradiation dose of 500 Gy. It is clear from Table 4 that decreases in specific conductivity due to oscillations are very small (maximum 0.41 %) as such this influence can be omitted. Therefore, we can conclude that the impact of collagen deformation on changes in specific conductivity were negligible. Additionally, it was shown that the radiation dose had no effect on specific electric conductivity (Table 4). Differences in specific electric conductivity, valid for samples irradiated by 500 Gy and zero dose are statistically insignificant. Irradiation apparently influences only consistency parameters as measured by oscillatory rheometry. Crosslinking that increases *G'* is not evidently capable of increasing electrical connections within the macromolecular structure of the studied collagen.

3.3 MACROMOLECULES SIZE

Figure 11 presents chromatographic results of the molecular mass of individual fractions and percentage of collagen content in those fractions. Percentages were calculated in milligrams per 100 mg of dry matter for irradiation doses of 0, 330, and 500 Gy. It can be seen from Figure 11 that the irradiation dose had practically no influence on molecular mass fractions (distribution of detected mass fractions). On the other hand, irradiation dose did influence the overall content of eluted collagen: 52 % for non-irradiated samples, 46 % for 330 Gy, and only 39 % at the maximum irradiation of 500 Gy. The percentage of macromolecule masses, largest to smallest (about 780 kDa and 547 kDa), regularly decreased with increasing irradiation dose (Figure 11).

4 CONCLUSIONS

Bovine collagen water solutions behave like a linearly viscoelastic material in the frequency range studied, i.e. as a viscoelastic gel-like body. Its rheological behavior can be characterized by two components from the Maxwell model. Its model parameters can be influenced by electron irradiation doses, namely elasticity modules G_1 and G_2. The higher the irradiation doses the higher the values of these parameters. All model parameters were correlated with temperature and electron irradiation

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dose and linear correlations were found, with only one exception, that being μ_1, which did not exhibit any statistically evident dependence on collagen temperature. Our results provide evidence that collagen electron irradiation can substantially influence rheological properties. Deformation of collagen was found to have a negligible effect on specific electric conductivity (variations). The same was true for the radiation dose on specific electric conductivity. Electron irradiation dose had practically no influence on molecular mass fractions but did influence the percentage of collagen content in the fractions. We hope that the results presented in this paper can be applied in the food and biochemical engineering industry in the near future. Knowledge of collagen solution properties, as influenced by electron irradiation, can help in innovation of biomedical technologies, e. g. production of artificial cartilage or veins, or in the food industry for novel methods of producing collagen casing.

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ETHICAL STATEMENT

This study did not involve any human or animal testing.

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